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Pharmacopeial Forum is covered in *Current Contents/Life Sciences* and in the *Science Citation Index (SCI)*, in *International Pharmaceutical Abstracts*, and in *Current Awareness in Biological Sciences*.

The United States Pharmacopeial Convention comprises representatives from colleges and national and state organizations of medicine and pharmacy. It publishes the *U.S. Pharmacopeia* and *National Formulary*, the legally recognized compendia of standards for drugs and products of other health care technologies. The *USP* and *NF* include assays and tests for the determination of strength, quality, and purity and requirements for packaging and labeling. **Pharmacopeial Forum's Public Review and Comment Process**

This section presents an overview of the public review and comment process, conducted through *Pharmacopeial Forum (PF)*, for the development of official standards in the *United States Pharmacopeia* and the *National Formulary (USP-NF)*.

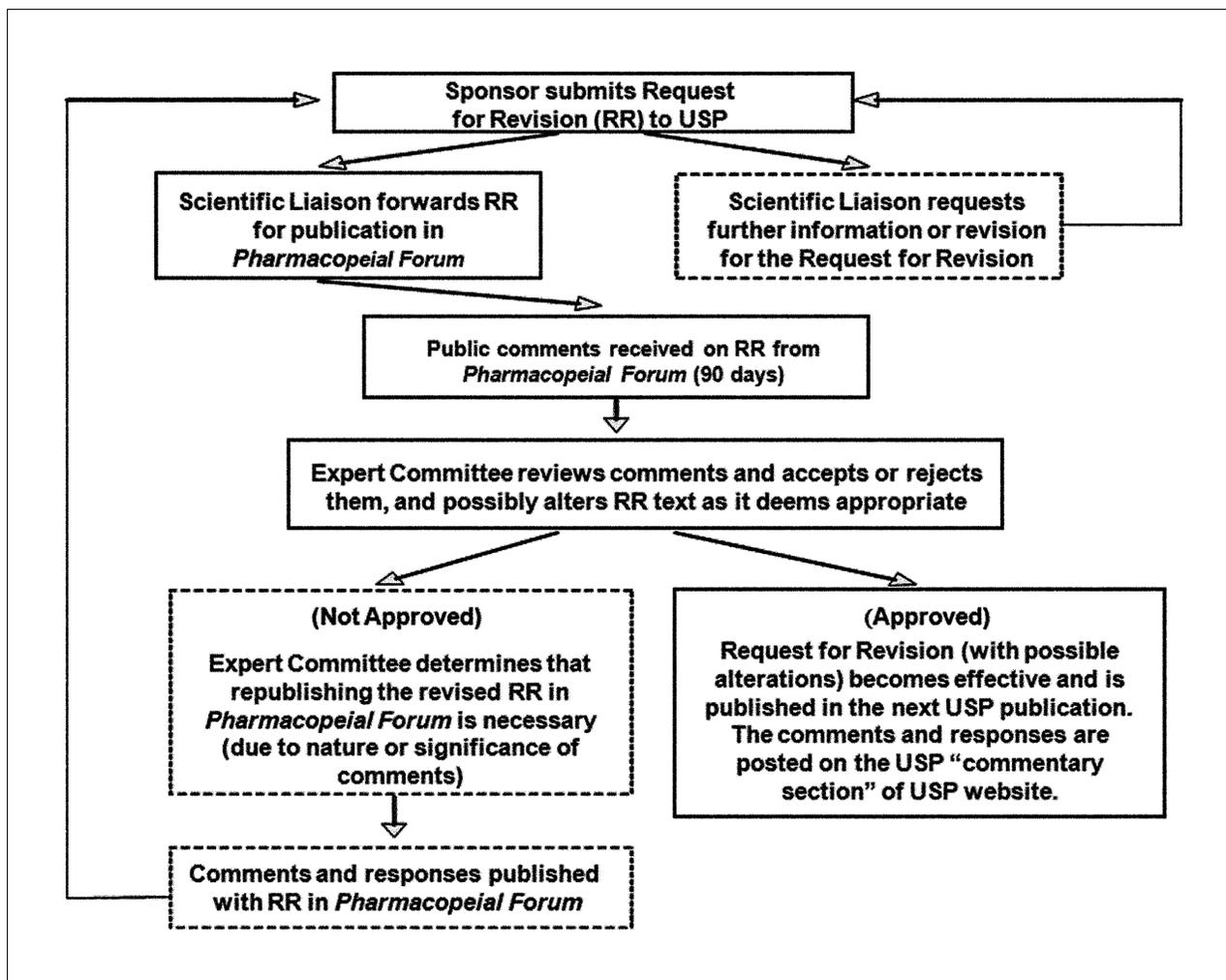
USP publishes *PF* on a bimonthly basis to provide an opportunity to review and comment on new or revised standards.

There are two types of proposed revisions in *PF*:

1. **Proposed Revisions**—New or revised standards targeted for adoption through USP's Standard Revision Process. USP's Revision Process calls for publication of a proposed revision in *PF* for a 90-day notice and comment period. After the comment period and subsequent review of comments and approval by the relevant USP Expert Committee, the official standard is published in the next available *USP-NF* or *Supplement*. If comments received are significant, or if the Expert Committee's consideration of comments results in significant additional changes, the Expert Committee may determine that republishing in *PF* is necessary prior to the revision becoming official. See the *In-Process Revision* section for current proposed revisions.
2. **Proposed Interim Revision Announcements**—New or revised standards that become official through an accelerated process in accordance with USP's Guideline on Accelerated Revisions (available on the USP Web site). *Interim Revision Announcements (IRAs)* allow for a revision to become official prior to the next *USP-NF* or *Supplement*. *IRAs* are first presented for a 90-day public comment period in the *Proposed Interim Revision Announcement* section of the *PF*. Note that final *IRAs*, as well as *Errata*, and *Revision Bulletins*, which also are defined in the Accelerated Revision Guideline, appear only on the USP Web site.

USP welcomes comments and data on proposed revisions. A summary of comments received, along with USP's responses, will be published in the *Revisions and Commentary* section of the USP website (<http://www.usp.org/USPNF/revisions/>).

The chart below shows the public review and comment process and its relationship to standards development.



Questions on the process should be addressed to the USP Executive Secretariat, United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852 (e-mail: execsec@usp.org).

HOW TO USE PF

The various sections of *PF* are briefly described below. A more detailed description of each section is provided at the beginning of that section. A general description of the types and amount of information expected in a *Request for Revision* is available in the *Guideline for Submitting Requests for Revision to the USP-NF* on the USP Web site (www.usp.org/USPNF/submitMonograph/subGuide.html). Note that the Expert Committee listing and the Scientific Staff Directory also are located on the USP Web site (see below for links).

Proposed and Adopted Revisions to the USP-NF

Section	Content	How Readers Can Respond
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<p>Proposed Interim Revision Announcements</p>	<p>Proposals for <i>Interim Revision Announcements (IRAs)</i> that will be published as official <i>USP</i> or <i>NF</i> standards BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Expert Committee and Scientific Liaisons who handled the monograph or general chapter.</p>	<p>Review material and send comments within 90 days of the <i>PF</i> publication in which the standard was proposed (or per the comment deadline listed in the Briefing section). Direct comments to the USP Scientific Liaison using the contact information provided at the end of each <i>Proposed IRA</i></p>
<p>In-Process Revision</p>	<p>Proposals for standards that will be published as official in a future <i>USP-NF</i> book or <i>Supplement</i>. BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Expert Committee and USP Scientific Liaisons who handle the monograph or general chapter.</p>	<p>Review material and send comments within 90 days of the <i>PF</i> publication in which the revision was proposed (or per the comment deadline listed in the Briefing section). Direct comments to the USP Scientific Liaison using the contact information provided at the end of each proposed revision. For general inquiries or in cases where a Scientific Liaison is not identified, use the general USP telephone number 301-881-0666 or fax number 301-998-6839 or stdsmonographs@usp.org.</p>

<p>Stage 4 Harmonization</p>	<p>Revision proposals from the Pharmacopoeial Discussion Group (PDG), which comprises the European Pharmacopoeia, the Japanese Pharmacopoeia, and USP. The Stage 4 draft and the briefing are published in the forum of each pharmacopoeia for public comment. The draft is published in its entirety. BRIEFING: Scientific rationale for the potential inclusion or change or for the proposed change.</p>	<p>Review material and provide comments to the USP Scientific Liaison using the contact information provided at the end of each Stage 4 Harmonization. Individuals who wish to correspond with the European and Japanese Pharmacopoeias concerning monographs in the Stage 4 period of international harmonization should address their comments to the coordinating pharmacopoeia, with a copy to USP.</p> <p>PhEur Secretariat Ms. Lynn Kelso-Eleuterio Central Secretariat European Pharmacopoeia Department European Directorate for the Health Care Council of Europe 7, Allée Kastner CS 30026 67081 Strasbourg France Tel: +33 (3) 88 41 31 48 Fax: +33 (3) 88 41 27 71 lynn.kelso@edqm.eu</p> <p>JP Secretariat Mr. Issei Takayama Technical Officer Pharmaceuticals and Medical Devices Agency (PMDA) Shin-kasumigaseki Building 3-3-2, Kasumigaseki, Chiyoda-ku Tokyo, 100-0013 Japan Phone: +81-3-3506-9431 Fax: +81-3-3506-9440 takayama-issei@pmda.go.jp</p>
<p>Stimuli to the Revision Process</p>	<p>Articles on standards development topics authored by the USP Council of Experts, USP staff, or other interested parties on which USP desires public input prior to further development.</p>	<p>Review material and provide comments to the recipient indicated (usually footnoted in each <i>Stimuli</i> article).</p>

Other Sections

Expert Committees

A listing of the 2010–2015 Expert Committees that work on the development of USP compendial standards

(<http://www.usp.org/aboutUSP/governance/councilOfExperts/expertCommittees.html>)

Staff Directory

Names and contact information of key USP scientific staff members who work on the development of USP compendial standards

(<http://www.usp.org/USPNF/devProcess/contactScientists.html>)

Proposed Interim Revision Announcements

This section includes proposals for *Interim Revision Announcements (IRAs)* that will be published as official *USP* or *NF* standards. There is a 90-day comment period for these proposals (see <http://www.usp.org/USPNF/pf/pfRedesign.html> for the *PF* comment schedule). Readers should review material in this section and provide comments to the Scientific Liaison using the contact information appearing at the end of each proposed *IRA*. Information on how to comment can be found under the "Participation" section of www.usp.org. It is important to send comments promptly so that the Expert Committee members can consider reader's input as they are deciding whether to advance standards to official status. The approved official text will be published under *IRAs* in the "New Official Text" section of USP's Web site.

Each proposal is preceded by a Briefing that indicates the proposed revisions.

Symbols—New text is enclosed in symbols and set off from the current official text as shown in the following example:

◆new text◆

Where the symbols appear together with no enclosed text, such as

◆◆

, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the date the proposal, if approved, will become official as an *IRA*. For example, ◆(IRA 1-Apr-2011)

BRIEFING

Sesame Oil, *NF 33* page 6858. Based on a request to accommodate certain grades used in injectable dosage forms that are not currently included in the monograph, it is proposed to make the following changes:

1. Revise the specification of *Specific Gravity* by decreasing the lower limit from 0.916 to 0.912.
2. In the *Labeling* section, add a statement to accommodate a special grade that is used in injectable dosage forms. The specific grade must meet the *Other Requirements* that will be added under the section of *Additional Requirements*.
3. Add the subsection for *Other Requirements* that are applied to the special grade of sesame oil, which is used in injectable dosage forms.

The comment period for the above revision ends March 31, 2015. In the absence of significant adverse comments, it is proposed to implement this revision via an *Interim Revision Announcement* to *USP 38–NF33*, with an official date of July 1, 2015.

Interested parties are encouraged to submit comments to Hong Wang, Ph.D., senior scientific liaison to the Excipient Expert Committees (301-816-8351 or hw@usp.org)

As part of the USP monograph modernization effort, the following revisions are proposed:

1. Add a specification for *Alkaline Impurities*.
2. Add a specification for *Peroxide Value*.
3. Add the specification for *Water Determination*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC: H. Wang.)

Correspondence Number—C149850; C152089

Comment deadline: March 31, 2015**Sesame Oil****DEFINITION**

Sesame Oil is the refined fixed oil obtained from the seed of one or more cultivated varieties of *Sesamum indicum* L. (Fam. Pedaliaceae). It may contain suitable antioxidants.

IDENTIFICATION**• A. Identity by Triglyceride Profile**

Analysis: Proceed as directed in the test for *Triglyceride Composition*

Acceptance criteria: The peak responses for the eight major triglycerides—LLL, OLL, PLL, OOL, POL, OOO, SOL, and POO—elute between 0 and about 40 min, in the order specified, and at relative retention times of about 0.55, 0.65, 0.69, 0.77, 0.82, 0.93, 0.97, and 1.0, respectively, as obtained in the chromatogram of the *Sample solution* in the test for *Triglyceride Composition*.

ASSAY**• Triglyceride Composition**

[Note—The fatty acid radicals are designated as linoleic (L), oleic (O), palmitic (P), and stearic (S), and the common abbreviations for triglycerides used are as follows: trilinolein (LLL), 1,2-dilinoleoyl-3-oleoyl-rac-glycerol (OLL), 1,2-dilinoleoyl-3-palmitoyl-rac-glycerol (PLL), 1,2-dioleoyl-3-linoleoyl-rac-glycerol (OOL), 1-palmitoyl-2-oleoyl-3-linoleoyl-rac-glycerol (POL), triolein (OOO), 1-linoleoyl-2-oleoyl-3-stearoyl-rac-glycerol (SOL), and 1,2-dioleoyl-3-palmitoyl-rac-glycerol (POO).]

Mobile phase: Acetonitrile and methylene chloride (60:40)

System suitability solution: 3.0 mg/mL of USP Sesame Oil Related Compound A RS and USP Sesame Oil Related Compound B RS in *Mobile phase*. [Note—USP Sesame Oil Related Compound A RS is OLL, and USP Sesame Oil Related Compound B RS is PLL.]

Sample solution: 20 mg/mL of Sesame Oil in *Mobile phase*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Refractive index

Columns: Two 4.6-mm × 25-cm in series; packings L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for OLL and PLL are about 0.93 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.8 between OLL and PLL

Relative standard deviation: NMT 1.5% determined from peak areas; NMT 2.2%

determined from the peak area ratio of OLL to PLL

Analysis

[Note—The relative retention times for the eight major triglyceride peaks are listed in *Table 1*.]

Sample: *Sample solution*

Calculate the percentage of each of these triglycerides in the portion of sample taken:

$$\text{Result} = (A/B) \times 100$$

A = peak area for each individual triglyceride

B = sum of the areas of all the peaks, excluding the solvent peak

Table 1

Triglyceride	Relative Retention Time	Composition (%)
LLL	0.55	7.0–19.0
OLL	0.65	13.0–30.0
PLL	0.69	5.0–9.0
OOL	0.77	14.0–25.0
POL	0.82	8.0–16.0
OOO	0.93	5.0–14.0
SOL	0.97	2.0–8.0
POO	1.0	2.0–8.0

IMPURITIES

Delete the following:

•

• **Heavy Metals, Method II** (231)

: NMT 10 µg/g •(Official 1-Dec-2015)

Add the following:

▲• Alkaline Impurities

Sample: 10 mL of Sesame Oil

Analysis: Mix 10 mL of freshly opened acetone and 0.3 mL of water, and add 0.05 mL of bromophenol blue TS. Add the *Sample*, shake, and allow to stand. Titrate with 0.01 N hydrochloric acid VS to change the color of the upper layer to yellow.

Acceptance criteria: NMT 0.1 mL of 0.01 N hydrochloric acid is required. ▲NF34

SPECIFIC TESTS

Change to read:

• **Specific Gravity** (841) : 0.916

•0.912•(IRA 1-Jul-2015)

-0.921

- **Fats and Fixed Oils, Acid Value (Free Fatty Acids) 〈 401 〉**

Sample: 10 g

Acceptance criteria: NMT 2.0 mL of 0.020 N sodium hydroxide is required for neutralization.

- **Fats and Fixed Oils, Iodine Value 〈 401 〉:** 103–116

- **Fats and Fixed Oils, Saponification Value 〈 401 〉:** 188–195

- **Fats and Fixed Oils, Solidification Temperature of Fatty Acids 〈 401 〉:** 20°–25°

Add the following:

- ▲● **Fats and Fixed Oils, Peroxide Value 〈 401 〉:** NMT 10.0 ▲NF34

- **Fats and Fixed Oils, Unsaponifiable Matter 〈 401 〉:** NMT 1.5%

- **Cottonseed Oil**

Sample: 5 mL

Analysis: Mix the *Sample* in a test tube with 5 mL of a mixture of equal volumes of amyl alcohol and a 10-mg/mL solution of sulfur in carbon disulfide. Warm the mixture carefully until the carbon disulfide is expelled, and immerse the tube to one-third of its depth in a boiling saturated solution of sodium chloride.

Acceptance criteria: No reddish color develops within 15 min.

Add the following:

- ▲● **Water Determination, Method Ic 〈 921 〉:** NMT 0.1% ▲NF34

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and prevent exposure to excessive heat.

Change to read:

- **Labeling:** Label it to indicate the name and quantity of any added antioxidant.

● Where Sesame Oil is intended for use in the manufacture of injectable dosage forms, it is so labeled. ●(IRA 1-Jul-2015)

Add the following:

- **Other Requirements:** For Sesame Oil intended for use in injectable dosage forms, which is specified in the labeling, the requirements must be met for *Unsaponifiable Matter, Acid Value, Peroxide Value, and Water, Method Ic*, under *Specific Tests* in the chapter *Injections and Implanted Drug Products 〈 1 〉, Vehicles and Added Substances, Nonaqueous Vehicles*. ●(IRA 1-Jul-2015)

- **USP Reference Standards 〈 11 〉**

USP Sesame Oil Related Compound A RS

USP Sesame Oil Related Compound B RS

BRIEFING

Aluminum Sulfate and Calcium Acetate for Topical Solution, *USP 38* page 2142. On the basis of comments received, the calculation for aluminum sulfate tetradecahydrate in the *Assay* is revised to correct the equation. The calculation for calcium acetate monohydrate in the *Assay* is also updated for consistency.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

The comment period for this revision ends on March 31, 2015. In the absence of significant comments, it is proposed to implement this revision through an *Interim Revision Announcement* to *USP 39–NF 34* with an official date of July 1, 2015.

(SM1: S. Shivaprasad.)

Correspondence Number—C149453; C151337

Comment deadline: March 31, 2015

Aluminum Sulfate and Calcium Acetate for Topical Solution

DEFINITION

Aluminum Sulfate and Calcium Acetate for Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum sulfate tetradecahydrate $[\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}]$ and calcium acetate monohydrate $(\text{C}_4\text{H}_6\text{CaO}_4 \cdot \text{H}_2\text{O})$.

IDENTIFICATION

- **A.**

Sample: 0.25 g of Aluminum Sulfate and Calcium Acetate for Topical Solution

Analysis: Place the *Sample* in a test tube. Add 10 mL of water and 0.25 g of calcium carbonate. Heat on a steam bath for 10 min, and filter. Add 3–4 drops of ferric chloride TS to the filtrate. [Note—After the addition of the ferric chloride TS, the solution may be heated for 1 min to speed the reaction.]

Acceptance criteria: A reddish-brown color or precipitate indicates acetate.

- **B. Identification Tests—General, Sulfate** 〈 191 〉 *and Calcium* 〈 191 〉

Sample solution: Suspend 2 g of sample in 50 mL of water and filter.

Acceptance criteria: Meets the requirements

ASSAY

Change to read:

- **Aluminum Sulfate Tetradecahydrate**

Sample solution: Transfer 10 g of Aluminum Sulfate and Calcium Acetate for Topical Solution to a 1000-mL volumetric flask. Add 100 mL of 1.2 N hydrochloric acid and 250 mL of water. Heat on a steam bath or hot plate until dissolved. Cool, and dilute with water to volume. Retain a portion of the *Sample solution* for the *Assay for Calcium Acetate Monohydrate*.

Blank: Water

Titrimetric system

Mode: Residual titration

Titrant: 0.02 M zinc sulfate VS

~~**Back-titrant:** 0.01 M edetate disodium VS~~

~~••(IRA 1-Jul-2015)~~

Endpoint detection: Visual

Analysis: Transfer a 5.0-mL aliquot of the *Sample solution* to a 250-mL conical flask. Add, in the order named, 40.0 mL of *Back-titrant*

~~•0.01 M edetate disodium VS•(IRA 1-Jul-2015)~~

and 20 mL of acetic acid-ammonium acetate buffer TS, and mix by swirling. Add 50 mL of alcohol and 2 mL of dithizone TS, and titrate the excess *Back-titrant*

~~•0.01 M edetate disodium VS•(IRA 1-Jul-2015)~~

with *Titrant* until the color changes from green-violet to a clear rose-pink. Perform a blank titration, substituting 5.0 mL of water for the *Sample solution*. Each mL of 0.01 M edetate disodium is equivalent to 2.972 mg of aluminum sulfate tetradecahydrate $[\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}]$.

Calculate the percentage of the labeled amount of aluminum sulfate tetradecahydrate $[\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}]$ taken:

$$\text{Result} = [D \times F \times M \times (V_B - V_U)] / (M_T \times W) \times 100$$

D = dilution factor, 1000/5.0

F = conversion factor (2.972 mg of sample per mL of 0.01 M edetate disodium)

M = actual molarity of the *Titrant*

V_B = blank titration volume (mL)

V_U = sample titration volume (mL)

M_T = theoretical molarity of the *Titrant*, 0.02

W = weight of the sample (mg)

•Calculate the percentage of aluminum sulfate tetradecahydrate $[\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}]$ in the portion of Aluminum Sulfate and Calcium Acetate for Topical Solution taken:

$$\text{Result} = \{ [D \times (V_B - V_S) \times M \times F] / W \} \times 100$$

D

= dilution factor, 1000/5.0

V_B

= blank titration volume (mL)

V_S

= sample titration volume (mL)

M

= molarity of the *Titrant* (mM/mL)

F

= equivalency factor, 297.2 mg/mM

W

= weight of sample used (mg)

•(IRA 1-Jul-2015)

Acceptance criteria: 90.0%–110.0%

•of the labeled amount of aluminum sulfate tetradecahydrate $[\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}]$ •(IRA 1-Jul-2015)

Change to read:

• **Calcium Acetate Monohydrate**

Sample: Transfer a 5.0-mL aliquot of the *Sample solution* retained from the *Assay* for *Aluminum Sulfate Tetradecahydrate* to a 250-mL conical flask.

Titrimetric system

Mode: Direct titration

Titrant: 0.01 M edetate disodium VS

Endpoint detection: Visual

Analysis: Add 1–2 mL of 50% triethanolamine to mask the aluminum, and mix well. With constant stirring, add to the *Sample*, in the order named, 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue, and titrate with *Titrant*. The indicator will change from purple to a clear blue color at the endpoint. ~~Each mL of 0.01 M edetate disodium is equivalent to 1.762 mg of calcium acetate monohydrate (C₄H₆CaO₄·H₂O).~~

~~Calculate the percentage of the labeled amount of calcium acetate monohydrate (C₄H₆CaO₄·H₂O) taken:~~

$$\text{Result} = [(D \times V_T \times F \times M)/(M_r \times W)] \times 100$$

~~D = dilution factor, 1000/5.0~~

~~V_T = sample titration volume (mL)~~

~~F = conversion factor (1.762 mg of sample per mL of 0.01 M edetate disodium)~~

~~M = actual molarity of the *Titrant*~~

~~M_r = theoretical molarity of the *Titrant*, 0.01~~

~~W = weight of the sample (mg)~~

• Calculate the percentage of calcium acetate monohydrate (C₄H₆CaO₄·H₂O) in the portion of Aluminum Sulfate and Calcium Acetate for Topical Solution taken:

$$\text{Result} = [(D \times V \times M \times F)/W] \times 100$$

D

= dilution factor, 1000/5.0

V

= sample titration volume (mL)

M

= molarity of the *Titrant* (mM/mL)

F

= equivalency factor, 176.2 mg/mM

W

= weight of sample used (mg)

*(IRA 1-Jul-2015)

Acceptance criteria: 90.0%–110.0%

*of the labeled amount of calcium acetate monohydrate (C₄H₆CaO₄·H₂O) *(IRA 1-Jul-2015)

SPECIFIC TESTS

- pH (791)

Sample solution: 1 g of Aluminum Sulfate and Calcium Acetate for Topical Solution in 200 mL of water

Acceptance criteria: 4.0–4.8

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-unit containers, and protect from excessive heat.

In-Process Revision

This section contains proposals for adoption as official *USP* or *NF* standards (either proposed *new* standards or proposed *revisions* of current *USP* or *NF* standards). These may be any of the following: (1) proposed revisions placed directly under *In-Process Revision*, or (2) modifications of revisions previously proposed under *In-Process Revision*. Readers should review material in this section and provide comments to the Scientific Liaison using the contact information appearing at the end of each proposal. Information on how to comment can be found under the "Participation" section of www.usp.org. It is important to send comments promptly, using the comment deadline listed after each title.

Briefings Each Proposal is preceded by a Briefing in the following format:

Briefing
Name of Item , citations of the most recent <i>USP</i> publications in which this item appeared. Rationale for the revision. Other relevant information. (For example, if a chromatographic method is being proposed, column specifications and retention times for compounds of interest.) Finally, the Expert Committee acronym (the name of the Scientific Liaison who handled the particular monograph or general chapter, and the <i>USP</i> tracking correspondence number, as shown in the example below:
(Expert Committee Acronym: Liaison Name.)
Correspondence Number—CXXXXX

Symbols Proposed revisions are shown with language proposed for deletion or replacement crossed out. All *USP–NF* revisions use the following symbols that indicate the final destination of the official text:

•new text•

if slated for an *IRA*;

▲new text▲

if slated for *USP–NF*;

■new text■

if slated for a *Supplement* to *USP–NF*. The same symbols indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as

• •

, or

■ ■

, or

▲▲

, it means that text has been deleted and no new text was proposed to replace it.

In all revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA*, *Supplement*, or the *USP* or *NF* as the publication where the revision will appear if approved. For example, ■

2S (*USP 34*) indicates that the proposed revision is slated for the *Second Supplement to USP 34*, and ▲

USP 35 and ▲*NF30* indicates that the revisions are proposed for *USP 35* and *NF 30*, respectively.

Official Title Changes Where the specification "**Monograph title change**" is found, it indicates that the official title stated after that specification will be substituted for the former title in the appropriate places throughout that monograph once this revision becomes official.

BRIEFING

General Notices and Requirements, USP 38–NF 33 page 1. The *General Notices and Requirements (GN)* section of the *United States Pharmacopeia* and the *National Formulary (USP–NF)* was most recently revised in the *Second Supplement to USP 37–NF 32* (official December 1, 2014). *GN* is regularly revised to refine and clarify its content. This current proposal has been informed by input from stakeholders on the General Notices Project Team, representatives of the U.S. Food and Drug Administration, and members of the Council of Experts Executive Committee, who discussed these revisions in their September 16–17, 2014 meeting. Outlined below are changes that are proposed for this round of *GN* revisions, which are being published for public notice and comment herein (*Pharmacopeial Forum* 41(1) [Jan.–Feb. 2015]) and, following subsequent consideration and balloting by the Council of Experts Executive Committee, are anticipated to be included in *USP 39–NF 34* (publishing November 1, 2015; official May 1, 2016).

Changes are proposed for the following *General Notices and Requirements* sections:

- 1. TITLE AND REVISION
 - Clarification of accelerated revision definitions and location
- 2. OFFICIAL STATUS AND LEGAL RECOGNITION
 - 2.10. *Official Text*
 - Specification of the prevailing publication format
 - Clarification that general chapter citations in the *NF* refer to the general chapters in the *USP*
- 3. CONFORMANCE TO STANDARDS
 - 3.10. *Applicability of Standards*
 - Clarification of the primacy of monographs over general chapters and *General Notices and Requirements*
 - Clarification of early adoption
 - Clarification that tests using multiple dosage units are not statistical

sampling plans with extrapolation to larger populations

3.20. *Indicating Conformance*

- Extension of included provisions to compounded preparations

• 4. MONOGRAPHS AND GENERAL CHAPTERS

4.10. *Monographs*

- Replacement of "interchangeability" with "substitutability"

4.10.10. *Applicability of Test Procedures*

- Simplification of text

• 5. MONOGRAPH COMPONENTS

5.20. *Added Substances* and 5.20.10. *Added Substances, Excipients, and Ingredients in Official Substances*

- Clarification of requirement to use a minimum quantity of added substances

5.50.10. *Units of Potency (Biological)*

- Clarification of the use of USP units and international units

• 6. TESTING PRACTICES AND PROCEDURES

6.50.20.1. *Adjustments to Solutions*

- Removal of the word "special"

• 8. TERMS AND DEFINITIONS

8.230.20. *Water in the Manufacture of Official Substances*

- Revision to requirements for water used in manufacturing

8.230.30. *Water in a Compendial Procedure*

- Clarification of references

8.240. *Weights and Measures*

- Clarification that the degree symbol ($^{\circ}$) is always interpreted as Celsius
- Revision of the unit of acceleration due to gravity in centrifugation as "g"

• 9. PRESCRIBING AND DISPENSING

9.10. *Use of Metric Units*

- Revision restricting the use of abbreviations or international units for prescription purposes

• 10. PRESERVATION, PACKAGING, STORAGE, AND LABELING

10.–10.40.100.1.

- Revision to remove from *General Notices and Requirements* information that is moving to general chapter *Labeling* 〈 7 〉

Additionally, minor editorial changes have been made to update the text to current *USP* style.

(COEEC: M. Sindaco.) Correspondence Number—C145488

Comment deadline: March 31, 2015

GENERAL NOTICES AND REQUIREMENTS

Change to read:

The *General Notices and Requirements* section (the *General Notices*) presents the basic assumptions, definitions, and default conditions for the interpretation and application of the *United States Pharmacopeia (USP)* and the *National Formulary (NF)*.

Requirements stated in these *General Notices* apply to all articles recognized in the *USP* and *NF* (the “compendia”) and to all general chapters unless specifically stated otherwise. ~~Where the requirements of an individual monograph differ from the *General Notices* or a general chapter, the monograph requirements apply and supersede the requirements of the *General Notices* or the general chapter, whether or not the monograph explicitly states the difference.~~

▲▲USP39

Change to read:

1. TITLE AND REVISION

The full title of this publication (consisting of four volumes and including its *Supplements*), is *The Pharmacopeia of the United States of America, Thirty-Ninth Revision* and the *National Formulary, Thirty-Fourth Edition*. These titles may be abbreviated to *USP 39*, to *NF 34*, and to *USP 39–NF 34*. The *United States Pharmacopeia, Thirty-Ninth Revision*, and the *National Formulary, Thirty-Fourth Edition*, supersede all earlier revisions. Where the terms “*USP*,” “*NF*,” or “*USP–NF*” are used without further qualification during the period in which these compendia are official, they refer only to *USP 39*, *NF 34*, and any *Supplement(s)* thereto. The same titles, with no further distinction, apply equally to print or electronic presentation of these contents. Although *USP* and *NF* are published under one cover and share these *General Notices*, they are separate compendia.

This revision is official beginning May 1, 2016, unless otherwise indicated in specific text. *Supplements* to *USP* and *NF* are published periodically.

~~*Interim Revision Announcements* are revisions to *USP* and *NF* that are published on the *USP* website. *Interim Revision Announcements* contain official revisions and their effective dates. Announcements of the availability of new *USP* Reference Standards and announcements of tests or procedures that are held in abeyance pending availability of required *USP* Reference Standards are also available on the “New Official Text” tab of *USP*'s website.~~

▲ Accelerated Revisions, published periodically on the *Official Text* section of *USP*'s website (<http://www.usp.org/usp-nf/official-text>), are designed to make revisions official more quickly than through the routine process for publishing standards in the *USP–NF*. *Interim Revision Announcements* are Accelerated Revisions to *USP* and *NF* that contain official revisions and their effective dates. ▲USP39

~~*Revision Bulletins* are revisions to official text or postponements that require expedited publication. They are published on the *USP* website and generally are official immediately unless otherwise specified in the *Revision Bulletin*.~~

▲*Revision Bulletins* are Accelerated Revisions to official text or postponements that require expedited publication. They generally are official immediately unless otherwise specified in the *Revision Bulletin*. ▲*USP39*

~~*Errata* are corrections to items erroneously published that have not received the approval of the Council of Experts and that do not reflect the official requirements.~~

▲*Errata* are Accelerated Revisions representing corrections to items erroneously published. Announcements of the availability of new USP Reference Standards and announcements of tests or procedures that are held in abeyance pending availability of required USP Reference Standards are also available on the "Official Text" tab of USP's website. ▲*USP39*

Change to read:

2. OFFICIAL STATUS AND LEGAL RECOGNITION

2.10. Official Text

~~*Official text* is text contained in *USP* and *NF*, including monographs, general chapters, and these *General Notices*. Revisions to official text are provided in *Supplements*, *Interim Revision Announcements*, and *Revision Bulletins*. General chapters numbered from 1000 to 1999 are considered interpretive and are intended to provide information on, give definition to, or describe a particular subject. They contain no mandatory requirements applicable to any official article unless specifically referenced in *General Notices*, a monograph, or a general chapter numbered below 1000. General chapters numbered above 2000 apply only to articles that are intended for use as dietary ingredients and dietary supplements.~~

▲*Official text* of the *USP* and *NF*, is published in the *USP-NF Online* (www.uspnf.com) in the edition identified as "CURRENTLY OFFICIAL" and in Accelerated Revisions that supersede the *USP-NF Online* as described below. ▲*USP39*

▲Routine revisions are published in the *USP-NF Online* and become official on the date indicated, usually six months after publication. Accelerated Revisions supersede the *USP-NF Online* and become official on the date indicated. Links to Accelerated Revisions on the USP website can be found in any superseded monograph or general chapter in the *USP-NF Online*.

Print and USB flash drive versions of the *USP* and *NF* also are available. Routine revisions are provided with the same timing as the *USP-NF Online*. Official text published in *Supplements* supersedes that in the previously published print or USB flash drive versions of *USP-NF*. These versions also are superseded by Accelerated Revisions as described above.

In the event of any disparity between the print or USB flash drive versions and the *USP-NF Online*, the *USP-NF Online* will be deemed to apply.

General chapters numbered from 1000 to 1999 are considered interpretive and are intended to provide information on, give definition to, or describe a particular subject. They contain no mandatory requirements applicable to any official article unless specifically referenced in *General Notices and Requirements*, a monograph, or a general chapter numbered below 1000. General chapter citations in *NF* monographs refer to *USP* general chapters. General chapters

numbered above 2000 apply only to articles that are intended for use as dietary ingredients and dietary supplements.

▲USP39

2.20. Official Articles

An *official article* is an article that is recognized in *USP* or *NF*. An article is deemed to be recognized and included in a compendium when a monograph for the article is published in the compendium and an official date is generally or specifically assigned to the monograph.

The title specified in a monograph is the *official title* for such article. Other names considered to be synonyms of the official titles may not be used as substitutes for official titles.

Official articles include both *official substances* and *official products*. An *official substance* is a drug substance, excipient, dietary ingredient, other ingredient, or component of a finished device for which the monograph title includes no indication of the nature of the finished form.

An *official product* is a drug product, dietary supplement, compounded preparation, or finished device for which a monograph is provided.

2.30. Legal Recognition

The *USP* and *NF* are recognized in the laws and regulations of many countries throughout the world. Regulatory authorities may enforce the standards presented in the *USP* and *NF*, but because recognition of the *USP* and *NF* may vary by country, users should understand applicable laws and regulations.

In the United States under the Federal Food, Drug, and Cosmetic Act (FDCA), both *USP* and *NF* are recognized as official compendia. A drug with a name recognized in *USP-NF* must comply with compendial identity standards or be deemed adulterated, misbranded, or both. See, e.g., FDCA § 501(b) and 502(e)(3)(b); also FDA regulations, 21 CFR § 299.5(a&b). To avoid being deemed adulterated, such drugs must also comply with compendial standards for strength, quality, and purity, unless labeled to show all respects in which the drug differs. See, e.g., FDCA § 501(b) and 21 CFR § 299.5(c). In addition, to avoid being deemed misbranded, drugs recognized in *USP-NF* must also be packaged and labeled in compliance with compendial standards. See FDCA § 502(g).

A dietary supplement represented as conforming to specifications in *USP* will be deemed a misbranded food if it fails to so conform. See FDCA § 403(s)(2)(D).

Enforcement of *USP* standards is the responsibility of FDA and other government authorities in the U.S. and elsewhere. *USP* has no role in enforcement.

Change to read:

3. CONFORMANCE TO STANDARDS

3.10. Applicability of Standards

~~Standards for an article recognized in the compendia (*USP-NF*) are expressed in the article's monograph, applicable general chapters, and *General Notices*. Unless specifically exempted elsewhere in a compendium, the identity, strength, quality, and purity of an article are determined by the official tests, procedures, and acceptance criteria, whether incorporated in the monograph itself, in the *General Notices*, or in the applicable general chapters. Early adoption of revised standards is allowed. Where revised standards for an existing article have been published as final approved "official text" (as approved in section 2.10) but are not yet official (six months after publication, unless otherwise specified; see "official date," section 2.20), compliance with the revised standard shall not preclude a finding or indication of conformance with compendial standards, unless *USP* specifies otherwise by prohibiting early adoption in a particular standard.~~

▲Standards for an article recognized in the compendia (*USP–NF*) are expressed in the article's monograph, applicable general chapters, and *General Notices and Requirements*. The identity, strength, quality, and purity of an article are determined by the official tests, procedures, and acceptance criteria, whether incorporated in the monograph, in applicable general chapters, or in the *General Notices and Requirements*. Where the requirements of a monograph differ from the *General Notices and Requirements* or an applicable general chapter, the monograph requirements apply and supersede the requirements of the *General Notices and Requirements* or applicable general chapters, whether or not the monograph explicitly states the difference.

▲*USP39*

Early adoption of revised standards

▲in advance of the official date▲*USP39*

is allowed

▲by USP unless specified otherwise at the time of publication.▲*USP39*

Where revised standards for an existing article have been published as final approved "official text" (as approved in section 2.10) but ~~are not yet official~~

▲have not yet reached the official date▲*USP39*

(six months after publication, unless otherwise specified; see "official date," section 2.20), compliance with the revised standard shall not preclude a finding or indication of conformance with compendial standards, unless USP specifies otherwise by prohibiting early adoption in a particular standard.

~~The standards in the relevant monograph, general chapter(s), and *General Notices* apply at all times in the life of the article from production to expiration. The manufacturer's specifications, and good manufacturing practices generally (including, e.g., Quality by Design initiatives), are developed and followed to ensure that the article will comply with compendial standards until its expiration date, when stored as directed. Thus, any official article is expected to meet the compendial standards if tested, and any official article actually tested as directed in the relevant monograph must meet such standards to demonstrate compliance.~~

▲The standards in the relevant monograph, general chapter(s), and *General Notices and Requirements* apply at all times in the life of the article from production to expiration. It is also noted that the manufacturer's specifications, and manufacturing practices (e.g., Quality by Design, Process Analytical Technology, and Real Time Release Testing initiatives), generally are developed and followed to ensure that the article will comply with compendial standards until its expiration date, when stored as directed. Every compendial article in commerce shall be so constituted that when examined in accordance with these assays and test procedures, it meets all applicable pharmacopeial requirements (*General Notices and Requirements*, monographs, and general chapters). Thus, any official article is expected to meet the compendial standards if tested, and any official article actually tested as directed in the relevant monograph must meet such standards to demonstrate compliance.▲*USP39*

~~At times, compendial standards take on the character of statistical procedures, with multiple units involved and perhaps a sequential procedural design to allow the user to determine that the tested article meets or does not meet the standard. The similarity to statistical procedures may seem to suggest an intent to make inference to some larger group of units, but in all~~

~~cases, statements about whether the compendial standard is met apply only to the units tested.~~

▲Some tests, such as those for Dissolution and Uniformity of Dosage Units, require multiple dosage units in conjunction with a decision scheme. These tests, albeit using a number of dosage units, are in fact one determination. These procedures should not be confused with statistical sampling plans. ▲*USP39*

Repeats, replicates, statistical rejection of outliers, or extrapolations of results to larger populations, as well as the necessity and appropriate frequency of batch testing, are neither specified nor proscribed by the compendia;

▲such decisions are based on the objectives of the testing. ▲*USP39*

Frequency of testing and sampling are left to the preferences or direction of those performing compliance testing, and other users of *USP-NF*, including manufacturers, buyers, or regulatory authorities.

Official products are prepared according to recognized principles of good manufacturing practice and from ingredients that meet *USP* or *NF* standards, where standards for such ingredients exist (for dietary supplements, see section 3.10.20).

Official substances are prepared according to recognized principles of good manufacturing practice and from ingredients complying with specifications designed to ensure that the resultant substances meet the requirements of the compendial monographs.

3.10.10. Applicability of Standards to Drug Products, Drug Substances, and Excipients

The applicable *USP* or *NF* standard applies to any article marketed in the United States that (1) is recognized in the compendium and (2) is intended or labeled for use as a drug or as an ingredient in a drug. Such articles (drug products, drug substances, and excipients) include both human drugs (whether dispensed by prescription, "over the counter," or otherwise), as well as animal drugs. The applicable standard applies to such articles whether or not the added designation "USP" or "NF" is used. The standards apply equally to articles bearing the official titles or names derived by transposition of the definitive words of official titles or transposition in the order of the names of two or more active ingredients in official titles, or where there is use of synonyms with the intent or effect of suggesting a significant degree of identity with the official title or name.

3.10.20. Applicability of Standards to Medical Devices, Dietary Supplements, and Their Components and Ingredients

An article recognized in *USP* or *NF* shall comply with the compendial standards if the article is a medical device, component intended for a medical device, dietary supplement, dietary ingredient, or other ingredient that is intended for incorporation into a dietary supplement, and is labeled as conforming to the *USP* or *NF*.

Generally, dietary supplements are prepared from ingredients that meet *USP*, *NF*, or *Food Chemicals Codex* standards. Where such standards do not exist, substances may be used in dietary supplements if they have been shown to be of acceptable food grade quality using other suitable procedures.

3.20. Indicating Conformance

A drug product, drug substance, or excipient may use the designation "USP" or "NF" in conjunction with its official title or elsewhere on the label only when (1) a monograph is provided in the specified compendium and (2) the article complies with the identity prescribed in the specified compendium.

When a drug product, drug substance,

▲compounded preparation, ▲*USP39*

or excipient differs from the relevant *USP* or *NF* standard of strength, quality, or purity, as determined by the application of the tests, procedures, and acceptance criteria set forth in the relevant compendium, its difference shall be plainly stated on its label.

When a drug product, drug substance,

▲compounded preparation, ▲*USP39*

or excipient fails to comply with the identity prescribed in *USP* or *NF* or contains an added substance that interferes with the prescribed tests and procedures, the article shall be designated by a name that is clearly distinguishing and differentiating from any name recognized in *USP* or *NF*.

A medical device, dietary supplement, or ingredient or component of a medical device or dietary supplement may use the designation "USP" or "NF" in conjunction with its official title or elsewhere on the label only when (1) a monograph is provided in the specified compendium and (2) the article complies with the monograph standards and other applicable standards in the compendium.

The designation "USP" or "NF" on the label may not and does not constitute an endorsement by USP and does not represent assurance by USP that the article is known to comply with the relevant standards. USP may seek legal redress if an article purports to be or is represented as an official article in one of USP's compendia and such claim is determined by USP not to be made in good faith.

The designation "USP-NF" may be used on the label of an article provided that the label also bears a statement such as "Meets *NF* standards as published by USP," indicating the particular compendium to which the article purports to apply.

When the letters "USP," "NF," or "USP-NF" are used on the label of an article to indicate compliance with compendial standards, the letters shall appear in conjunction with the official title of the article. The letters are not to be enclosed in any symbol such as a circle, square, etc., and shall appear in capital letters.

If a dietary supplement does not comply with all applicable compendial requirements but contains one or more dietary ingredients or other ingredients that are recognized in *USP* or *NF*, the individual ingredient(s) may be designated as complying with *USP* or *NF* standards or being of *USP* or *NF* quality provided that the designation is limited to the individual ingredient(s) and does not suggest that the dietary supplement complies with *USP* standards.

Change to read:

4. MONOGRAPHS AND GENERAL CHAPTERS

4.10. Monographs

Monographs set forth the article's name, definition, specification, and other requirements related to packaging, storage, and labeling. The specification consists of tests, procedures, and acceptance criteria that help ensure the identity, strength, quality, and purity of the article. For general requirements relating to specific monograph sections, see section 5, *Monograph Components*.

Because monographs may not provide standards for all relevant characteristics, some official substances may conform to the *USP* or *NF* standard but differ with regard to nonstandardized properties that are relevant to their use in specific preparations. To assure interchangeability

▲substitutability ▲*USP39*

in such instances, users may wish to ascertain functional equivalence or determine such characteristics before use.

4.10.10. Applicability of Test Procedures

~~A single monograph may include several different tests, procedures, and/or acceptance criteria that reflect attributes of different manufacturers' articles. Such alternatives may be presented for different polymorphic forms, impurities, hydrates, and dissolution cases. Monographs indicate the tests, procedures, and/or acceptance criteria to be used and the required labeling.~~

▲A single monograph may include more than one test, procedure, and/or acceptance criterion for the same attribute. Unless otherwise specified in the monograph, all tests are requirements. In some cases, monograph instructions allow the selection of tests that reflect attributes of different manufacturers' articles, such as different polymorphic forms, impurities, hydrates, and dissolution. Monograph instructions indicate the tests, procedures, and/or acceptance criteria to be used and the required labeling. ▲USP39

~~A test in a monograph may contain and require multiple procedures. However, multiple procedures may be included in particular monographs specifically for the purpose of assuring the availability of an appropriate procedure for a particular product. In such cases, a labeling statement to indicate the appropriate application of the procedure(s) will be included in the monograph. A labeling statement is not required if Test 1 is used.~~

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4.10.11. Dissolution, Disintegration, and Drug Release Tests

▲▲USP39

~~Multiple Dissolution, Disintegration, or Drug Release tests may be present in the monograph.~~

▲▲USP39

The order in which the tests are listed in the monograph is based on the order in which they are approved by the relevant Expert Committee for inclusion in the monograph. Test 1 is not necessarily the test for the innovator or for the reference product. ~~Compliance with any of the tests does not assure bioequivalence or bioavailability.~~

▲Depending on monograph instructions, a labeling statement is not typically required if Test 1 is used. ▲USP39

4.10.20. Acceptance Criteria

The acceptance criteria allow for analytical error, for unavoidable variations in manufacturing and compounding, and for deterioration to an extent considered acceptable under practical conditions. The existence of compendial acceptance criteria does not constitute a basis for a claim that an official substance that more nearly approaches 100 percent purity "exceeds" compendial quality. Similarly, the fact that an article has been prepared to tighter criteria than those specified in the monograph does not constitute a basis for a claim that the article "exceeds" the compendial requirements.

An official product shall be formulated with the intent to provide 100 percent of the quantity of each ingredient declared on the label. Where the minimum amount of a substance present in a

dietary supplement is required by law to be higher than the lower acceptance criterion allowed for in the monograph, the upper acceptance criterion contained in the monograph may be increased by a corresponding amount.

The acceptance criteria specified in individual monographs and in the general chapters for compounded preparations are based on such attributes of quality as might be expected to characterize an article compounded from suitable bulk drug substances and ingredients, using the procedures provided or recognized principles of good compounding practice, as described in these compendia.

4.20. General Chapters

Each general chapter is assigned a number that appears in angle brackets adjacent to the chapter name (e.g., *Chromatography* 〈621〉). General chapters may contain the following:

- Descriptions of tests and procedures for application through individual monographs,
- Descriptions and specifications of conditions and practices for pharmaceutical compounding,
- General information for the interpretation of the compendial requirements,
- Descriptions of general pharmaceutical storage, dispensing, and packaging practices, or
- General guidance to manufacturers of official substances or official products.

When a general chapter is referenced in a monograph, acceptance criteria may be presented after a colon.

Some chapters may serve as introductory overviews of a test or of analytical techniques. They may reference other general chapters that contain techniques, details of the procedures, and, at times, acceptance criteria.

Change to read:

5. MONOGRAPH COMPONENTS

5.10. Molecular Formula

The use of the molecular formula for the active ingredient(s) named in defining the required strength of a compendial article is intended to designate the chemical entity or entities, as given in the complete chemical name of the article, having absolute (100 percent) purity.

5.20. Added Substances

Added substances are presumed to be unsuitable for inclusion in an official article and therefore prohibited, if ~~:(1) they exceed the minimum quantity required for providing their intended effect;~~ ~~(2)~~

▲▲USP39

their presence impairs the bioavailability, therapeutic efficacy, or safety of the official article; or ~~(3)~~

▲▲USP39

they interfere with the assays and tests prescribed for determining compliance with the compendial standards

▲(see 3.20 *Indicating Conformance*).▲USP39

The air in a container of an official article may, where appropriate, be evacuated or be replaced by carbon dioxide, helium, argon, or nitrogen, or by a mixture of these gases. The use of such gas need not be declared in the labeling.

5.20.10. Added Substances, ~~Excipients, and Ingredients~~

▲▲USP39

in Official Substances

Official substances may contain only the specific added substances that are permitted by the individual monograph.

▲Such added substances shall not exceed the quantity required for providing their intended effect. ▲USP39

Where such addition is permitted, the label shall indicate the name(s) and amount(s) of any added substance(s).

5.20.20. Added Substances, ~~Excipients, and Ingredients~~

▲(Excipients and Ingredients) ▲USP39

in Official Products

Suitable substances and excipients such as antimicrobial agents, pharmaceutical bases, carriers, coatings, flavors, preservatives, stabilizers, and vehicles may be added to an official product to enhance its stability, usefulness, or elegance, or to facilitate its preparation, unless otherwise specified in the individual monograph.

Added substances and excipients employed solely to impart color may be incorporated into official products other than those intended for parenteral or ophthalmic use, in accordance with the regulations pertaining to the use of colors issued by the U.S. Food and Drug Administration (FDA), provided such added substances or excipients are otherwise appropriate in all respects. (See also *Added Substances under Injections and Implanted Drug Products (Parenterals)*—

Product Quality Tests { 1 } .)

The proportions of the substances constituting the base in ointment and suppository products and preparations may be varied to maintain a suitable consistency under different climatic conditions, provided that the concentrations of active ingredients are not varied and provided that the bioavailability, therapeutic efficacy, and safety of the preparation are not impaired.

5.20.20.1. In Compounded Preparations

Compounded preparations for which a complete composition is given shall contain only the ingredients named in the formulas unless specifically exempted herein or in the individual monograph. Deviation from the specified processes or methods of compounding, although not from the ingredients or proportions thereof, may occur provided that the finished preparation conforms to the relevant standards and to preparations produced by following the specified process.

Where a monograph for a compounded preparation calls for an ingredient in an amount expressed on the dried basis, the ingredient need not be dried before use if due allowance is made for the water or other volatile substances present in the quantity taken.

Specially denatured alcohol formulas are available for use in accordance with federal statutes and regulations of the Internal Revenue Service. A suitable formula of specially denatured alcohol may be substituted for Alcohol in the manufacture of official preparations intended for internal or topical use, provided that the denaturant is volatile and does not remain in the finished product. A preparation that is intended for topical application to the skin may contain specially denatured alcohol, provided that the denaturant is either a usual ingredient in the preparation or a permissible added substance; in either case the denaturant shall be identified on the label of the topical preparation. Where a process is given in the individual monograph, any preparation compounded using denatured alcohol shall be identical to that prepared by the

monograph process.

5.20.20.2. In Dietary Supplements

Additional ingredients may be added to dietary supplement products provided that the additional ingredients: (1) comply with applicable regulatory requirements; and (2) do not interfere with the assays and tests prescribed for determining compliance with compendial standards.

5.30. Description and Solubility

Only where a quantitative solubility test is given in a monograph and is designated as such is it a test for purity.

A monograph may include information regarding the article's description. Information about an article's "description and solubility" also is provided in the reference table *Description and Relative Solubility of USP and NF Articles*. The reference table merely denotes the properties of articles that comply with monograph standards. The reference table is intended primarily for those who use, prepare, and dispense drugs and/or related articles. Although the information provided in monographs and the information in the reference table may indirectly assist in the preliminary evaluation of an article, it is not intended to serve as a standard or test for purity. The approximate solubility of a compendial substance is indicated by one of the following descriptive terms:

Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1,000
Very slightly soluble	From 1,000 to 10,000
Practically insoluble, or Insoluble	Greater than or equal to 10,000

5.40. Identity

A compendial test titled *Identity* or *Identification* is provided as an aid in verifying the identity of articles as they are purported to be, e.g., those taken from labeled containers, and to establish whether it is the article named in *USP-NF*. The *Identity* or *Identification* test for a particular article may consist of one or more procedures. When a compendial test for *Identity* or *Identification* is undertaken, all requirements of all specified procedures in the test must be met to satisfy the requirements of the test. Failure of an article to meet all the requirements of a prescribed *Identity* or *Identification* test (i.e., failure to meet the requirements of all of the specified procedures that are components of that test) indicates that the article is mislabeled and/or adulterated.

5.50. Assay

Assay tests for compounded preparations are not intended for evaluating a compounded preparation before dispensing, but instead are intended to serve as the official test in the event of a question or dispute regarding the preparation's conformance to official standards.

5.50.10. Units of Potency (Biological)

For substances that cannot be completely characterized by chemical and physical means, it

~~may be necessary to express quantities of activity in biological units of potency, each defined by an authoritative, designated reference standard.~~

▲or physical means or that need confirmation of functionality or tertiary structure, it may be necessary to express quantities of biological activity in units of biological potency, each defined by an authoritative, designated reference standard. In cases where international reference materials have been discontinued, international units of potency may be defined in terms of molecular mass, such as in the cases of vitamins A, D, and E. ▲*USP39*

~~Units of biological potency defined by the World Health Organization (WHO) for International Biological Standards and International Biological Reference Preparations are termed International Units (IU). Monographs refer to the units defined by USP Reference Standards as "USP Units." For biological products, units of potency are defined by the corresponding U.S. Standard established by FDA, whether or not International Units or USP Units have been defined (see *Biologics* ~~(1041)~~).~~

▲Where available, World Health Organization (WHO) international biological standards define the International Units (IU). *USP* monographs refer to the units assigned by USP Reference Standards either directly as International Units (IU) or as "USP Units." For some biological products, units of potency are value assigned against a corresponding U.S. Standard established by FDA, whether or not International Units or USP Units have been defined (see *Biologics* ~~(1041)~~). Note that product-related labeling, e.g., on containers, need not use the full phrase "USP [product name] Units" that appears in many *USP* monograph labeling sections. The term "USP units" can be used on product labeling consistent with USP compendial requirements, provided it is clear from the context that the volume is stated in terms of USP [product name] Units. In such circumstances it should be clear that "USP Units" and "USP [product name] Units" share the same meaning. ▲*USP39*

5.60. Impurities and Foreign Substances

Tests for the presence of impurities and foreign substances are provided to limit such substances to amounts that are unobjectionable under conditions in which the article is customarily employed (see also *Impurities in Drug Substances and Drug Products* ~~(1086)~~). Nonmonograph tests and acceptance criteria suitable for detecting and controlling impurities that may result from a change in the processing methods or that may be introduced from external sources should be employed in addition to the tests provided in the individual monograph, where the presence of the impurity is inconsistent with applicable good manufacturing practices or good pharmaceutical practice.

5.60.10. Other Impurities in *USP* and *NF* Articles

If a *USP* or *NF* monograph includes an assay or organic impurity test based on chromatography, other than a test for residual solvents, and that monograph procedure does not detect an impurity present in the substance, the amount and identity of the impurity, where both are known, shall be stated in the labeling (certificate of analysis) of the official substance, under the heading *Other Impurity(ies)*.

The presence of any unlabeled other impurity in an official substance is a variance from the standard if the content is 0.1% or greater. The sum of all *Other Impurities* combined with the

monograph-detected impurities may not exceed 2.0% (see *Ordinary Impurities* 〈 466 〉), unless otherwise stated in the monograph.

The following categories of drug substances are excluded from *Other Impurities* requirements:

- Fermentation products and semi-synthetics derived therefrom,
- Radiopharmaceuticals,
- Biologics,
- Biotechnology-derived products,
- Peptides,
- Herbals, and
- Crude products of animal or plant origin.

Any substance known to be toxic shall not be listed under *Other Impurities*.

5.60.20. Residual Solvents in USP and NF Articles

All *USP* and *NF* articles are subject to relevant control of residual solvents, even when no test is specified in the individual monograph. If solvents are used during production, they must be of suitable quality. In addition, the toxicity and residual level of each solvent shall be taken into consideration, and the solvents limited according to the principles defined and the requirements specified in *Residual Solvents* 〈 467 〉, using the general methods presented therein or other suitable methods.

5.60.30 Elemental Impurities in USP Drug Products and Dietary Supplements

[Note—General chapter 〈 232 〉 was published June 1, 2012, in the *Second Supplement to USP 35–NF 30* and became official on February 1, 2013. General chapter 〈 2232 〉 was published February 1, 2013, in the *First Supplement to USP 36–NF 31* and became official on August 1, 2013. The date of their applicability to articles recognized in *USP–NF*, however, is December 1, 2015, the date on which this *General Notices* provision becomes official.]

Elemental impurities are controlled in official drug products according to the principles defined and requirements specified in *Elemental Impurities—Limits* 〈 232 〉. Also see 〈 232 〉 for information related to drug substances and excipients. Elemental contaminants are controlled in official dietary supplements according to the principles defined and requirements specified in *Elemental Contaminants in Dietary Supplements* 〈 2232 〉.
(Official December 1, 2015)

5.70. Performance Tests

Where content uniformity determinations have been made using the same analytical methodology specified in the *Assay*, with appropriate allowances made for differences in sample preparation, the average of all of the individual content uniformity determinations may be used as the *Assay* value.

5.80. USP Reference Standards

USP Reference Standards are authentic specimens that have been approved as suitable for use as comparison standards in *USP* or *NF* tests and assays. (See *USP Reference Standards* 〈 11 〉.) Where *USP* or *NF* tests or assays call for the use of a *USP Reference Standard*, only those results obtained using the specified *USP Reference Standard* are conclusive. Where a procedure calls for the use of a compendial article rather than for a *USP Reference Standard* as a material standard of reference, a substance meeting all of the compendial monograph requirements for

that article shall be used. If any new *USP* or *NF* standard requires the use of a new *USP* Reference Standard that is not yet available, that portion of the standard containing the requirement shall not be official until the specified *USP* reference material is available. Unless a reference standard label bears a specific potency or content, assume the reference standard is 100.0% pure in the official application. Unless otherwise directed in the procedure in the individual monograph or in a general chapter, *USP* Reference Standards are to be used in accordance with the instructions on the label of the Reference Standard.

Change to read:

6. TESTING PRACTICES AND PROCEDURES

6.10. Safe Laboratory Practices

In performing compendial procedures, safe laboratory practices shall be followed, including precautionary measures, protective equipment, and work practices consistent with the chemicals and procedures used. Before undertaking any procedure described in the compendia, the analyst should be aware of the hazards associated with the chemicals and the techniques and means of protecting against them. These compendia are not designed to describe such hazards or protective measures.

6.20. Automated Procedures

Automated and manual procedures employing the same basic chemistry are considered equivalent.

6.30. Alternative and Harmonized Methods and Procedures

Alternative methods and/or procedures may be used if they provide advantages in terms of accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction, or in other special circumstances. Such alternative procedures and methods shall be validated as described in the general chapter *Validation of Compendial Procedures* (1225) and must be shown to give equivalent or better results. Only those results obtained by the methods and procedures given in the compendium are conclusive.

Alternative procedures should be submitted to *USP* for evaluation as a potential replacement or addition to the standard (see section 4.10, *Monographs*).

Certain general chapters contain a statement that the text in question is harmonized with the corresponding text of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia* and that these texts are interchangeable. Therefore, if a substance or preparation is found to comply with a requirement using an interchangeable method or procedure from one of these pharmacopoeias, it should comply with the requirements of the *USP*. When a difference appears, or in the event of dispute, only the result obtained by the method and/or procedure given in the *USP* is conclusive.

6.40. Dried, Anhydrous, Ignited, or Solvent-Free Basis

All calculations in the compendia assume an "as-is" basis unless otherwise specified.

Test procedures may be performed on the undried or unignited substance and the results calculated on the dried, anhydrous, or ignited basis, provided a test for *Loss on Drying*, or *Water Determination*, or *Loss on Ignition*, respectively, is given in the monograph. Where the presence of moisture or other volatile material may interfere with the procedure, previous drying of the substance is specified in the individual monograph and is obligatory.

The term "solvent-free" signifies that the calculation shall be corrected for the presence of known solvents as determined using the methods described in *Residual Solvents* (467) unless

a test for limit of organic solvents is provided in the monograph.

The term "previously dried" without qualification signifies that the substance shall be dried as directed under *Loss on Drying* 〈 731 〉 or *Water Determination* 〈 921 〉 (gravimetric determination).

Where drying in vacuum over a desiccant is directed, a vacuum desiccator, a vacuum drying pistol, or other suitable vacuum drying apparatus shall be used.

6.40.10. Ignite to Constant Weight

"Ignite to constant weight" means that ignition shall be continued at $800 \pm 25^{\circ}$, unless otherwise indicated, until two consecutive weighings, the second of which is taken after an additional period appropriate to the nature and quantity of the residue, do not differ by more than 0.50 mg per g of substance taken.

6.40.20. Dried to Constant Weight

"Dried to constant weight" means that drying shall be continued until two consecutive weighings, the second of which is taken after an additional drying period appropriate to the nature and quantity of the residue, do not differ by more than 0.50 mg per g of substance taken.

6.50. Preparation of Solutions

6.50.10. Filtration

Where a procedure gives direction to "filter" without further qualification, the liquid shall be passed through suitable filter paper or equivalent device until the filtrate is clear. Due to the possibility of filter effects, the initial volumes of a filtrate may be discarded.

6.50.20. Solutions

Unless otherwise specified, all solutions shall be prepared with Purified Water. Solutions for quantitative measures shall be prepared using accurately weighed or accurately measured analytes (see section 8.20, *About*).

An expression such as "(1 in 10)" means that 1 part *by volume* of a liquid shall be diluted with, or 1 part *by weight* of a solid shall be dissolved in, (ERR 1-Aug-2014)

a sufficient quantity of the diluent or solvent to make the volume of the finished solution 10 parts *by volume*. An expression such as "(20:5:2)" means that the respective numbers of parts, by volume, of the designated liquids shall be mixed, unless otherwise indicated.

6.50.20.1. Adjustments to Solutions

When a specified concentration is called for in a procedure, a solution of other normality or molarity may be used, provided that allowance is made for the difference in concentration and that the change does not increase the error of measurement.

Proportionately larger or smaller quantities than the specified weights and volumes of assay or test substances and Reference Standards may be taken, provided the measurement is made with at least equivalent accuracy.

Unless otherwise indicated, analyte concentrations shall be prepared to within ten percent (10%) of the indicated value. In the special

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case in which a procedure is adapted to the working range of an instrument, solution concentrations may differ from the indicated value by more than ten percent (10%), with

appropriate changes in associated calculations. Any changes shall fall within the validated range of the instrument.

When adjustment of pH is indicated with either an acid or base and the concentration is not indicated, appropriate concentrations of that acid or base may be used.

6.50.20.2. Test Solutions

Information on Test Solutions (TS) is provided in the *Test Solutions* portion of the *Reagents, Indicators, and Solutions* section of the *USP-NF*. Use of an alternative Test Solution or a change in the Test Solution used may require validation.

6.50.20.3. Indicator Solutions

Where a procedure specifies the use of an indicator TS, approximately 0.2 mL, or 3 drops, of the solution shall be added unless otherwise directed.

6.60. Units Necessary to Complete a Test

Unless otherwise specified, a sufficient number of units to ensure a suitable analytical result shall be taken.

6.60.10. Tablets

Where the procedure of a Tablet monograph directs to weigh and finely powder not fewer than a given number of Tablets, a counted number of Tablets shall be weighed and reduced to a powder. The portion of the powdered Tablets taken shall be representative of the whole Tablets and shall, in turn, be weighed accurately.

6.60.20. Capsules

Where the procedure of a Capsule monograph gives direction to remove, as completely as possible, the contents of not fewer than a given number of the Capsules, a counted number of Capsules shall be carefully opened and the contents quantitatively removed, combined, mixed, and weighed accurately. The portion of mixed Capsules contents taken shall be representative of the contents of the Capsules and shall, in turn, be weighed accurately.

6.70. Reagents

The proper conduct of the compendial procedures and the reliability of the results depend, in part, upon the quality of the reagents used in the performance of the procedures. Unless otherwise specified, reagents conforming to the specifications set forth in the current edition of *Reagent Chemicals* published by the American Chemical Society (ACS) shall be used. Where such ACS reagent specifications are not available or where the required purity differs, compendial specifications for reagents of acceptable quality are provided (see the *Reagents, Indicators, and Solutions* section of the *USP-NF*). Reagents not covered by any of these specifications should be of a grade suitable to the proper performance of the method of assay or test involved.

Listing of these reagents, including the indicators and solutions employed as reagents, in no way implies that they have therapeutic utility; furthermore, any reference to *USP* or *NF* in their labeling shall include also the term "reagent" or "reagent grade." *USP* may supply reagents if they otherwise may not be generally commercially available.

6.80. Equipment

Unless otherwise specified, a specification for a definite size or type of container or apparatus in a procedure is given solely as a recommendation. Other dimensions or types may be used if they are suitable for the intended use.

6.80.10. Apparatus for Measurement

Where volumetric flasks or other exact measuring, weighing, or sorting devices are specified, this or other equipment of at least equivalent accuracy shall be employed.

6.80.10.1. Pipet/Pipette

Where a pipet/pipette is specified, a suitable buret may be substituted. Where a "to contain" pipet/pipette is specified, a suitable volumetric flask may be substituted.

6.80.10.2. Light Protection

Where low-actinic or light-resistant containers are specified, either containers specially treated to protect contents from light or clear containers that have been rendered opaque by application of a suitable coating or wrapping may be used.

6.80.20. Instrumental Apparatus

An instrument may be substituted for the specified instrument if the substitute uses the same fundamental principles of operation and is of equivalent or greater sensitivity and accuracy. These characteristics shall be qualified as appropriate. Where a particular brand or source of a material, instrument, or piece of equipment, or the name and address of a manufacturer or distributor, is mentioned (ordinarily in a footnote), this identification is furnished solely for informational purposes as a matter of convenience, without implication of approval, endorsement, or certification.

6.80.20.1. Chromatographic Tubes and Columns

The term "diameter" refers to internal diameter (ID).

6.80.20.2. Tubing

The term "diameter" refers to outside diameter (OD).

6.80.20.3. Steam Bath

Where use of a steam bath is directed, use actively flowing steam or another regulated heat source controlled at an equivalent temperature.

6.80.20.4. Water Bath

A water bath requires vigorously boiling water unless otherwise specified.

6.80.30. Temperature Reading Devices

Temperature reading devices suitable for Pharmacopeial tests conform to specifications that are traceable to an NIST standard or equivalent. Temperature reading devices may be of the liquid-in-glass type or an analog or digital temperature indicator type, such as a resistance temperature device, thermistor, or thermocouple. Standardization of thermometers is performed on an established testing frequency with a temperature standard traceable to NIST. For example, refer to the current issue of ASTM standards E1 for liquid-in-glass thermometers.

7. TEST RESULTS**7.10. Interpretation of Requirements**

Analytical results observed in the laboratory (or calculated from experimental measurements) are compared with stated acceptance criteria to determine whether the article conforms to compendial requirements.

The reportable value, which often is a summary value for several individual determinations, is compared with the acceptance criteria. The reportable value is the end result of a completed measurement procedure, as documented.

Where acceptance criteria are expressed numerically herein through specification of an upper and/or lower limit, permitted values include the specified values themselves, but no values outside the limit(s). Acceptance criteria are considered significant to the last digit shown.

7.10.5. Nominal Concentrations in Equations

Where a "nominal concentration" is specified, calculate the concentration based on the label claim. In assay procedures, water correction is typically stated in the Definition and on the label of the USP Reference Standard. For other procedures, correction for assayed content, potency, or both is made prior to using the concentration in the equation provided in the monograph.

7.10.10. Equivalence Statements in Titrimetric Procedures

The directions for titrimetric procedures conclude with a statement of the weight of the analyte that is equivalent to each mL of the standardized titrant. In such an equivalence statement, the number of significant figures in the concentration of the titrant should be understood to correspond to the number of significant figures in the weight of the analyte. Corrections to calculations based on the blank determination are to be made for all titrimetric assays where appropriate (see *Titrimetry* 〈 541 〉).

7.20. Rounding Rules

The observed or calculated values shall be rounded off to the number of decimal places that is in agreement with the limit expression. Numbers should not be rounded until the final calculations for the reportable value have been completed. Intermediate calculations (e.g., slope for linearity) may be rounded for reporting purposes, but the original (not rounded) value should be used for any additional required calculations. Acceptance criteria are fixed numbers and are not rounded.

When rounding is required, consider only one digit in the decimal place to the right of the last place in the limit expression. If this digit is smaller than 5, it is eliminated and the preceding digit is unchanged. If this digit is equal to or greater than 5, it is eliminated and the preceding digit is increased by 1.

Illustration of Rounding Numerical Values for Comparison with Requirements			
Compendial Requirement	Unrounded Value	Rounded Result	Conforms
Assay limit \geq 98.0%	97.96%	98.0%	Yes
	97.92%	97.9%	No
	97.95%	98.0%	Yes
Assay limit \leq 101.5%	101.55%	101.6%	No
	101.46%	101.5%	Yes
	101.45%	101.5%	Yes
Limit test \leq 0.02%	0.025%	0.03%	No
	0.015%	0.02%	Yes
	0.027%	0.03%	No
Limit test \leq 3 ppm	3.5 ppm	4 ppm	No
	3.4 ppm	3 ppm	Yes
	2.5 ppm	3 ppm	Yes

Change to read:**8. TERMS AND DEFINITIONS****8.10. Abbreviations**

- RS refers to a USP Reference Standard.
- CS refers to a Colorimetric Solution.
- TS refers to a Test Solution.
- VS refers to a Volumetric Solution that is standardized in accordance with directions given in the individual monograph or in the *Reagents, Indicators, and Solutions* section of *USP-NF*.

8.20. About

"About" indicates a quantity within 10%.

If the measurement is stated to be "accurately measured" or "accurately weighed," follow the statements in the general chapters *Volumetric Apparatus* 〈 31 〉 and *Balances* 〈 41 〉, respectively.

8.30. Alcohol Content

Percentages of alcohol, such as those under the heading *Alcohol Content*, refer to percentage by volume of C₂H₅OH at 15.56°. Where a formula, test, or assay calls for alcohol, ethyl alcohol, or ethanol, the *USP* monograph article Alcohol shall be used. Where reference is made to "C₂H₅OH," absolute (100 percent) ethanol is intended. Where a procedure calls for dehydrated alcohol, alcohol absolute, or anhydrous alcohol, the *USP* monograph article Dehydrated Alcohol shall be used.

8.40. Atomic Weights

Atomic weights used in computing molecular weights and the factors in the assays and elsewhere are those established by the IUPAC Commission on Atomic Weights and Isotopic Abundances.

8.50. Blank Determinations

Where it is directed that "any necessary correction" be made by a blank determination, the determination shall be conducted using the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under assay or test, but with the substance itself omitted.

8.60. Concomitantly

"Concomitantly" denotes that the determinations or measurements are to be performed in immediate succession.

8.70. Desiccator

The instruction "in a desiccator" indicates use of a tightly closed container of suitable size and design that maintains an atmosphere of low moisture content by means of a suitable desiccant such as anhydrous calcium chloride, magnesium perchlorate, phosphorus pentoxide, or silica gel. See also section 8.220, *Vacuum Desiccator*.

8.80. Logarithms

Logarithms are to the base 10.

8.90. Microbial Strain

A microbial strain cited and identified by its ATCC catalog number shall be used directly or, if

subcultured, shall be used not more than five passages removed from the original strain.

8.100. Negligible

"Negligible" indicates a quantity not exceeding 0.50 mg.

8.110. NLT/NMT

"NLT" means "not less than." "NMT" means "not more than."

8.120. Odor

"Odorless," "practically odorless," "a faint characteristic odor," and variations thereof indicate evaluation of a suitable quantity of freshly opened material after exposure to the air for 15 minutes. An odor designation is descriptive only and should not be regarded as a standard of purity for a particular lot of an article.

8.130. Percent

"Percent" used without qualification means:

- For mixtures of solids and semisolids, percent weight in weight;
- For solutions or suspensions of solids in liquids, percent weight in volume;
- For solutions of liquids in liquids, percent volume in volume;
- For solutions of gases in liquids, percent weight in volume.

For example, a 1 percent solution is prepared by dissolving 1 g of a solid or semisolid, or 1 mL of a liquid, in sufficient solvent to make 100 mL of the solution.

8.140. Percentage Concentrations

Percentage concentrations are expressed as follows:

- *Percent Weight in Weight (w/w)* is defined as the number of g of a solute in 100 g of solution.
- *Percent Weight in Volume (w/v)* is defined as number of g of a solute in 100 mL of solution.
- *Percent Volume in Volume (v/v)* is defined as the number of mL of a solute in 100 mL of solution.

8.150. Pressure

Pressure is determined by use of a suitable manometer or barometer calibrated in terms of the pressure exerted by a column of mercury of the stated height.

8.160. Reaction Time

Reaction time is 5 minutes unless otherwise specified.

8.170. Specific Gravity

Specific gravity is the weight of a substance in air at 25^o divided by the weight of an equal volume of water at the same temperature.

8.180. Temperatures

Temperatures are expressed in centigrade (Celsius) degrees, and all measurements are made at 25^o unless otherwise indicated. Where moderate heat is specified, any temperature not higher than 45^o (113^o F) is indicated.

8.190. Time

Unless otherwise specified, rounding rules, as described in section 7.20, *Rounding Rules*, apply

to any time specified.

8.200. Transfer

"Transfer" indicates a quantitative manipulation.

8.210. Vacuum

"Vacuum" denotes exposure to a pressure of less than 20 mm of mercury (2.67 kPas), unless otherwise indicated.

8.220. Vacuum Desiccator

"Vacuum desiccator" indicates a desiccator that maintains a low-moisture atmosphere at a reduced pressure of not more than 20 mm of mercury (2.67 kPas) or at the pressure designated in the individual monograph.

8.230. Water

8.230.10. Water as an Ingredient in an Official Product

As an ingredient in an official product, water meets the requirements of the appropriate water monograph in *USP* or *NF*.

8.230.20. Water in the Manufacture of Official Substances

When used in the manufacture of official substances, water ~~may meet the requirements for drinking water as set forth in the regulations of the U.S. Environmental Protection Agency (potable water).~~

▲shall meet the requirements for drinking water as set forth in the U.S. Environmental Protection Agency National Primary Drinking Water Regulations or in the drinking water regulations of the European Union or of Japan, or in the World Health Organization's Guidelines for Drinking Water Quality. Additional specifications may be required in monographs.▲*USP39*

8.230.30. Water in a Compendial Procedure

When water is called for in a compendial procedure, the USP article Purified Water shall be used unless otherwise specified. Definitions for ~~High-Purity Water and Carbon Dioxide-Free Water~~ are provided in ~~Containers—Glass <660>~~. Definitions of other types of water are provided in ~~Water for Pharmaceutical Purposes <1231>~~.

▲other types of water are provided in *Reagents, Indicators, and Solutions* and in *Water for Pharmaceutical Purposes <1231>*.▲*USP39*

8.240. Weights and Measures

In general, weights and measures are expressed in the International System of Units (SI) as established and revised by the *Conférence générale des poids et mesures*. For compendial purposes, the term "weight" is considered to be synonymous with "mass."

Molality is designated by the symbol *m* preceded by a number that represents the number of moles of the designated solute contained in 1 kilogram of the designated solvent.

Molarity is designated by the symbol *M* preceded by a number that represents the number of moles of the designated solute contained in an amount of the designated solvent that is

sufficient to prepare 1 liter of solution.

Normality is designated by the symbol N preceded by a number that represents the number of equivalents of the designated solute contained in an amount of the designated solvent that is sufficient to prepare 1 liter of solution.

▲The symbol for degrees ($^{\circ}$) without a qualifying unit of measure represents degrees Celsius.

▲USP39

Chart of Symbols and Prefixes commonly employed for SI metric units and other units:

	Units	Symbol	Notes
Length			
	meter	m	
	centimeter	cm	
	millimeter	mm	
	micrometer	μm	Previously referred to as a micron
	nanometer	nm	Previously the symbol $\text{m}\mu$ (for millimicron) was used
	Ångström	Å	Equal to 0.1 nm
Mass			
	kilogram	kg	
	gram	g	
	milligram	mg	
	microgram	μg	The symbol μg is used in the <i>USP</i> and <i>NF</i> to represent micrograms, but micrograms may be represented as "mcg" for labeling and prescribing purposes. The term "gamma," symbolized by γ , frequently is used to represent micrograms in biochemical literature.
	nanogram	ng	
	picogram	pg	
	dalton	Da	Also referred to as the unified atomic mass unit and is equal to 1/12 times the mass of the free carbon 12 atom.
	kilodalton	kDa	
Time			
	second	s	
	minute	min	
	hour	h	
Volume			
	liter	L	1 L is equal to 1000 cm^3 (cubic centimeters)
	deciliter	dL	
	milliliter	mL	1 mL is equal to 1 cm^3 , sometimes referred to as cc
	microliter	μL	
Temperature			
	Celsius	$^{\circ}\text{C}$	

Amount of Substance			
	mole	mol	Historically referred to as gram-molecular weight or gram-atomic weight
	millimole	mmol	
	micromole	μmol	
	femtomole	fmol	
	equivalent	Eq	Also referred to as gram-equivalent weight. It is used in the calculation of substance concentration in units of normality. This unit is no longer preferred for use in analytical chemistry or metrology.
	milliequivalent	mEq	
	osmole	Osmol	Osmotic pressure of a solution, related to substance concentration.
	milliosmole	mOsmol	
Pressure			
	pascal	Pa	
	kilopascal	kPa	
	pounds per square inch	psi	
	millimeter of mercury	mmHg	Equal to 133.322 Pa
Electrical units			
	ampere	A	
	volt	V	
	millivolt	mV	
	hertz	Hz	Unit of frequency
	kilohertz	kHz	
	megahertz	MHz	
	electron volt	eV	
	kilo-electron volt	keV	
	mega-electron volt	MeV	
Radiation			
	becquerel	Bq	SI unit of activity for radionuclides
	kilobecquerel	kBq	
	megabecquerel	MBq	
	gigabecquerel	GBq	
	curie	Ci	Non-SI unit of activity for radionuclides
	millicurie	mCi	
	microcurie	μCi	
	nanocurie	nCi	
Other			

	acceleration due to gravity	g_n ▲▲USP39
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Used to express rate of centrifugation revolutions per minute rpm Used to express rate of centrifugation

Selected SI Prefixes

Name	Symbol	Factor
giga	G	10^9
mega	M	10^6
kilo	k	10^3
deci	d	10_{-1}
centi	c	10_{-2}
milli	m	10_{-3}
micro	μ	10_{-6}
nano	n	10_{-9}
pico	p	10_{-12}
femto	f	10_{-15}

Change to read:

9. PRESCRIBING AND DISPENSING

9.10 Use of Metric Units

Prescriptions for compendial articles shall be written to state the quantity and/or strength desired in metric units unless otherwise indicated in the individual monograph (see also *Units of Potency [Biological]*, section 5.50.10 above). If an amount is prescribed by any other system of measurement, only an amount that is the metric equivalent of the prescribed amount shall be dispensed.

▲Abbreviations for units or International Units shall not be used for labeling or prescribing purposes. ▲USP39

Apothecary unit designations on labels and labeling shall not be used.

9.20 Changes in Volume

In the dispensing of prescription medications, slight changes in volume owing to variations in room temperatures may be disregarded.

Change to read:

10. PRESERVATION, PACKAGING, STORAGE, AND LABELING

[Note—Storage and packaging-related provisions previously addressed in the *General Notices and Requirements* have been omitted, except for the brief provision ~~proposed to be established~~ below in 10.10; see the new general chapter ~~(659)~~ for packaging components and storage conditions. Labeling-related provisions are also in the process of being moved to a new general chapter ~~(7)~~, and will be similarly omitted in the future.

▲previously established below in 10.10; see the general chapter *Packaging and Storage Requirements* 〈 659 〉 for packaging and storage requirements. Labeling-related provisions also previously addressed in the *General Notices and Requirements* are also now proposed for deletion, except for the brief provision proposed to be established below in 10.20; see the new general chapter *Labeling* 〈 7 〉 for labeling requirements. General chapter 〈 7 〉 is scheduled to become official on the same date these changes to *General Notices* become official.▲*USP39*

]

10.10. Packaging and Storage

All articles in *USP* or *NF* are subject to the packaging and storage requirements specified in general chapter *Packaging and Storage Requirements* 〈 659 〉, unless different requirements are provided in a specific

▲an individual▲*USP39*

monograph.

▲10.20. Labeling

All articles in *USP* or *NF* are subject to the labeling requirements specified in general chapter 〈 7 〉, unless different requirements are provided in an individual monograph.▲*USP39*

~~10.40. Labeling~~

~~The term "labeling" designates all labels and other written, printed, or graphic matter upon an immediate container of an article or upon, or in, any package or wrapper in which it is enclosed, except any outer shipping container. The term "label" designates that part of the labeling upon the immediate container.~~

~~A shipping container containing a single article, unless such container is also essentially the immediate container or the outside of the consumer package, is labeled with a minimum of product identification (except for controlled articles), lot number, expiration date, and conditions for storage and distribution.~~

~~Articles in these compendia are subject to compliance with such labeling requirements as may be promulgated by governmental bodies in addition to the compendial requirements set forth for the articles.~~

~~10.40.10. Amount of Ingredient Per Dosage Unit~~

~~The strength of a drug product is expressed on the container label in terms of micrograms or milligrams or grams or percentage of the therapeutically active moiety or drug substance, whichever form is used in the title, unless otherwise indicated in an individual monograph. Both the active moiety and drug substance names and their equivalent amounts are then provided in the labeling.~~

~~Official articles in capsule, tablet, or other unit dosage form shall be labeled to express the quantity of each active ingredient or recognized nutrient contained in each such unit; except that, in the case of unit dose oral solutions or suspensions, whether supplied as liquid~~

~~preparations or as liquid preparations that are constituted from solids upon addition of a designated volume of a specific diluent, the label shall express the quantity of each active ingredient or recognized nutrient delivered under the conditions prescribed in *Deliverable Volume* (698). Official drug products not in unit dosage form shall be labeled to express the quantity of each active ingredient in each milliliter or in each gram, or to express the percentage of each such ingredient (see 8.140, *Percentage Concentrations*), except that oral liquids or solids intended to be constituted to yield oral liquids may, alternatively, be labeled in terms of each 5-mL portion of the liquid or resulting liquid. Unless otherwise indicated in a monograph or chapter, such declarations of strength or quantity shall be stated only in metric units. See also 5.50.10, *Units of Potency (Biological)*.~~

~~10.40.20. Use of Leading and Terminal Zeros~~

~~To help minimize the possibility of errors in the dispensing and administration of drugs, the quantity of active ingredient when expressed in whole numbers shall be shown without a decimal point that is followed by a terminal zero (e.g., express as 4 mg [not 4.0 mg]). The quantity of active ingredient when expressed as a decimal number smaller than 1 shall be shown with a zero preceding the decimal point (e.g., express as 0.2 mg [not .2 mg]).~~

~~10.40.30. Labeling of Salts of Drugs~~

~~It is an established principle that official articles shall have only one official title. For purposes of saving space on labels, and because chemical symbols for the most common inorganic salts of drugs are well known to practitioners as synonymous with the written forms, the following alternatives are permitted in labeling official articles that are salts: HCl for hydrochloride; HBr for hydrobromide; Na for sodium; and K for potassium. The symbols Na and K are intended for use in abbreviating names of the salts of organic acids, but these symbols are not used where the word Sodium or Potassium appears at the beginning of an official title (e.g., Phenobarbital Na is acceptable, but Na Salicylate is not to be written).~~

~~10.40.40. Labeling Vitamin-Containing Products~~

~~The vitamin content of an official drug product shall be stated on the label in metric units per dosage unit. The amounts of vitamins A, D, and E may be stated also in USP Units. Quantities of vitamin A declared in metric units refer to the equivalent amounts of retinol (vitamin A alcohol). The label of a nutritional supplement shall bear an identifying lot number, control number, or batch number.~~

~~10.40.50. Labeling Botanical-Containing Products~~

~~The label of an herb or other botanical intended for use as a dietary supplement bears the statement, "If you are pregnant or nursing a baby, seek the advice of a health professional before using this product."~~

~~10.40.60. Labeling Parenteral and Topical Preparations~~

~~The label of a preparation intended for parenteral or topical use states the names of all added substances (see 5.20, *Added Substances* and see *Labeling* under *Injections* (1)), and, in the case of parenteral preparations, also their amounts or proportions, except that for substances added for adjustment of pH or to achieve isotonicity, the label may indicate only their presence and the reason for their addition.~~

~~10.40.70. Labeling Electrolytes~~

~~The concentration and dosage of electrolytes for replacement therapy (e.g., sodium chloride or potassium chloride) shall be stated on the label in milliequivalents (mEq). The label of the product shall indicate also the quantity of ingredient(s) in terms of weight or percentage concentration.~~

~~10.40.80. Labeling Alcohol~~

~~The content of alcohol in a liquid preparation shall be stated on the label as a percentage (v/v) of C₂H₅OH.~~

~~10.40.90. Special Capsules and Tablets~~

~~The label of any form of Capsule or Tablet intended for administration other than by swallowing intact bears a prominent indication of the manner in which it shall be used.~~

~~10.40.100. Expiration Date and Beyond-Use Date~~

~~The label of an official drug product or nutritional or dietary supplement product shall bear an expiration date. All articles shall display the expiration date so that it can be read by an ordinary individual under customary conditions of purchase and use. The expiration date shall be prominently displayed in high contrast to the background or sharply embossed, and easily understood (e.g., "EXP 6/08," "Exp. June 08," or "Expires 6/08"). [Note—For additional information and guidance, refer to the Consumer Healthcare Products Association's *Voluntary Codes and Guidelines of the Self-Medication Industry*.]~~

~~The monographs for some preparations state how the expiration date that shall appear on the label shall be determined. In the absence of a specific requirement in the individual monograph for a drug product or nutritional supplement, the label shall bear an expiration date assigned for the particular formulation and package of the article, with the following exception: the label need not show an expiration date in the case of a drug product or nutritional supplement packaged in a container that is intended for sale without prescription and the labeling of which states no dosage limitations, and which is stable for not less than 3 years when stored under the prescribed conditions.~~

~~Where an official article is required to bear an expiration date, such article shall be dispensed solely in, or from, a container labeled with an expiration date, and the date on which the article is dispensed shall be within the labeled expiry period. The expiration date identifies the time during which the article may be expected to meet the requirements of the compendial monograph, provided it is kept under the prescribed storage conditions. The expiration date limits the time during which the article may be dispensed or used. Where an expiration date is stated only in terms of the month and the year, it is a representation that the intended expiration date is the last day of the stated month. The beyond-use date is the date after which an article shall not be used. The dispenser shall place on the label of the prescription container a suitable beyond-use date to limit the patient's use of the article based on any information supplied by the manufacturer and the *General Notices*. The beyond-use date placed on the label shall not be later than the expiration date on the manufacturer's container.~~

~~For articles requiring constitution before use, a suitable beyond-use date for the constituted product shall be identified in the labeling.~~

~~For all other dosage forms, in determining an appropriate period of time during which a~~

prescription drug may be retained by a patient after its dispensing, the dispenser shall take into account, in addition to any other relevant factors, the nature of the drug; the container in which it was packaged by the manufacturer and the expiration date thereon; the characteristics of the patient's container, if the article is repackaged for dispensing; the expected storage conditions to which the article may be exposed; any unusual storage conditions to which the article may be exposed; and the expected length of time of the course of therapy. The dispenser shall, on taking into account the foregoing, place on the label of a multiple-unit container a suitable beyond-use date to limit the patient's use of the article. Unless otherwise specified in the individual monograph, or in the absence of stability data to the contrary, such beyond-use date shall be not later than (a) the expiration date on the manufacturer's container, or (b) 1 year from the date the drug is dispensed, whichever is earlier. For nonsterile solid and liquid dosage forms that are packaged in single-unit and unit-dose containers, the beyond-use date shall be 1 year from the date the drug is packaged into the single-unit or unit-dose container or the expiration date on the manufacturer's container, whichever is earlier, unless stability data or the manufacturer's labeling indicates otherwise.

The dispenser shall maintain the facility where the dosage forms are packaged and stored, at a temperature such that the mean kinetic temperature is not greater than 25°. The plastic material used in packaging the dosage forms shall afford better protection than polyvinyl chloride, which does not provide adequate protection against moisture permeation. Records shall be kept of the temperature of the facility where the dosage forms are stored, and of the plastic materials used in packaging.

10.40.100.1. Compounded Preparations

The label on the container or package of an official compounded preparation shall bear a beyond-use date. The beyond-use date is the date after which a compounded preparation is not to be used. Because compounded preparations are intended for administration immediately or following short-term storage, their beyond-use dates may be assigned based on criteria different from those applied to assigning expiration dates to manufactured drug products.

The monograph for an official compounded preparation typically includes a beyond-use requirement that states the time period following the date of compounding during which the preparation, properly stored, may be used. In the absence of stability information that is applicable to a specific drug and preparation, recommendations for maximum beyond-use dates have been devised for nonsterile compounded drug preparations that are packaged in tight, light-resistant containers and stored at controlled room temperature unless otherwise indicated (see *Stability Criteria and Beyond-Use Dating* in the general test chapter *Pharmaceutical Compounding—Nonsterile Preparations* ~~(795)~~).

▲▲USP39

BRIEFING

《 21 》 **Thermometers**, USP 38 page 98. It is proposed to omit this general chapter from USP because it does not have the structure nor content of a typical enforceable chapter. The updated relevant content has been introduced into the USP *General Notices and Requirements* under 6.80.30. *Temperature Reading Devices*.

Chapter 《 21 》 is referenced in the following general chapters and monographs: *Congeeing*

Temperature 〈 651 〉, *Monitoring Devices—Time, Temperature, and Humidity* 〈 1118 〉, and *Emulsifying Wax*. The omission of 〈 21 〉 does affect the citations within these chapters or monographs. These citations will be replaced when the general chapter is officially omitted.

(GCPA: A. Hernandez-Cardoso.)
Correspondence Number—C153848

Comment deadline: March 31, 2015

Delete the following:

▲ 〈 21 〉 THERMOMETERS

~~Temperature reading devices suitable for Pharmacopeial tests conform to specifications that are traceable to a NIST standard. Temperature reading devices may be of the liquid-in-glass type or an analog or digital temperature indicator type, such as a resistance temperature device, thermistor, or thermocouple.~~

~~An analog or digital temperature indicator consists of a temperature probe, which houses a sensor. The probe is attached to a meter capable of translating a signal in ohms or millivolts into a temperature reading. The temperature probe portion of the analog or digital temperature indicator that is submerged in the medium whose temperature is being measured must be made of inert material. Standardization of analog and digital temperature indicator devices is performed on an established testing frequency with a temperature standard traceable to NIST. In the selection of a temperature reading device, careful consideration of the condition under which it is to be used is essential.~~

~~Liquid-in-glass thermometers may be standardized for total immersion, partial immersion, or full immersion. Insofar as practicable, each thermometer should be employed according to the condition of immersion under which it was standardized. Standardization of thermometers is performed on an established testing frequency with a temperature standard traceable to NIST. Refer to the current issue of ASTM standards E1. Standardization of liquid-in-glass thermometers for total immersion involves immersion of the thermometer to the top of the liquid column, with the remainder of the stem and the upper expansion chamber exposed to ambient temperature. Standardization for partial immersion involves immersion of the thermometer to the indicated immersion line etched on the front of the thermometer, with the remainder of the stem exposed to ambient temperature. Standardization for full immersion involves immersion of the entire thermometer, with no portion of the stem exposed to ambient temperature. For use under other conditions of immersion, an emergent stem correction is necessary to obtain correct temperature readings. ▲USP39~~

BRIEFING

〈 321 〉 **Drug Product Assay Tests—Organic Chemical Medicines.** U.S. Pharmacopeia (USP) is engaged in the ongoing challenge of obtaining information needed to create and sustain up-to-date drug product quality monographs for drugs marketed under the Food and Drug Administration (FDA) over-the-counter (OTC) drug monograph system (21 CFR Part 330). This general chapter will facilitate assay testing in drug products by providing chromatographic procedures to address the development of quality standards for this category of drug products. The first procedure, *Procedure 1*, introduced in this chapter,

covers drug products containing 1 or more of 11 different active ingredients associated with oral dosage forms. *Procedure 1* provides a high-performance liquid chromatographic assay procedure using photo-diode array ultraviolet spectroscopic detection for the identification and quantitation of acetaminophen, aspirin, brompheniramine maleate, dextromethorphan hydrobromide, chlorpheniramine maleate, doxylamine succinate, diphenhydramine citrate, diphenhydramine hydrochloride, pseudoephedrine hydrochloride, phenylephrine hydrochloride, and caffeine in drug products. The proposed procedure uses an Acquity UPLC HSS T3 brand of L1 column manufactured by Waters. This analytical procedure and future procedures will be reflective of currently marketed drug product active ingredient combinations, their routes of administration, and/or their dosage forms. The *Assay* and *Identification* procedures in the individual drug product monographs will be modernized by cross-referencing the procedures in this general chapter, unless there are circumstances for which an individual product must have a separate procedure to ensure its identity, quality, purity, or strength. New drug product monographs proposed for this category will also cross-reference the procedures in this general chapter. Further information can be found in the *Stimuli* article *Medicines Marketed under the Food and Drug Administration's Over-the-Counter (FDA OTC) System Regulations: Strategy for Developing Compendial Quality Standards*, also published in this issue of *Pharmacopeial Forum (PF)*. This article describes in detail the rationale for development of this chapter and other related chapters, as well as the associated impact on drug product monographs. USP plans to continue with this initiative to revise or establish standards for OTC drug products.

The following is a list of USP drug product monographs that are being proposed to be modernized in this *PF* by replacing the current official *Assay* test procedure, all of which use outdated techniques and methodologies, with the proposed procedure, *Procedure 1*.

- *Aspirin Capsules*
- *Brompheniramine Maleate Oral Solution*
- *Brompheniramine Maleate Tablets*
- *Chlorpheniramine Maleate Tablets*

(SM2: A. Potts.)

Correspondence Number—C150881

Comment deadline: March 31, 2015

Add the following:

▲〈 321 〉 DRUG PRODUCT ASSAY TESTS—ORGANIC CHEMICAL MEDICINES

INTRODUCTION

The general procedures set forth in the following sections of this general chapter are applicable to the quantitation and identification of active ingredients in drug products.

ASSAY

• **Procedure 1**

Procedure 1 provides a high-performance liquid chromatographic assay procedure using ultraviolet spectroscopic detection for acetaminophen, aspirin, brompheniramine maleate, caffeine, chlorpheniramine maleate, dextromethorphan hydrobromide, diphenhydramine

citrate, diphenhydramine hydrochloride, doxylamine succinate, phenylephrine hydrochloride, and pseudoephedrine hydrochloride containing drug products.

Solution preparation: All solution preparations containing drug substances should be protected from light and should be stored only for as long as can be supported by solution stability data acquired during verification under actual conditions of use.

Solution A: 0.15% Trifluoroacetic acid in water

Solution B: 0.02% Trifluoroacetic acid in a mixture of acetonitrile and methanol (75:25)

Mobile phase: See *Table 1*. Return to original conditions and equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
0.6	90	10
2.3	77	23
5.5	77	23
7.4	57	43
8.0	57	43
12.5	1	99
13.5	1	99

Diluent A: 0.2% Formic acid in a mixture of methanol and water (25:75). Add 2.0 mL of formic acid and dilute to 1000 mL for each liter.

Diluent B: 0.2% Formic acid in a mixture of acetonitrile and water (5:95). Add 2.0 mL of formic acid and dilute to 1000 mL for each liter.

System suitability solution: 0.05 mg/mL each of USP Caffeine RS, USP Doxylamine Succinate RS, USP Pseudoephedrine Hydrochloride RS, USP Dextromethorphan Hydrobromide RS, and USP Diphenhydramine Hydrochloride RS in *Diluent A*

Standard solution: The *Standard solution* may be limited to the USP Reference Standards that correspond to the active ingredients specified in the monograph of the Article under test. Unless otherwise stated in the monograph, *Table 2* provides the concentration and diluent information for each Reference Standard solution. Reference Standards within the same category may be combined into a single solution.

Table 2

Category	Reference Standard	Concentration (mg/mL)	Diluent
1	USP Acetaminophen RS USP Caffeine RS USP Chlorpheniramine Maleate RS USP Brompheniramine Maleate RS USP Dextromethorphan Hydrobromide RS USP Diphenhydramine Citrate RS USP Diphenhydramine Hydrochloride RS USP Pseudoephedrine Hydrochloride RS	0.1	<i>Diluent A</i>
1	USP Doxylamine Succinate RS	0.05	<i>Diluent A</i>
2	USP Phenylephrine Hydrochloride RS	0.1	<i>Diluent B</i>
3	USP Aspirin RS	0.2	<i>Diluent A</i>

Transfer an amount of the USP RS into a suitable flask. Dissolve in the specified diluent, with sonication if necessary. Dilute to final volume with the specified diluent.

Sample solution: (unless otherwise stated in the monograph) Prepare a solution in *Diluent A* containing an amount of drug product necessary to provide a final concentration that is similar to the concentration of the drug in the *Standard solution* prepared as follows. Transfer a suitable portion of drug product to a suitable volumetric flask. Add *Diluent A* equivalent to about two-thirds of the flask volume. Dissolve and make to volume with *Diluent A*. Sonicate solutions for Aspirin analysis for 5 min, otherwise sonicate for 15 min. Pass a portion through a suitable filter of 0.2- μ m pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Variable wavelength, UV, see *Table 3*. Unless otherwise stated in the monograph, use a photo-diode array detector (190–400 nm) when specified for Identification test.

Column: 2.1-mm \times 15-cm; 1.8- μ m packing L1

Column temperature: 40 $^{\circ}$

Flow rate: 0.4 mL/min

Injection volume: 2 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The approximate retention times are given in *Table 3*.]

Suitability requirements

Relative standard deviation: NMT 1.0%, *Standard solution*

Tailing: NMT 2.0, *Standard solution*

Resolution: Evaluate the *System suitability solution* at 254 nm.

The resolution, *R*, between caffeine and doxylamine is NLT 1.

The resolution, *R*, between doxylamine and pseudoephedrine is NLT 2.0.

The resolution, *R*, between dextromethorphan and diphenhydramine is NLT 1.

Analysis

Samples: Analyze the *Standard solution* and *Sample solution* at the wavelengths given in *Table 3*.

Table 3

Name	Wavelength (nm)	Retention Time (min)
Phenylephrine	225	1.9
Acetaminophen	254	2.7
Caffeine	254	3.9
Doxylamine	264	4.0
Pseudoephedrine	210	4.1
Chlorpheniramine	225	6.1
Brompheniramine	264	7.0
Aspirin	276	7.5
Dextromethorphan	225	10.1
Diphenhydramine	225	10.2

If present, calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$), aspirin ($C_9H_8O_4$), caffeine ($C_8H_{10}N_4O_2$), chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), brompheniramine maleate ($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$), dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$), diphenhydramine citrate ($C_{17}H_{21}NO \cdot C_6H_8O_7$), diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$), doxylamine succinate ($C_{17}H_{22}N_2O \cdot C_4H_6O_4$), phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$), or pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of drug product taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of the respective active ingredient from the *Sample solution*

r_S peak response of the respective active ingredient from the *Standard solution*

C_S concentration of the respective Reference Standard in the *Standard solution* (mg/mL)

C_U nominal concentration of the respective active ingredient from the *Sample solution* (mg/mL)

Chromatographic adjustments: The mobile phase gradient can be adjusted to achieve specificity for a given product matrix. This allowance supersedes provisions in $\langle 621 \rangle$ for adjusting chromatographic conditions and is intended to provide a measure of flexibility when needed. Suggestions for changing the gradient program are given in *Table 4*.

Table 4

Condition Change
Solution B (%): NMT 30% relative change may be made at the gradient times 0, 2.3, and 7.4 min to effect the %B/min gradient ramp rate.

Adjustments to the chromatographic procedure may lead to a need for verification or validation of the new conditions. See *Validation of Compendial Procedures* $\langle 1225 \rangle$ and *Verification of Compendial Procedures* $\langle 1226 \rangle$ for guidance. The need for adjustments does not change the need to meet the *System suitability requirements*.

ADDITIONAL REQUIREMENTS

• USP Reference Standards $\langle 11 \rangle$

- USP Acetaminophen RS
- USP Aspirin RS
- USP Brompheniramine Maleate RS
- USP Caffeine RS
- USP Chlorpheniramine Maleate RS
- USP Dextromethorphan Hydrobromide RS
- USP Diphenhydramine Citrate RS
- USP Diphenhydramine Hydrochloride RS
- USP Doxylamine Succinate RS
- USP Phenylephrine Hydrochloride RS
- USP Pseudoephedrine Hydrochloride RS

▲USP39

BRIEFING

《 327 》 **Drug Product Impurities Tests.** U.S. Pharmacopeial Convention (USP) is engaged in the ongoing challenge of obtaining information needed to create and sustain up-to-date drug product quality monographs for drugs marketed under the Food and Drug Administration's Over-the-Counter (FDA OTC) drug monograph system (21 CFR Part 330). This general chapter will facilitate testing for specified impurities in drug products by providing chromatographic procedures to address the development of quality standards for this category of drug products. The first procedure, *Salicylic Acid in Aspirin-containing Drug Products*, introduced in this chapter, provides a liquid chromatographic assay procedure using ultraviolet spectroscopic detection for the quantitation of salicylic acid, the principal degradation product, in aspirin-containing drug products. The proposed procedure uses an Acquity UPLC HSS T3 brand of L1 column manufactured by Waters and gives retention times for aspirin and salicylic acid at approximately 7.5 and 8.6 min, respectively.

This procedure and any future procedures will be reflective of additional specified impurities that need to be controlled in currently marketed drug products. The *Organic Impurities* procedures found in individual drug product monographs may be modernized by replacing those procedures with the procedures in this general chapter, unless there are circumstances for which an individual product must have a separate procedure to ensure identity, quality, purity, or strength. Further information can be found in the *Stimuli* article *Medicines Marketed under the Food and Drug Administration's Over-the-Counter (FDA OTC) System Regulations: Strategy for Developing Compendial Quality Standards*, also published in this issue of *Pharmacoepial Forum (PF)*. This article describes in detail the rationale for development of this chapter and other related chapters, as well as the associated impact on drug product monographs. USP plans to continue with this initiative to revise or establish standards for OTC drug products.

The following is a USP drug product monograph wherein the proposed procedure will replace the current *Limit of Free Salicylic Acid* test procedure.

- *Aspirin Capsules*

A revision to this monograph is published in this same issue of *PF*.

(SM2: A. Potts.)

Correspondence Number—C150882

Comment deadline: March 31, 2015

Add the following:

▲《 327 》 DRUG PRODUCT IMPURITIES TESTS

INTRODUCTION

The procedures set forth in the following sections of this general chapter are applicable to the specified organic impurities in drug products.

IMPURITIES

- **Salicylic Acid in Aspirin-Containing Drug Products**

This general test provides a procedure for salicylic acid, the principal degradation product that is formed by hydrolysis of aspirin. The typical relative retention time between aspirin and salicylic acid is approximately 1.1.

Solution A: 0.15% Trifluoroacetic acid in water

Solution B: 0.02% Trifluoroacetic acid in a mixture of acetonitrile and methanol (75:25)

Mobile phase: See *Table 1*. Return to original conditions and equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
0.6	90	10
2.3	77	23
5.5	77	23
7.4	57	43
8.0	57	43
12.5	1	99
13.5	1	99

Diluent: 0.2% Formic acid in a mixture of methanol and water (25:75). Add 2.0 mL of formic acid, and dilute to 1000 mL for each liter. Chill in an ice bath.

System suitability solution A: 0.05 mg/mL each of USP Caffeine RS, USP Doxylamine Succinate RS, USP Dextromethorphan Hydrobromide RS, and USP Diphenhydramine Hydrochloride RS in *Diluent*

System suitability solution B: 0.2 µg/mL of USP Salicylic Acid RS in *Diluent*

Standard solution: 6 µg/mL of USP Salicylic Acid RS in *Diluent*

Sample solution: Nominally 0.2 mg/mL of aspirin. Prepare a solution in *Diluent* containing an amount of drug product necessary to provide a final concentration of 0.2 mg/mL of aspirin. Samples should be injected immediately following preparation.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV

Analytical wavelength: 304 nm

Column: 2.1-mm × 15-cm; 1.8 µm packing L1

Column temperature: 40°

Flow rate: 0.4 mL/min

Autosampler: 2°–8°

Injection volume: 2 µL

System suitability

Samples: *System suitability solution A*, *System suitability solution B*, and *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0% of the salicylic acid peak, *Standard solution*

Resolution: NLT 1 between caffeine and doxylamine and NLT 1 between dextromethorphan and diphenhydramine, *System suitability solution A*

Tailing factor: NMT 1.5 for the salicylic acid peak, *Standard solution*

Signal-to-noise ratio: NLT 10 for the salicylic acid peak, *System suitability solution B*

Analysis

Samples: *Standard solution* and *Sample solution*.

Calculate the percentage of salicylic acid ($C_7H_6O_3$), relative to aspirin, in the portion of drug product taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of salicylic acid from the *Sample solution*

r_S peak response of salicylic acid from the *Standard solution*

C_S concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

C_U concentration of aspirin in the *Sample solution* (mg/mL)

ADDITIONAL REQUIREMENTS

• USP Reference Standards { 11 }

USP Caffeine RS

USP Dextromethorphan Hydrobromide RS

USP Diphenhydramine Hydrochloride RS

USP Doxylamine Succinate RS

USP Salicylic Acid RS

▲USP39

BRIEFING

{ 856 } **Near-Infrared Spectroscopy.** This new general chapter is being proposed as part of the broader initiative to revise the general chapters related to spectroscopy. This chapter provides calibration and qualification criteria for near-infrared (NIR) spectroscopic instrumentation as well as the acceptable procedure validation criteria. This chapter is complemented by general information chapter *Near-Infrared Spectroscopy—Theory and Practice* { 1119 }; a proposed revision to { 1119 }, including a title and number change, also appears in this issue of *PF*. Sections related to qualification, calibration, and validation that appeared in { 1119 } have been moved to the new chapter { 856 }; therefore, these sections now contain mandatory standards. The NIR technique can be used for both qualitative and quantitative analyses of liquids, powders, and solid materials. A *Stimuli* article published in *PF* 40(1) entitled *An Alignment of Concepts and Content across the Spectroscopy General Chapters in the United States Pharmacopeia–National Formulary (USP-NF)* provides insight into the rationale for the revisions to the chapters related to spectroscopy. This chapter follows the same format as the other spectroscopy chapters described in the *Stimuli* article and previously published in *PF*.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCCA: K. Zaidi.)

Correspondence Number—C142378

Comment deadline: March 31, 2015

Add the following:**▲〈 856 〉 Near-Infrared Spectroscopy**

1. Introduction
2. Qualification of NIR Spectrometers
 - 2.1 Installation Qualification
 - 2.2 Operational Qualification
 - 2.3 Performance Qualification
3. Procedure
4. Validation and Verification
 - 4.1 Validation
 - 4.2 Verification

1. INTRODUCTION

Near-infrared (NIR) spectroscopy covers the electromagnetic field wavelength range from 780 to 2500 nm, with the frequencies expressed in wavenumbers (from 12,821 to 4,000 cm^{-1}). NIR spectroscopy is a vibrational spectroscopic technique that measures the absorption of radiation in this wavelength range that is resonant with frequencies from molecular dipole changes in the sample; thus, NIR spectroscopy is related to infrared (IR) spectroscopy. The NIR spectroscopic region is dominated by overtone and combination frequencies of the molecular fundamental harmonic frequencies in the mid-IR region. The molar absorptivities in the NIR region tend to be much lower than those in the IR region, and the radiation can penetrate several millimeters into the material, giving rise to a wavelength-dependent scattering signal overlapping the molecular absorbance signals.

Not all materials absorb NIR radiation, and materials such as glass are relatively transparent. These attributes of NIR spectroscopy have been shown to be useful in the area of pharmaceutical analysis for both qualitative and quantitative applications. For further discussion of the theory and applications, see *Near-Infrared Spectroscopy—Theory and Practice* 〈 1119 〉, which may be a helpful, but not mandatory, resource.

The instrument qualification tests and acceptance criteria provided in this chapter may not be appropriate for some instrument configurations. In such cases, alternative instrument qualification and performance checks should be scientifically justified and documented before use. In addition, validation parameters discussed in this chapter may not be appropriate for all applications of NIR spectroscopy. Validation parameters characterized for a specific NIR-spectroscopic application should demonstrate the suitability of the application for its intended use.

2. QUALIFICATION OF NIR SPECTROMETERS

Qualification of NIR spectrometers is divided into three components: 1) *Installation*

Qualification, 2) *Operational Qualification*, and 3) *Performance Qualification*. For further discussion on qualification, see the general chapter *Analytical Instrument Qualification* (1058)

2.1 Installation Qualification

The installation qualification (IQ) requirements elicit evidence that the hardware and software are properly installed in the desired location.

2.2 Operational Qualification

In operational qualification (OQ), an instrument's performance is characterized using standards to verify that the system operates within target specifications. The purpose of OQ is to demonstrate that instrument performance is suitable. Because there are so many different approaches for measuring NIR spectra, OQ using standards with known spectral properties is recommended. The use of external, traceable reference standard materials does not justify omitting the instrument's internal quality control procedures. As is the case with any spectroscopic device, wavelength uncertainty, photometric linearity, and noise characteristics of NIR instruments must be qualified against target specifications for the intended application. The OQ tests described in the sections that follow are typical examples only. Other tests and samples can be used to establish specifications for OQ. Instrument vendors often have samples and test parameters available as part of the IQ/OQ package. The acceptance specifications given in this section are applicable for general use specifically, whereas specifications for particular instruments and applications can vary, depending on the analytical method used and the desired accuracy of the final result.

2.2.1 CHARACTERIZING INSTRUMENT PERFORMANCE

Use the apparatus according to the manufacturer's instructions, and carry out the prescribed verification at regular intervals. For in-line and on-line applications, the use of alternative means of control of instrument performance must be scientifically justified. For example, use the manufacturer's internal standards or use separate channels/probes, with scientific justification, to demonstrate instrument performance. System suitability tests may be required prior to sample scanning, and the instrument attributes with the most variation (typically photometric noise and wavelength accuracy) must be tested. The frequency at which each performance test is conducted must be assessed for risk, depending on the instrument type and its environment. For example, instruments placed in harsh environments, such as environments with changing temperature and humidity, will need frequent performance testing. Wherever possible, in the procedures for determining wavelength accuracy and linearity, analysts must use certified reference materials (CRMs) rather than laboratory-prepared solutions. These CRMs should be obtained from a recognized, accredited source and should include independently verified, traceable value assignments with associated calculated uncertainty. CRMs are to be kept clean and free from dust. Recertification is to be performed periodically to maintain the validity of the certification.

2.2.2 WAVELENGTH ACCURACY

Wavelength uncertainty: NIR spectra from sample and/or reference standard materials can be used to demonstrate an instrument's suitable wavelength-dispersion performance against target

specifications. Typically, wavelength uncertainty is characterized from a single spectrum (collected with the same spectral resolution used to obtain the standard value) using a minimum of three peaks that cover a suitable spectral range of the instrument.

Verification of wavelength scale (except for filter apparatus): Verify the wavelength scale utilized, which is generally in the region between about 780 and about 2,500 nm (about 12,800 cm^{-1} to about 4,000 cm^{-1}) or in the intended spectral range using one or more suitable wavelength standards that have characteristic maxima or minima within the range of methodology wavelengths to be used. The USP Near IR System Suitability RS can be used for wavelength verification. Suitable materials for demonstrating wavelength dispersion performance must be used. With appropriate justification, alternative standards may be used. Additional information on calibration considerations may be found in $\langle 1119 \rangle$.

Typical tolerances for agreement with standard values are ± 1.0 nm from approximately 700 to 2,000 nm and ± 1.5 nm above 2,000 nm to approximately 2,500 nm (± 8 cm^{-1} below 5,000 cm^{-1} and ± 4 cm^{-1} from 5,000 cm^{-1} to approximately 14,000 cm^{-1}). For the reference material used, apply the tolerance for the nearest wavelength (or wavenumber) from the above information for each peak used. For diode-array instruments, the pixel resolution (wavelength between pixels) can be as large as 10 nm. The pixel resolution must be adapted to match the spectral resolution. The peak-finding algorithms are critical to wavelength accuracy. From a practical standpoint, ± 2 nm is appropriate for peak wavelength accuracy when using such instrumentation. Alternatively, refer to the manufacturer's specifications for acceptance.

2.2.3 PHOTOMETRIC LINEARITY AND RESPONSE STABILITY

Photometric linearity is typically characterized in the range from 10% to 90% reflection (or transmission). NIR applications based on measuring an absorbance larger than 1.0 may require standards with reflectivity properties between 2% and 5% reflection (or transmission) for characterizing instrument performance at low reflectance. The purpose is to demonstrate a linear relationship between NIR reflectance and/or transmittance and instrument response across the scanning range of the instrument. Subsequent verifications of the photometric model can use the initial observed absorbance values as the established values.

Measure four photometric standards across the working method absorbance range. Typical tolerances for a linear relationship are 1.00 ± 0.05 for the slope and 0.00 ± 0.05 for the intercept of a plot of the measured photometric response versus the standard photometric response. Alternative tolerances may be used when justified for specific applications. A tolerance of $\pm 2\%$ is acceptable for long-term stability.

2.2.4 PHOTOMETRIC NOISE

NIR instrument software may include built-in procedures to automatically determine system noise and to provide a statistical report of noise or signal-to-noise ratio (S/N) over the instrument's operating range. In addition, it may be desirable to supplement such checks with measurements that do not rely directly on manufacturer-supplied procedures. Typical procedures involve measuring spectra of traceable reference materials with both high and low reflectance. Determine the photometric noise using suitable reflectance standards. Tolerances for these procedures should demonstrate suitable S/N for the intended application. Follow the

manufacturer's methodology and specifications.

High-flux noise: Instrument noise is evaluated at high-light flux by measuring reflectance or transmittance of the reference standard, with the reference material (e.g., 99% reflection standard) acting as both the sample and the background reference.

Low-flux noise: The same procedure may be used with a lower-reflectivity reference material (e.g., 10% reflectance standard) to determine system noise at reduced-light flux. The source, optics, detector, and electronics make significant contributions to the noise under these conditions.

2.3 Performance Qualification

The objective of performance qualification (PQ) is to ensure that the instrument is performing within specified limits with respect to wavelength and photometric precision. In certain cases, when the instrument has been set up for a specific measurement, it might no longer be possible or desirable to measure the wavelength and photometric (signal) qualification reference standards used in the OQ. Provided that the instrument operational qualification has shown that the equipment is fit for use, a single external performance verification standard can be used on a continuing basis (e.g., daily or before use). The performance verification standard must match the format of the samples in the current analysis as closely as possible and must use similar spectral acquisition parameters. Quantitative measurements of an external performance verification standard can be used to check both the wavelength accuracy and the photometric precision. Favorable comparison of a series of performance verification spectra demonstrates proper continued operation of the instrument.

Specific procedures, acceptance criteria, and time intervals for characterizing NIR instrument performance are selected based on the instrument and intended application. Many NIR applications use previously validated models that relate NIR spectral response to a physical or chemical property of interest. Demonstrating stable instrument performance over extended periods of time provides some assurance that reliable measurements can be taken from sample spectra using previously validated chemometric models.

3. PROCEDURE

NIR spectra can be obtained from several sample presentations including solids, powders, slurries, gels, liquids, films, and gases. In addition, measurement can be performed through glass or plastic films that are normally used for containment. Typically, NIR spectroscopy does not require any sample preparation because the detectors have a wide dynamic range, and the collection time can be adjusted by the user. It is important to note that specific parameters such as spectrometer noise, limits of detection (LOD), limits of quantification (LOQ), and acceptable spectral bandwidth for any given application must be included as part of the analytical procedure. Equally important, data pretreatment must be properly implemented such that the intended measurement sensitivity is not subsequently degraded. Specific values for tests such as spectrometer noise and bandwidth will be dependent on the instrument chosen and the purpose required. For this reason, specific instrument tests for these parameters are not indicated here. Additional details can be found in 〈 1119 〉.

4. VALIDATION AND VERIFICATION

The objective of NIR procedure validation, as is the case with validation of any analytical process, is to demonstrate that the measurement is suitable for its intended purpose. NIR spectroscopy is somewhat different from conventional vibrational spectroscopic techniques, because it is not a primary technique. Validation can be achieved by the assessment of variability (precision) and comparison to a reference standard or the result of a validated primary method (accuracy). The procedure for validation is related to the fundamental validation characteristics required for any analytical procedure. Data pretreatment is often a vital step in the chemometric analysis of NIR spectral data. Many suitable data pretreatments exist; the selection should be based on sound scientific judgment and suitability for the intended application.

4.1 Validation

Validation is required when a NIR spectroscopic procedure is intended for use as an alternative to the monograph procedure for testing an official article. The objective of procedure validation is to demonstrate that the measurement is suitable for its intended purpose, including: quantitative determination of the main component in a drug substance or drug product (Category I assays); quantitative determination of impurities or limit tests (Category II); and identification tests (Category IV; see *Table 2* in *Validation of Compendial Procedures* 〈1225〉). Depending on the category of the test, the analytical procedure validation process for a NIR spectroscopic method requires the testing of linearity, range, accuracy, specificity, precision, detection limit, quantitation limit, and robustness. Note that validated NIR spectroscopy procedures may not be transferrable to all configurations of NIR spectrometers because of differences in their inherent performance characteristics.

Chapter 〈1225〉 provides definitions and general guidance on analytical procedures validation without indicating specific validation criteria for each characteristic. The following sections are intended to provide the user with specific validation criteria that represent the minimum expectations for this technology, assuming use of the typical Category 1 USP specifications of 98.0%–102.0% for a drug substance and 90.0%–110.0% for a drug product. The actual validation performance characteristics will depend on the specifications in place and must provide adequate evidence that the measurement capability is sufficient for those specifications. For certain particular applications, tighter criteria may be needed in order to demonstrate suitability for the intended use.

4.1.1 ACCURACY

Accuracy is demonstrated by showing the closeness of agreement between the value that is obtained and either a conventional true value or an accepted reference value from a reference procedure. Accuracy can be determined by direct comparison between validation results and actual or accepted reference values. Suitable agreement with reference values is based on required measurement capability for a specific application. The purpose is to demonstrate a linear relationship between the NIR spectroscopic results and actual values. If a chemometric model is used, accuracy can be determined by agreement between the standard error of prediction (SEP) and the standard error of the reference procedure for validation. The error of the reference procedure may be known: 1) on the basis of historical data, 2) through validation

results specific to the reference procedure, or 3) by calculating the standard error of the laboratory (SEL). Suitable agreement between SEP and SEL is based on required measurement capability for a specific application.

For Category I and II procedures, accuracy can be determined by conducting recovery studies with the appropriate matrix spiked with known concentrations of the analyte. It is also an acceptable practice to compare assay results obtained using the NIR spectroscopy procedure under validation with those obtained from an established alternative analytical method.

Validation criteria: 98.0%–102.0% recovery for a drug substance, 95.0%–105.0% recovery for a drug product assay, and 70.0%–150.0% recovery for an impurity analysis. These criteria must be met throughout the intended range.

4.1.2 PRECISION

The precision of a NIR spectroscopic procedure expresses the closeness of agreement among a series of measurements under prescribed conditions. Precision measurements are typically expressed as the relative standard deviation of a series of results and should be suitable for the intended application. Two levels of precision should be considered for NIR spectroscopic procedures: *Repeatability* and *Intermediate precision*.

Repeatability: Repeatability can be demonstrated by either: 1) statistical evaluation of a number of replicate measurements of the sample without repositioning the sample between the individual spectral acquisitions, or 2) statistical evaluation of multiple NIR procedure results, with each result being from a replicate analysis of a sample after repositioning between spectral acquisitions. The analytical procedure can be assessed by measuring the concentrations of six independently prepared sample preparations at 100% of the assay test concentration. Alternatively, it can be based on measurements of three replicates of three separate sample solutions at different concentrations. The three concentrations should be close enough that the repeatability is constant across the concentration range. If this is done, the repeatability at the three concentrations may be pooled for comparison to the acceptance criteria.

Validation criteria—The relative standard deviation is NMT 1.0% for a drug substance, NMT 2.0% for a drug product, and NMT 20.0% for an impurity analysis.

Intermediate precision: Analysts must test the effects of changes in variables, such as performing the analysis on different days, using different instrumentation, or having the method performed by two or more analysts in the *Repeatability* study. At a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision.

Validation criteria—The relative standard deviation is NMT 1.0% for a drug substance, NMT 3.0% for a drug product assay, and NMT 25.0% for an impurity analysis.

4.1.3 SPECIFICITY

The extent of specificity testing depends on the intended application. Specificity is typically demonstrated by using the following approaches.

Qualitative: Identification testing is a common application of qualitative NIR spectroscopy. Identification is achieved by comparing a sample spectrum to a reference spectrum or spectra. The specificity of the NIR identification procedure is demonstrated by obtaining positive

identification from samples, coupled with negative results from materials that should not meet the criteria for positive identification. Materials to demonstrate specificity should be selected on the basis of sound scientific judgment.

Quantitative: Quantitative applications of NIR spectroscopy typically first establish a mathematical relationship (chemometric model) between NIR spectral response and a physical or chemical property of interest. Demonstration of specificity against a physical or chemical property of interest is based on interpreting both NIR spectral attributes and chemometric parameters in terms of the intended application. The demonstration of specificity may include the following:

- Spectral regions can be correlated to the property of interest.
- Wavelengths used by regression analysis for the calibration (e.g., for multiple linear regression models) or the loading vector for each factor (e.g., for partial least squares or principal component regression models) can be examined to verify relevant spectroscopic information that is used for the mathematical model.
- Variation in spectra from samples for calibration should be examined and interpreted.
- Variation in material composition and sample matrix may be shown to have no significant effect on quantification of the property of interest within the specified procedure range.

For Category I and II procedures, the specificity is demonstrated by meeting the accuracy requirements.

For Category IV procedures, the identity of the analyte must be confirmed by comparison with appropriate reference substances.

4.1.4 QUANTITATION LIMIT

A measurement of a representative sample matrix spiked at the estimated quantitation limit (QL) concentration must be performed to confirm accuracy. The QL can be estimated by calculating the standard deviation, or the QL can be determined from the error of the intercept from a calibration curve or by determining that the S/N is >10 . Other suitable approaches can be used (see $\langle 1225 \rangle$).

A measurement of a test sample prepared from a representative sample matrix spiked at the required QL concentration must be performed to confirm sufficient sensitivity and adequate precision. The observed S/N at the required QL must be >10 .

Validation criteria: For the estimated QL to be considered valid, the measured concentration must be accurate and precise at a level $\leq 50\%$ of the specification.

4.1.5 LINEARITY

Quantitative procedures generally attempt to demonstrate a linear relationship between NIR spectral response and the property of interest. Although demonstrating a linear response is not required for all NIR applications, the model chosen should properly represent the relationship. Linearity of NIR spectroscopic procedures depends on variables such as matrix effects and data pretreatment. Validation of linearity in NIR procedures may be accomplished by examining NLT five samples that span the range and by plotting the NIR spectral responses versus actual or accepted values for the property of interest. Many applications may require models of higher order, and various statistical procedures are available for evaluation of the goodness of fit.

Applicable statistics and graphical procedures may be used as appropriate.

The correlation coefficient, R , may not be an informative measure of linearity. The square of the (Pearson) correlation coefficient is a measure of the fraction of the data's variation that is adequately modeled. Linearity depends on the standard error of the calibration equation (and hence the reference procedure) and on the range of the calibration data. Thus, although values very near 1.00, such as 0.99 or greater, typically indicate a linear relationship, lower values do not distinguish between nonlinearity and variability.

Validation criteria: The correlation coefficient (R) is NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests.

4.1.6 RANGE

The specified range of a NIR spectroscopic procedure depends on the specific application. The range typically is established by confirming suitable measurement capability (accuracy and precision) at the extreme limits. Controls must be used to ensure that results outside of the validated range are not accepted. In certain circumstances, it may not be possible or desirable to extend the validated range to include sample variability outside of the validated range. Extending the range of a NIR spectroscopic procedure requires demonstration of suitable measurement capability within the limits of the expanded range. Examples of situations in which only a limited sample range may be available are samples from a controlled manufacturing process and in-process samples. A limited procedure range does not preclude the use of a NIR spectroscopic procedure.

Validation criteria: For Category I tests, the validation range for 100.0% centered acceptance criteria is 80.0%–120.0%. For noncentered acceptance criteria, the validation range is 10.0% below the lower limit to 10.0% above the upper limit. For content uniformity, the range is 70.0%–130.0%. For Category II tests, the validation range covers 50.0%–120.0% of the acceptance criteria.

4.1.7 ROBUSTNESS

The reliability of an analytical measurement must be demonstrated by making deliberate changes to experimental parameters. NIR spectroscopic measurement parameters selected to demonstrate robustness will vary depending on the application and the sample's interface with the instrument. Critical measurement parameters associated with robustness often are identified and characterized during method development. Typical measurement parameters include the following:

- Effect of environmental conditions (e.g., temperature, humidity, and vibration)
- Effect of sample temperature
- Sample handling (e.g., fiber-optic probe depth, compression of material, sample depth/thickness)
- Influence of instrument changes (e.g., lamp change, warm-up time)

4.2 Verification

U.S. Current Good Manufacturing Practices regulations [21 CFR 211.194(a)(2)] indicate that users of analytical procedures described in *USP-NF* are not required to validate these procedures if provided in a monograph. Instead, they must simply verify their suitability under actual conditions of use.

The objective of method verification is to demonstrate that the procedure, as prescribed in specific monographs, is being executed with suitable accuracy, sensitivity, and precision.

General chapter *Verification of Compendial Procedures* 〈 1226 〉 notes that, if the verification of the compendial procedure, according to the monograph, is not successful, the procedure may not be suitable for use with the article under test. It may be necessary to develop and validate an alternative procedure as allowed in *General Notices and Requirements*.

Although complete revalidation of a compendial procedure is not required, verification of the compendial NIR spectroscopic procedure includes the determination of certain validation parameters. When the method being verified is for identification purposes, specificity is the only parameter required. For quantitative applications, additional validation parameters, such as accuracy, precision, and QL, must be studied as indicated in *Validation* in 〈 1225 〉, as appropriate.

USP Reference Standards 〈 11 〉

USP Near IR System Suitability RS ▲*USP39*

BRIEFING

〈 1119 〉 **Near-Infrared Spectroscopy**, *USP 37* page 953. A title change is proposed for general chapter *Near-Infrared Spectroscopy* 〈 1119 〉; the proposed new chapter title is *Near-Infrared Spectroscopy* 〈 1856 〉. In addition, it is proposed to move the content on qualification, calibration, and validation to another new general chapter, *Near-Infrared Spectroscopy* 〈 856 〉, and to move the chemometric-analysis terms and definitions to a future general chapter on chemometrics. Chapter 〈 1856 〉 (renamed from 〈 1119 〉) complements chapter 〈 856 〉 by providing information on the theory of near-infrared (NIR) spectroscopy and the acceptable practices for consistent analysis and interpretation of spectroscopic data. The NIR technique can be used for both qualitative and quantitative analysis of liquids, powders, and solid materials. Chapter 〈 1856 〉 also provides practical information on topics including sample preparation, instrumentation, qualitative NIR analysis, and quantitative NIR analysis. A *Stimuli* article published in *PF 40(1)* entitled *An Alignment of Concepts and Content across the Spectroscopy General Chapters in the United States Pharmacopeia–National Formulary (USP–NF)* provides insight into the rationale for the revisions to the chapters related to spectroscopy. This chapter follows the same format as the other new spectroscopy chapters.

(GCCA: K. Zaidi.)

Correspondence Number—C143741

Comment deadline: March 31, 2015

Change to read:

~~〈 1119 〉 NEAR-INFRARED SPECTROSCOPY~~

▲ 〈 1856 〉 **NEAR-INFRARED SPECTROSCOPY—THEORY AND PRACTICE** ▲*USP39*

Change to read:

INTRODUCTION

Near-infrared (NIR) spectroscopy is a branch of vibrational spectroscopy that shares many of the principles that apply to other spectroscopic measurements. The NIR spectral region comprises two subranges associated with detectors used in the initial development of NIR instrumentation. The short-wavelength (Herschel or silicon region) extends from approximately 780 to 1100 nm ($12,821$ – 9000 cm^{-1}); and longer wavelengths, between 1100 and 2500 nm, compose the traditional (lead sulfide) NIR region. Applications of NIR spectroscopy use spectra displayed in either wavelength or wavenumber units. As is the case with other spectroscopy measurements, interactions between NIR radiation and matter provide information that can be for both qualitative and quantitative assessment of the chemical composition of samples. In addition, qualitative and quantitative characterization of a sample's physical properties can be made because of the sample's influence on NIR spectra. Measurements can be made directly on samples in situ in addition to applications during standard sampling and testing procedures.

Applications of qualitative analysis include identification of raw material, in-process sample, or finished product. These applications often involve comparing an NIR spectrum from a sample to reference spectra and assessing similarities against acceptance criteria developed and validated for a specific application. In contrast, applications of quantitative analysis involve the development of a predictive relationship between NIR spectral attributes and sample properties. These applications typically use numerical models to quantitatively predict chemical and/or physical properties of the sample on the basis of NIR spectral attributes.

Vibrational spectroscopy in the NIR region is dominated by overtones and combinations that are much weaker than the fundamental mid-IR vibrations from which they originate. Because molar absorptivities in the NIR range are low, radiation can penetrate several millimeters into materials, including solids. Many materials, such as glass, are relatively transparent in this region. Fiber-optic technology is readily implemented in the NIR range, which allows monitoring of processes in environments that might otherwise be inaccessible.

The instrument qualification tests and acceptance criteria provided in this chapter may not be appropriate for all instrument configurations. In such cases, alternative instrument qualification and performance checks should be scientifically justified and documented. In addition, validation parameters discussed in this chapter may not be applicable for all applications of NIR spectroscopy. Validation parameters characterized for a specific NIR application should demonstrate suitability of the NIR application for its intended use.

Transmission and Reflection

The most common measurements performed in the NIR spectral range are transmission and reflection spectroscopy. Incident NIR radiation is absorbed or scattered by the sample and is measured as transmittance or reflectance, respectively. Transflection spectrometry is a hybrid of transmission and reflection wherein a reflector is placed behind the sample so that the optical path through the sample and back to the detector is doubled compared to a transmission measurement of a sample of the same thickness. Transflection is used to describe any double-pass transmission technique. The light may be reflected from a diffuse or specular (mirror) reflector placed behind the sample. This configuration can be adapted to share instrument geometry with certain reflection or fiber-optic probe systems in which the source and the detector are on the same side of the sample.

transmittance, T , is a measure of the decrease in radiation intensity as a function of

wavelength when radiation is passed through a sample. The sample is placed in the optical beam between the source and the detector. The results of both transmission and transreflection measurements are usually presented directly in terms of absorbance, i.e., $\log_{10}(1/T)$.

reflectance, R , is a measure of the ratio of the intensity of light reflected from the sample, I , to that reflected from a background or reference reflective surface, I_R . Most reflection measurements in the NIR are made of scattering samples such as powders and slurries. For such materials NIR radiation can penetrate a substantial distance into the sample, where it can be absorbed when the wavelength of the radiation corresponds to a transition between the ground vibrational state of the analyte and either a harmonic of a given vibrational mode (an *overtone*) or the sum of two or more different modes (a *combination band*). Nonabsorbed radiation is scattered back from the sample to the detector. NIR reflection spectra are accessed by calculating and plotting $\log(1/R)$ versus wavelength. This logarithmic form is the pseudo-absorbance of the material and is commonly called absorbance.

Factors That Affect NIR Spectra

The following list is not exhaustive, but it includes many of the major factors that affect NIR spectra:

Sample Temperature—Sample temperature influences spectra obtained from aqueous solutions and other hydrogen-bonded liquids, and a difference of a few degrees may result in significant spectral changes. Temperature may also affect spectra obtained from less polar liquids, as well as solids that contain solvents and/or water.

Moisture and Solvent—Moisture and solvent present in the sample material and analytical system may change the spectrum of the sample. Both absorption by moisture and solvent and their influence on hydrogen bonding of the APIs and excipients can change the NIR spectrum.

Sample Thickness—Sample thickness is a known source of spectral variability and must be understood and/or controlled. The sample thickness in transmission mode is typically controlled by using a fixed optical path length for the sample. In diffuse reflection mode, the sample thickness is typically controlled by using samples that are “infinitely thick” relative to the detectable penetration depth of NIR light into a solid material. Here “infinite thickness” implies that the reflection spectrum does not change if the thickness of the sample is increased.

Sample Optical Properties—In solids, both surface and bulk scattering properties of calibration standards and analytical samples must be taken into account. Surface morphology and refractive index properties affect the scattering properties of solid materials. For powder materials, particle size and bulk density influence scattering properties and the NIR spectrum.

Polymorphism—Variation in crystalline structure (polymorphism) from materials with the same chemical composition can influence NIR spectral response. Different polymorphs and amorphous forms of solid material may be distinguished from one another on the basis of their NIR spectral properties. Similarly, different crystalline hydration or solvation states of the same material can display different NIR spectral properties.

Age of Samples—Samples may exhibit changes in their chemical, physical, or optical properties over time. Care must be taken to ensure that both samples and standards used for NIR analysis are suitable for the intended application.

INSTRUMENTATION

Apparatus

All NIR measurements are based on exposing material to incident NIR light radiation and measuring the attenuation of the emerging (transmitted, scattered, or reflected) light. Several spectrophotometers are available; they are based on different operating principles—for example: filters, grating-based dispersive, acousto-optical tunable filter (AOTF), Fourier-transform NIR (FT-NIR), and liquid crystal tunable filter (LCTF). Silicon, lead sulfide, indium gallium arsenide, and deuterated triglycine sulfate are common detector materials. Conventional cuvette sample holders, fiber optic probes, transmission dip cells, and spinning or traversing sample holders are common examples of sample interfaces for introducing the sample to the optical train of a spectrometer.

The selection of specific NIR instrumentation and sampling accessories should be based on the intended application, and particular attention should be paid to the suitability of the sampling interface for the type of sample that will be analyzed.

Near-Infrared Reference Spectra

NIR references, by providing known stable measurements to which other measurements can be compared, are used to minimize instrumental variations that would affect the measurement.

Transmittance—The measurement of transmittance requires a background reference spectrum for determining the absorption by the sample relative to the background. Suitable transmittance reference materials depend on the specific NIR application and include air, an empty cell, a solvent blank, or a reference sample.

Reflectance—The measurement of reflectance requires the measurement of a reference reflection spectrum to determine the attenuation of reflected light relative to the unattenuated incident beam. The reflectance spectrum is calculated as the ratio of the single-beam spectrum of the sample to that of the reference material. Suitable reflectance reference materials depend on the specific NIR application and include ceramic, perfluorinated polymers, gold, and other suitable materials.

Qualification of NIR Instruments

Qualification—Qualification of an NIR instrument can be divided into three elements: Installation Qualification (IQ); Operational Qualification (OQ); and Performance Qualification (PQ). For further discussion, see general information chapter *Analytical Instrument Qualification* (1058):

Installation Qualification—The IQ requirements help ensure that the hardware and software are installed to accommodate safe and effective use of the instrument at the desired location.

Operational Qualification—In operational qualification, an instrument's performance is characterized using standards to verify that the system operates within target specifications. The purpose of operational qualification is to demonstrate that instrument performance is suitable. Because there are so many different approaches for measuring NIR spectra, operational qualification using standards with known spectral properties is recommended. Using external traceable reference standard materials does not justify omitting the instrument's internal quality control procedures. As is the case with any spectroscopic device, wavelength uncertainty, photometric linearity, and noise characteristics of NIR instruments should be

qualified against target specifications for the intended application.

Performance Qualification—Performance qualification demonstrates that the NIR measurement consistently operates within target specifications defined by the user for a specific application; it is often referred to as *system suitability*. Performance qualification for NIR measurements can include comparing a sample or standard spectrum to previously recorded spectra. Comparisons of spectra taken over time from identical and stable samples or reference standard materials can form the basis for evaluating the long-term stability of an NIR measurement system. The objective is to demonstrate that no abnormal wavelength shift or change in detector sensitivity has occurred during ongoing analysis.

Characterizing Instrument Performance—Specific procedures, acceptance criteria, and time intervals for characterizing NIR instrument performance depend on the instrument and intended application. Many NIR applications use previously validated models that relate NIR spectral response to a physical or chemical property of interest. Demonstrating stable instrument performance over extended periods of time provides some assurance that reliable measurements can be taken from sample spectra using previously validated NIR models.

Wavelength Uncertainty—NIR spectra from sample and/or reference standard materials can be used to demonstrate an instrument's suitable wavelength dispersion performance against target specifications. The USP Near IR System Suitability Reference Standard or the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2036 for reflectance measurement and NIST SRM 2035 for transmittance measurement can be used for wavelength verification. Suitable materials for demonstrating wavelength dispersion performance include polystyrene, mixtures of rare earth oxides, and absorption by water vapor for instruments that use an interferometer for wavelength dispersion. With appropriate justification, alternative standards may be used. Wavelength uncertainty typically is characterized from a single spectrum (collected with the same spectral resolution to obtain the standard value) using a minimum of three peaks that cover a suitable spectral range of the instrument. Typical tolerances for agreement with standard values are ± 1.0 nm below 2000 nm and ± 1.5 nm from 2000 nm to 2500 nm. Alternative tolerances may be used when justified for specific applications.

Photometric Linearity and Response Stability—NIR spectra from samples and/or reference standard materials with known relative transmittance or reflectance can be used to demonstrate a suitable relationship between NIR light attenuation (due to absorption) and instrument response. For reflectance measurements, commercially available reflectance standards with known reflectance properties are often used. Spectra obtained from reflection standards are subject to variability as a result of the difference between the experimental conditions under which they were factory calibrated and those under which they are subsequently put to use. Hence, the reflectance values supplied with a set of calibration standards may not be useful in the attempt to establish an "absolute" calibration for a given instrument. Provided that (1) the standards do not change chemically or physically, (2) the same reference background is also used to obtain the standard values, and (3) the instrument measures each standard under identical conditions (including precise sample positioning), the reproducibility of the photometric scale will be established over the range of standards. Subsequent measurements on the identical set of standards give information on long-term stability. Photometric linearity is typically characterized using a minimum of four reference standards in the range from 10% to 90% reflection (or transmission). NIR applications based on

measuring an absorbance larger than 1.0 may require standards with reflectivity properties between 2% and 5% reflection (or transmission) for characterizing instrument performance at low reflectance. The purpose is to demonstrate a linear relationship between NIR reflectance and/or transmittance and instrument response over the scanning range of the instrument. Typical tolerances for a linear relationship are 1.00 ± 0.05 for the slope and 0.00 ± 0.05 for the intercept of a plot of the measured photometric response versus standard photometric response. Alternative tolerances may occur when justified for specific applications.

Spectroscopic Noise—NIR instrument software may include built-in procedures to automatically determine system noise and to provide a statistical report of noise or S/N over the instrument's operating range. In addition, it may be desirable to supplement such checks with measurements that do not rely directly on manufacturer-supplied procedures. Typical procedures involve measuring spectra of traceable reference materials with high and low reflectance. Tolerances for these procedures should demonstrate suitable S/N for the intended application.

high-flux noise—Instrument noise is evaluated at high light flux by measuring reflectance or transmittance of the reference standard, with the reference material (e.g., 99% reflection standard) acting as both the sample and the background reference.

low-flux noise—The same procedure may be used with a lower reflectivity reference material (e.g., 10% reflectance standard) to determine system noise at reduced light flux. The source, optics, detector, and electronics make significant contributions to the noise under these conditions.

METHOD VALIDATION

Introduction

The objective of NIR method validation, as is the case with the validation of any analytical procedure, is to demonstrate that the measurement is suitable for its intended purpose. NIR spectroscopy is somewhat different from conventional analytical techniques because validation of the former generally is achieved by the assessment of chemometric parameters, but these parameters can still be related to the fundamental validation characteristics required for any analytical method.

Data pretreatment is often a vital step in the chemometric analysis of NIR spectral data. Data pretreatment can be defined as the mathematical transformation of NIR spectral data to enhance spectral features and/or remove or reduce unwanted sources of variation prior to using the spectrum. *Calibration* is the process of developing a mathematical relationship between NIR spectral response and properties of samples. Many suitable chemometric algorithms for data pretreatment and calibration exist; the selection should be based on sound scientific judgment and suitability for the intended application.

Validation Parameters

Performance characteristics that demonstrate the suitability of NIR methods are similar to those required for any analytical procedure. A discussion of the applicable general principles is found in *Validation of Compendial Procedures* (1225). These principles should be considered typical for NIR procedures, but exceptions should be dealt with on a case-by-case basis. For qualitative NIR methods, see chapter (1225), *Data Elements Required for Validation*,

Category IV assays. For quantitative NIR methods, see chapter 1225, *Data Elements Required for Validation, Category I and Category II* assays. Specific acceptance criteria for each validation parameter must be consistent with the intended use of the method. The samples for validation should be independent of the calibration set.

Specificity—The extent of specificity testing depends on the intended application. Demonstration of specificity in NIR methods is typically accomplished by using the following approaches:

Qualitative—Identification testing is a common application of qualitative NIR spectroscopy. Identification is achieved by comparing a sample spectrum to a reference spectrum or a library of reference spectra. The specificity of the NIR identification method is demonstrated by obtaining positive identification from samples coupled with negative results from materials that should not meet criteria for positive identification. Materials to demonstrate specificity should be based on sound scientific judgment and can include materials similar in visual appearance, chemical structure, or name.

Quantitative—Quantitative applications of NIR spectroscopy typically involve establishing a mathematical relationship between NIR spectral response and a physical or chemical property of interest. Demonstrating specificity against a physical or chemical property of interest is based on interpreting both NIR spectral attributes and chemometric parameters in terms of the intended application and may include the following:

- Spectral regions in the calibration model can be correlated to a known NIR spectral response associated with the property of interest.
- Wavelengths used by regression analysis for the calibration (e.g., for multiple linear regression [MLR] models) or the loading vector for each factor (e.g., for partial least squares [PLS] or principal component regression [PCR] models) can be examined to verify relevant spectroscopic information that is used for the mathematical model.
- Variation in spectra from samples for calibration can be examined and interpreted as expected spectral observations.
- Variation in material composition and sample matrix may be shown to have no significant effect on quantification of the property of interest within the specified method range.

Linearity—Quantitative NIR methods generally attempt to demonstrate a linear relationship between NIR spectral response and the property of interest. Although demonstrating a linear response is not required for all NIR applications, the model chosen, whether linear or not, should properly represent the relationship.

Validation of linearity in NIR methods may be accomplished by examining a plot of NIR spectral response versus actual or accepted values for the property of interest. Many statistical methods are available for evaluation of the goodness of fit of the linear relationship. Other applicable statistics and graphical methods may be as appropriate.

The correlation coefficient, r , may not be an informative measure of linearity. The square of the (Pearson) correlation coefficient is a measure of the fraction of the data's variation that is adequately modeled by the equation. Linearity depends on the standard error of the calibration equation (and hence the reference method) and on the range of the calibration data. Thus, although values very near 1.00, such as 0.99 or greater, typically indicate a linear relationship, lower values do not distinguish between nonlinearity and variability around the line.

Range—The specified range of an NIR method depends on the specific application. The range

typically is established by confirming that the NIR method provides suitable measurement capability (accuracy and precision) when applied to samples within extreme limits of the NIR measurement. Controls must be used to ensure that results outside the validated range are not accepted. In certain circumstances, it may not be possible or desirable to extend the validated range to include sample variability outside the validated range. Extending the range of an NIR method requires demonstration of suitable measurement capability within the limits of the expanded range. Examples of situations in which only a limited sample range may be available are samples from a controlled manufacturing process and in-process samples. A limited method range does not preclude the use of an NIR method.

Accuracy—Accuracy in NIR methods is demonstrated by showing the closeness of agreement between the value that is accepted as either a conventional true value or an accepted reference value. Accuracy can be determined by direct comparison between NIR validation results and actual or accepted reference values. Suitable agreement between NIR and reference values is based on required measurement capability for a specific application. The purpose is to demonstrate a linear relationship between NIR results and actual values. Accuracy can be determined by agreement between the standard error of prediction (SEP) and the standard error of the reference method for validation. The error of the reference method may be known on the basis of historical data, through validation results specific to the reference method, or by calculating the standard error of the laboratory (SEL). Suitable agreement between SEP and SEL is based on required measurement capability for a specific application.

Precision—The precision of an NIR method expresses the closeness of agreement between a series of measurements under prescribed conditions. Two levels of precision should be considered: repeatability and intermediate precision. The precision of an NIR method typically is expressed as the relative standard deviation of a series of NIR method results and should be suitable for the intended application. Demonstration of precision in NIR methods may be accomplished using the following approaches:

Repeatability—Repeatability can be demonstrated by the following:

- Statistical evaluation of a number of replicate measurements of the sample without repositioning the sample between each individual spectral acquisition, or
- Statistical evaluation of multiple NIR method results, each result from a replicate analysis of a sample subsequent to repositioning between spectral acquisitions

Intermediate Precision—Intermediate precision can be shown by the following:

- Statistical evaluation of a number of replicate NIR measurements of the same or similar samples in the *Repeatability* study by different analysts on different days.

Robustness—NIR measurement parameters selected to demonstrate robustness will vary depending on the application and the sample's interface with the NIR instrument. Critical measurement parameters associated with robustness often are identified and characterized during method development. Typical measurement parameters include the following:

- Effect of environmental conditions (e.g., temperature, humidity, and vibration)
- Effect of sample temperature
- Sample handling (e.g., probe depth, compression of material, sample depth/thickness, sample presentation)
- Influence of instrument changes (e.g., lamp change, warm-up time)

Ongoing Method Evaluation

~~Validated NIR methods should be subject to ongoing performance evaluation, which may include monitoring accuracy, precision, and other suitable method parameters. If performance is unacceptable, corrective action is necessary. It involves conducting an investigation to identify the cause of change in method performance and may indicate that the NIR method is not suitable for continued use. Improving the NIR method to meet measurement suitability criteria may require additional method development and documentation of validation experiments demonstrating that the improved method is suitable for the intended application. The extent of revalidation required depends on the cause of change in method performance and the nature of corrective action required in order to establish suitable method performance. Appropriate change controls should be implemented to document ongoing method improvement activities.~~

~~Revalidation of a qualitative model may be necessary as a result of the following:~~

- ~~• Addition of a new material to the spectral reference library~~
- ~~• Changes in the physical properties of the material~~
- ~~• Changes in the source of material supply~~
- ~~• Identification of previously unknown critical attribute(s) of material(s)~~

~~Revalidation of a quantitative model may be necessary as a result of the following:~~

- ~~• Changes in the composition of the test sample or finished product~~
- ~~• Changes in the manufacturing process~~
- ~~• Changes in the sources or grades of raw materials~~
- ~~• Changes in the reference analytical method~~
- ~~• Major changes in instrument hardware~~

~~**Outliers**—Sample spectra that produce an NIR response that differs from the qualitative or quantitative calibration model may produce an outlier. This does not necessarily indicate an out-of-specification result; but rather an outlier indicates that further testing of the sample may be required and is dependent on the particular NIR method. If subsequent testing of the sample by an appropriate method indicates that the property of interest is within specifications, then the sample meets its specifications. Outlier samples may be incorporated into an updated calibration model subsequent to execution and documentation of suitable validation studies.~~

Method Transfer

~~Controls and measures for demonstrating the suitability of NIR method performance following method transfer are similar to those required for any analytical procedure. Exceptions to general principles for conducting method transfer for NIR methods should be justified on a case-by-case basis. The transfer of an NIR method is often performed by using an NIR calibration model on a second instrument that is similar to the primary instrument used to develop and validate the method. When a calibration model is transferred to another instrument, procedures and criteria must be applied to demonstrate that the calibration model meets suitable measurement criteria on the second instrument. The selection of an appropriate calibration model transfer procedure should be based on sound scientific judgment.~~

GLOSSARY

~~absorbance, A , is represented by the equation:~~

$$~~A = -\log T = \log(1/T)~~$$

~~where T is the transmittance of the sample. Absorbance is also frequently given as:~~

$$A = -\log(1/R)$$

where R is the reflectance of the sample.

background spectrum is used for generating a sample spectrum with minimal contributions from instrument response. It is also referred to as a *reference spectrum* or *background reference*. The ratio of the sample spectrum to the background spectrum produces a transmittance or reflectance spectrum dominated by NIR spectral response associated with the sample. In reflection measurements, a highly reflective diffuse standard reference material is for the measurement of the background spectrum. For transmission measurement, the background spectrum may be measured with no sample present in the spectrometer or using a cell with the solvent blank or a cell filled with appropriate reference material.

calibration model is a mathematical expression to relate the response from an analytical instrument to the properties of samples.

diffuse reflectance is the ratio of the spectrum of radiated light penetrating the sample surface, interacting with the sample, passing back through the sample's surface, and reaching the detector to the background spectrum. This is the component of the overall reflectance that produces the absorption spectrum of the sample.

fiber optic probes consist of two components: optical fibers that may vary in length and in the number of fibers and a terminus, which contains specially designed optics for examination of the sample matrix.

installation qualification is the documented collection of activities necessary to establish that an instrument is delivered as designed and specified, is properly installed in the selected environment, and that this environment is suitable for the instrument's intended purpose.

instrument bandwidth or resolution is a measure of the ability of a spectrometer to separate radiation of similar wavelengths.

multiple linear regression is a calibration algorithm to relate the response from an analytical instrument to the properties of samples. The distinguishing feature of this algorithm is the use of a limited number of independent variables. Linear least squares calculations are performed to establish a relationship between these independent variables and the properties of the samples.

operational qualification is the process by which it is demonstrated and documented that an instrument performs according to specifications and that it can perform the intended task. This process is required following any significant change such as instrument installation, relocation, or major repair.

overall reflectance is the sum of diffuse and specular reflectance.

partial least squares (pls) is a calibration algorithm to relate instrument responses to the properties of samples. The distinguishing feature of this algorithm is that data concerning the properties of the samples for calibration are used in the calculation of the factors to describe instrument responses.

performance qualification is the process of using one or more well characterized and stable reference materials to verify consistent instrument performance. Performance qualification may employ the same or different standards for different performance characteristics.

photometric linearity, also referred to as *photometric verification*, is the process of verifying

the response of the photometric scale of an instrument.

principal component regression (pcr) is a calibration algorithm to relate the response from an analytical instrument to the properties of samples. This algorithm, which expresses a set of independent variables as a linear combination of factors, is a method of relating these factors to the properties of the samples for which the independent variables were obtained.

pseudo-absorbance, A , is represented by the equation:

$$A = -\log R = \log(1/R)$$

where R is the diffuse reflectance of the sample.

reference spectrum—See *Background Spectrum*.

reflectance is described by the equation:

$$R = I/I_R$$

in which I is the intensity of radiation reflected from the surface of the sample and I_R is the intensity of radiation reflected from a background reference material and its incorporated losses due to solvent absorption, refraction, and scattering.

root-mean-square (rms) noise is calculated by the equation:

$$\text{RMS} = \sqrt{\frac{1}{N} \sum_{i=1}^N (A_i - \bar{A})^2}$$

in which A_i is the absorbance for each data point; \bar{A} is the mean absorbance over the spectral segment; and N is the number of points per segment.

spectral reference library is a collection of spectra of known materials for comparison with unknown materials. The term is commonly used in connection with qualitative methods of spectral analysis (e.g., identification of materials).

specular (surface) reflectance is the reflectance of the front surface of the sample.

standard error of calibration (sec) is a measure of the capability of a model to fit reference data. SEC is the standard deviation of the residuals obtained from comparing the known values for each of the calibration samples to the values that are calculated from the calibration. SEC should not be used as an assessment tool for the expected method accuracy (trueness and precision of prediction) of the predicted value of future samples. The method accuracy should generally be verified by calculating the standard error of prediction (SEP), using an independent validation set of samples. An accepted method is to mark a part of the calibration set as the validation set. This set is not fully independent but can be used as an alternative for the determination of the accuracy.

standard error of cross-validation (secv) is the standard deviation calculated using the leave-one-out method. In this method, one calibration sample is omitted from the calibration, and the

difference is found between the value for this sample calculated from its reference value and the value obtained from the calibration calculated from all the other samples in the set. This process is repeated for all samples in the set, and the SECV is the standard deviation of the differences calculated for all the calibration samples. This procedure can also be performed with a group of samples. Instead of leaving the sample out, a group of samples is left out. The SECV is a measure of the model accuracy that one can expect when measuring future samples if not enough samples are available for the SEP to be calculated from a completely independent validation set.

standard error of the laboratory (sel) is a calculation based on repeated readings of one or more samples to estimate the precision and/or accuracy of the reference laboratory method, depending on how the data were collected.

standard error of prediction (sep) is a measure of model accuracy of an analytical method based on applying a given calibration model to the spectral data from a set of samples different from but similar to those used to calculate the calibration model. SEP is the standard deviation of the residuals obtained from comparing the values from the reference laboratory to those from the method under test for the specified samples. SEP provides a measure of the model accuracy expected when one measures future samples.

surface reflectance, also known as *specular reflection*, is that portion of the radiation not interacting with the sample but simply reflecting back from the sample surface layer (sample-air interface).

transflection is a transmittance measurement technique in which the radiation traverses the sample twice. The second time occurs after the radiation is reflected from a surface behind the sample.

transmittance is represented by the equation:

$$T = I/I_0 \text{ or } T = 10^{-A}$$

in which I is the intensity of the radiation transmitted through the sample; I_0 is the intensity of the radiant energy incident on the sample and includes losses due to solvent absorption, refraction, and scattering; and A is the absorbance.

- ▲
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8. Appendix: Glossary

1. THEORY

Near-infrared (NIR) spectroscopy is a technique with broad and varied applications in pharmaceutical analysis. The NIR spectral region lies between the UV-visible and infrared regions, and at one time was considered the forgotten region of the electromagnetic spectrum. It is a branch of vibrational spectroscopy that shares many of the principles that apply to other spectroscopic techniques. The NIR spectral region comprises two sub-ranges (see *Figure 1*) associated with detectors used in instrumentation. The short-wavelength (Herschel or silicon) region extends from approximately 780 to 1,100 nm ($12,821$ – $9,000$ cm^{-1}), and the longer wavelengths, which fall between 1,100 and 2,500 nm ($9,000$ – $4,000$ cm^{-1}), compose the traditional (lead sulfide) NIR spectral region. It is common to express the wavelength (λ) in nanometers (nm) and the frequency (ν) in reciprocal centimeters (wavenumber) as acquired by the instrument. Usually, Fourier-transform (FT) spectrometers report the wavelength in wavenumber (cm^{-1}), whereas a dispersive, monochromator-based instrument will show the wavelength in nm.

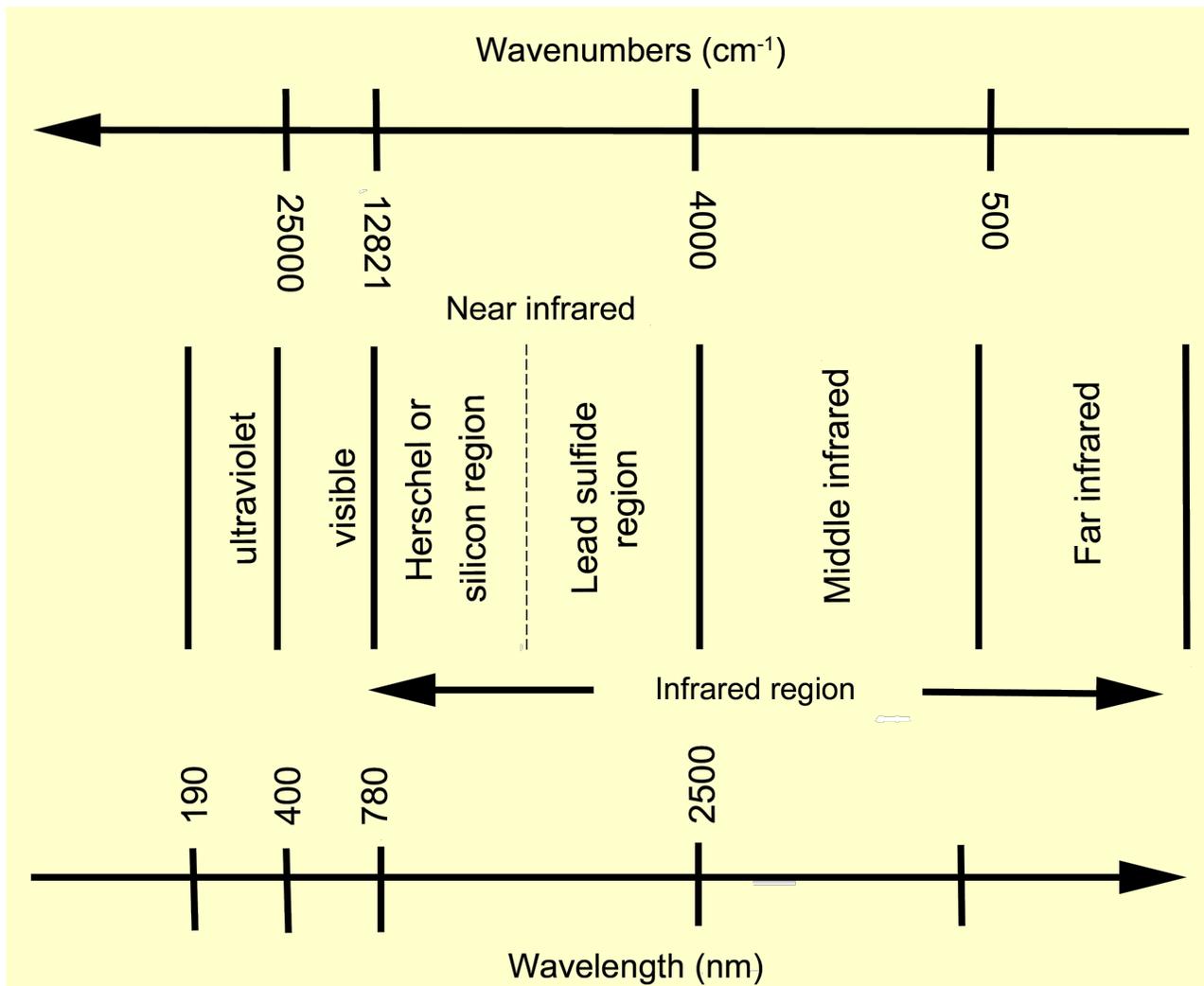


Figure 1. NIR region of the electromagnetic radiation spectrum.

NIR spectra are dominated by C-H, N-H, O-H, and S-H overtone resonances and combinations of fundamental mid-infrared vibrational modes from which they originate. Because molar absorptivities in the NIR range are low, radiation can penetrate several millimeters into materials, including solids. Many materials, such as glass and plastic film, are relatively transparent in this region. Fiber-optic technology is readily implemented in the NIR range, which allows real-time, or near real-time, monitoring of processes in environments that might otherwise be inaccessible. As is the case with other spectroscopy measurements, interactions between NIR radiation and matter provide information that can be useful for both qualitative and quantitative assessment of the chemical composition of samples. In addition, qualitative and quantitative characterization of a sample's physical properties can be made because of the sample's influence on NIR spectra.

Measurements can be made directly on samples *in situ* in addition to applications during standard sampling and testing procedures. Applications of qualitative analysis include identification of raw material, in-process control testing, and finished-product release testing. NIR measurements can be performed off-line, but also at-line, in-line, and on-line for process analytical technology. These applications often involve comparing a NIR spectrum from a sample to a reference spectrum and assessing similarities against acceptance criteria developed

and validated for a specific application. In contrast, applications of quantitative analysis involve the development of a predictive relationship between NIR spectral attributes and sample properties. Because of the highly covariant nature of the NIR signal, these applications typically use chemometrics models to quantitatively predict chemical and/or physical properties of the sample on the basis of its spectral attributes.

2. TRANSMISSION, REFLECTION, AND TRANSFLECTION MODES

The most common measurements performed in the NIR spectral range are transmission and reflection spectroscopy. Incident NIR radiation is absorbed or scattered by the sample and is measured as transmittance or reflectance, respectively.

2.1 Transmission Mode

Transmittance (T) is the intensity ratio of the transmitted radiation (I) to the incident radiation (I_0), which represents the decrease in intensity at given wavelengths when radiation is passed through the sample. The sample is placed in the optical beam between the source and the detector. The arrangement is analogous to that in many conventional spectrophotometers, and the result can be presented directly in terms of transmittance and/or absorbance (A). NIR spectra are usually measured in absorbance (A).

$$T = \frac{I}{I_0} \quad \text{or} \quad T = 10^{-A}$$

I = intensity of transmitted radiation

I_0 = intensity of incident radiation

The measurement of transmittance is dependent on a background transmittance spectrum for its calculation. Examples of background references include air, a polymeric disc, an empty cell, a solvent blank, or in special cases, a reference sample. The method generally applies to liquids (diluted or undiluted), dispersions, solutions, and solids including tablets and capsules. For transmittance measurements of solids, a suitable sample accessory is to be used. Tablets and capsules can be analyzed using suitably engineered holders, thereby ensuring reproducible measurements. Liquid samples are examined in a cell of suitable path length (typically 0.5–4 mm) that is transparent to NIR radiation, or alternatively by immersion of a fiber-optic probe of a suitable configuration.

2.2 Reflectance Modes

2.2.1 REFLECTANCE

Reflectance (R) is the ratio of the intensity of light reflected from the sample, I , to that reflected from a background or reference reflective surface, I_R .

It is composed of both the specular (mirror) and the diffuse components (see *Figure 2*).

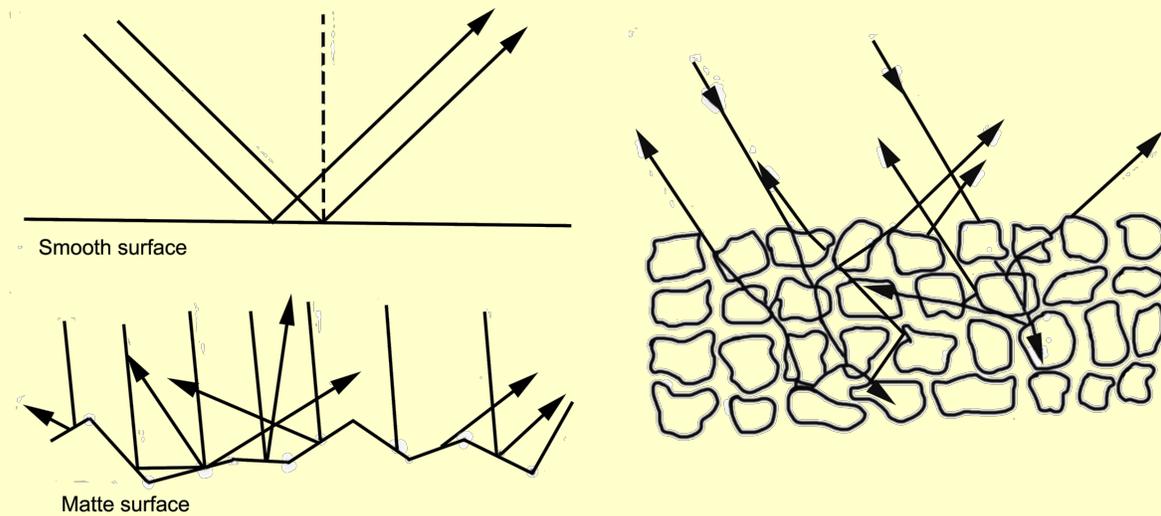


Figure 2.

The determination of reflectance requires the measurement of a reference reflection spectrum to determine the attenuation of reflected light relative to the unattenuated incident beam. The reflectance spectrum is calculated as the ratio of the single-beam spectrum of the sample to that of the reference material.

$$R = \frac{I}{I_R}$$

I = intensity of light diffusively reflected from the sample

I_R = intensity of light reflected from the background or reference reflective surface

Most reflection measurements in the NIR spectral region are made of scattering samples such as powders and slurries. For such materials, NIR radiation can penetrate a substantial distance into the sample, where it can be absorbed when the wavelength of the radiation corresponds to a transition between the ground vibrational state of the analyte and either a harmonic of a given vibrational mode (an overtone) or the sum of two or more different modes (a combination band). Nonabsorbed radiation is scattered back from the sample to the detector. NIR reflectance spectra are accessed by calculating and plotting $\log(1/R)$ versus wavelength. This logarithmic form is the pseudo-absorbance of the material and is commonly called absorbance.

2.2.2 DIFFUSE REFLECTION

The diffuse reflection mode gives a measure of reflectance (R), (penetrating the sample surface, interacting with the sample, and passing back through the sample's surface) which is the ratio of the intensity of light reflected from the sample (I) to that reflected from a background or reference reflective surface (I_r), where by using careful instrumental design, the specular component is often excluded. NIR radiation can penetrate a substantial distance into the sample, where it can be absorbed by vibrational combinations and overtone resonances of the analyte species present in the sample. Nonabsorbed radiation is partially reflected back from the sample to the detector.

$$R = \frac{I}{I_r}$$

I = intensity of light diffusively reflected from the sample

I_r = intensity of light reflected from the background or reference reflective surface

This mode generally applies to solids. The sample is examined in a suitable device. For process monitoring, material can be analyzed through a polished glass (e.g., sapphire) window interface, or using an in-line probe. Care must be taken to ensure that the measuring conditions are as reproducible as possible from one sample to another. The reflected radiation of a background reference is scanned to obtain the baseline, and then the reflectance of one or more analytical samples is measured. Common reflectance references include ceramic tiles, thermoplastic resins, and gold. Other suitable materials may be used. In some process-analysis situations, it may be impossible to remove a probe for reference background data collection; in these cases, consider various options including internal referencing; measurement of a background reference using a second detector; and others. Only spectra measured against a background possessing the same optical properties can be compared directly with one another.

2.3 Transflection Mode

The term transflection (T^*) is used to describe any double-pass transmission technique. Transflection spectrometry is a hybrid of transmission and reflection wherein a reflector is placed behind the sample so that the optical path through the sample and back to the detector is doubled, compared with a transmission measurement of a sample of the same thickness. Nonabsorbed radiation is reflected back from the sample to the detector. The light may be reflected from a diffuse or specular (mirror) reflector placed behind the sample.

$$T^* = \frac{I}{I_{T^*}}$$

I = intensity of translected radiation measured with the sample

I_{T^*} = intensity of translected radiation of the reference material as background

This mode generally applies to liquids and clear plastic materials. This configuration can be adapted to share the same instrument geometry with reflectance and fiber-optic probe systems where the source and the detector are on the same side of the sample. The sample is examined through a cell with a mirror or a suitable diffusive reflector made of either metal or an inert substance (e.g., dried titanium dioxide) not absorbing in the NIR region. Liquids can also be measured using in-line transfectance probes to increase intensity levels for more sensitive applications.

3. FACTORS THAT AFFECT NIR SPECTRA

NIR spectroscopy is advantageous because, as a result of lower molar absorptivities in this region of the electromagnetic spectrum, accurate measurements can often be made quickly without destroying the sample, and with minimal or no sample preparation; this applies whether the samples are solid, semi-solid, liquid, or gas. The NIR spectrum contains information on overtone resonances and fundamental vibrational modes of the sample that can yield both sample and process understanding. Samples may be analyzed directly through packaging or directly in media that is transparent to wavelengths in the NIR spectral region. In addition, by definition NIR spectra are produced using the corresponding NIR radiation, and therefore

standard glass/quartz and fiber optics may be used.

The following discussion is not exhaustive, but it includes many of the major factors that affect NIR spectra.

3.1 Environmental Factors

The environmental temperature and humidity must be considered before carrying out measurements. Ideally, ambient conditions will be controlled to meet the operating specifications of the instrument manufacturer. However, tighter controls may not always be practical to the degree necessary for the application, and as such, another justifiable means of accounting for the influence of the environment (e.g., spectral pre-processing) should be utilized, with frequent verification. This is particularly relevant when considering the effect of temperature when using fiber-optic probes.

3.2 Sampling Factors

Depending on the measurement mode, sample preparation and presentation can vary. The following requirements must be met for all sampling techniques:

- Find the best suitable measurement mode for the intended application (transmission, diffuse reflection, or transflection);
- Find the best suitable accessory (e.g., transmission or immersion probes);
- Optimize path length in transmission and transflection modes;
- Find a suitable spectroscopic background reference sample;
- Show that the background reference sample is reliable over time and that the measurement of the background is reproducible and stable over time;
- When measuring moving materials or samples (for process-related measurements) it is important to obtain a representative spectrum (e.g., by adjusting the measuring time or number of scans, by co-adding individual spectra, or by increasing the beam size);
- Ensure that there is not fouling of the sensor, for example with build-up of material or contamination;
- When measuring through packaging material, consider the variability of content and thickness.

3.2.1 SAMPLE PRESENTATION AREA

The sample presentation area or probe end must be clean and free of residue prior to the measurement. Similarly, the in-line or on-line interface to the sample should not have significant product or contamination build-up, which would interfere with the desired measurement.

3.2.2 SAMPLE TEMPERATURE

Sample temperature influences spectra obtained from aqueous solutions and other hydrogen-bonded liquids, and a difference of a few degrees may result in significant spectral changes. Temperature may also affect spectra obtained from less polar liquids, as well as solids that contain solvents and/or water.

3.2.3 MOISTURE AND SOLVENT

Moisture and solvent that are present in the sample material and analytical system may change the spectrum of the sample. Both absorption by moisture and solvent and their influence on hydrogen bonding of the active pharmaceutical ingredients and excipients can change the NIR spectrum.

3.2.4 SAMPLE THICKNESS

Sample thickness is a known source of spectral variability and must be understood and/or controlled, particularly for tablet and capsule analysis in transmittance mode. The sample thickness in transmission mode is typically controlled by using a fixed optical path length for the sample. In diffuse reflection mode, the sample thickness is typically controlled by using samples that are "infinitely thick" relative to the detectable penetration depth of NIR light into a solid material. Here, the term infinite thickness implies that the reflection spectrum does not change if the thickness of the sample is increased. For the measurement of compressed powders, an infinite thickness is typically reached after 5 mm of sample depth (e.g., in a filled vial).

3.2.5 SAMPLE OPTICAL PROPERTIES

With solids, both surface and bulk scattering properties of calibration standards and analytical samples must be taken into account. Surface morphology and refractive index properties affect the scattering properties of solid materials. For powder materials, particle size and bulk density influence scattering properties and the NIR spectrum. The spectra of physically, chemically, or optically heterogeneous samples may require sample averaging, examination of multiple samples, or spinning the sample to obtain a representative spectrum of the sample. Certain factors, such as differing degree of compaction or particle size in powdered materials and surface finish, can cause significant spectral differences.

3.2.6 SOLID-STATE FORMS

The variations in solid-state forms (polymorphs, hydrates, solvates, and amorphous forms) influence vibrational spectra. Hence, different crystalline forms as well as the amorphous form of a solid may be distinguished from one another on the basis of their NIR spectra. Where multiple crystalline forms are present, care must be taken to ensure that the calibration samples have a distribution of forms relevant to the intended application.

3.2.7 AGE OF SAMPLES

Samples may exhibit changes in their chemical, physical, or optical properties over time. Depending on the storage conditions, solid samples may either absorb or desorb water, and portions of amorphous materials may crystallize. Materials used for NIR calibration are representative of future samples and their matrix variables. Hence, care must be taken to ensure that samples for NIR analysis are representative.

4. PRETREATMENT OF NIR SPECTRAL DATA

NIR spectral data to be used in qualitative or quantitative applications often need pre-processing to attenuate environmental or sampling factors, enhance a certain signal, or for other purposes that may include transformation, normalization, or other mathematical treatment. Pre-processing of samples (rows of data) may include mean or median centering, scaling, and other procedures. NIR responses (columns of data) also can be transformed, centered, or scaled. Because of the highly covariant nature of the NIR signal, it is common to remove linear or polynomial trends or apply filtering or smoothing techniques such as derivatives, wavelets, or a Savitzky-Golay filter. Spectral pretreatment techniques may include the numerical calculation of the first- or second-order derivative of the spectrum. Higher-order derivatives are not recommended because of increased spectral noise.

5. INSTRUMENTATION

5.1 Apparatus

All NIR measurements result from exposing material to incident NIR light radiation and measuring the attenuation of the emerging (transmitted, scattered, or reflected) light.

Spectrophotometers for measurement in the NIR region consist of a suitable light source, such as a highly stable quartz-tungsten lamp, a monochromator or interferometer, and a detector. Common monochromators are acousto-optical tunable filters, gratings, or prisms. Traditionally, many NIR instruments have had a single-beam design, although some process instruments use internal referencing and can therefore be dual beam (for example, in diode-array instruments). Silicon, lead sulfide, and indium gallium arsenide are examples of detector materials. Examples of sampling devices include conventional cuvette sample holders, fiber-optic probes, transmission dip cells, neutral borosilicate vials, and spinning or traversing sample holders. The choice is made on the basis of the intended application, with particular attention paid to the suitability of the sampling system for the type of sample to be analyzed. Suitable data processing and evaluation units (e.g., software and computer) are usually part of the system.

5.2 Specialized Techniques

In addition to conventional NIR spectrometry, there are several more specialized NIR-based techniques.

5.2.1 IMAGING TECHNIQUES

Within a given material, the distribution of the various compounds can be characterized three dimensionally by using imaging. Use of NIR imaging allows for the collection of detailed chemical information. NIR microscopy techniques have the following advantages:

- No preparation of the sample required
- Good spatial resolution
- Clear image quality
- Outstanding chemical differentiation

It is possible to collect a signal from the entire sample (e.g., a tablet), disperse it into a spectrum using a spectrometer, and detect the spectrum using a multi-channel detector such as a charge couple detector or photo-diode array. In this case, instead of obtaining trivial cumulative information about the spot signal intensity, one can obtain a signal spectrum which can be transformed into detailed information about the chemical composition of the given spot on a sample. The three-dimensional array of data sets, two spatial and one spectral, recorded in such an imaging measurement has become known as a hypercube or data cube. These microscopy systems are capable of determining a spatial resolution down to approximately 1–5 μm .

NIR imaging systems are capable of characterizing the structure and distribution of the active components, and excipients within formulations, dosage forms, and delivery devices, to name a few. For example, chemical mapping for homogeneity testing of solid oral dosage forms, creams, and ointments may be useful in select applications.

5.3 Calibration Considerations

NIR instrument calibration involves three components: wavelength accuracy and uncertainty

(x-axis); photometric linearity and response stability (y-axis); and photometric noise. *Table 1* provides an overview of performance verification criteria for a wide range of NIR systems.

5.3.1 WAVELENGTH ACCURACY AND UNCERTAINTY (X-AXIS)

In the case of FT-NIR instruments, primary wavelength-axis calibration is maintained, at least to a first approximation, with an internal helium–neon laser. Suitable materials for demonstrating wavelength-dispersion performance include methylene chloride, talc *R*, spectral calibration lamps, polystyrene, mixtures of rare earth oxides, and absorption by water vapor for instruments that use an interferometer for wavelength dispersion. A certified traceable standard [Standard Reference Material (SRM) 2035a] is available from the National Institute of Standards and Technology (NIST) for transmittance measurements and can be used for wavelength verification. Other suitable standards may also be used. Instrument manufacturers may use polystyrene films (thickness of 0.75–2 mm) as an internal standard for wavelength qualification and control. However, because of the weak asymmetric signals emanating from polystyrene at short wavelengths, this material may not be suitable as a reference standard across the whole NIR range. When choosing an appropriate reference standard, it is important to confirm that the operating parameters (e.g., resolution, band width, and others) are within the limits of the certified material.

The effect of temperature on assignment of wavelength must be taken into account. Typically, standards are measured in environments near 25^o, and the temperature at which certified values were taken will be indicated. The effect of resolution can also have an impact on the measured values. Resolution for NIR spectrometers is typically 8 cm⁻¹, but modern spectrometers are capable of higher resolutions (2 cm⁻¹ or 1 cm⁻¹).

Standard certificates will indicate resolution and possibly even peak assessment algorithms. The choice of reference standard for a performance test should always match the resolution and environmental conditions expected for the execution of the test. Additional standards may be necessary, depending on the range of wavelengths or reference standards meeting these conditions.¹ Refer to *Table 1* for additional information.

5.3.2 PHOTOMETRIC LINEARITY AND RESPONSE STABILITY (Y-AXIS)

Calibration of the photometric axis can be critical for successful quantification. Both FT-NIR and dispersive NIR spectrometers should undergo similar calibration procedures. The tolerance of photometric precision acceptable for a given measurement should be assessed during the method development and validation stages.

NIR spectra from samples and/or reference standard materials with known relative transmittance or reflectance can be used to demonstrate a suitable relationship between NIR light attenuation (resulting from absorption) and instrument response. Verification of photometric linearity and verification of photometric noise are not required for instruments using methods to perform simple identifications that do not use the photometric absorbance as part of the model strategy (e.g., simple correlation with absorbing wavelengths). The USP Near IR System Suitability Reference Standard or the NIST SRM 2036 are recommended, but other certified traceable standards exist in the market and may be used. For reflectance measurements, commercially available reflectance standards with known reflectance properties are often used.

Spectra obtained from reflection standards are subject to variability because the experimental

conditions under which they were factory calibrated differ from those under which they are subsequently put to use. Hence, the reflectance values supplied with a set of calibration standards may not be useful in the attempt to establish an "absolute" calibration for a given instrument. The reproducibility of the photometric scale will be established over the range of standards provided that 1) the standards do not change chemically or physically, 2) the same reference background is also used to obtain the standard values, and 3) the instrument measures each standard under identical conditions (including precise sample positioning). Subsequent measurements on the identical set of standards provide information on long-term stability.

Photometric linearity is demonstrated by using a set of transmission or reflection standards with known values for percentage transmittance or reflectance. For reflectance measurements, carbon-doped polymer standards are available. It is important to ensure that the absorbance of the materials used is relevant to the intended linear working range of the method. Nonlinear calibration models, which are acceptable, may be used, as long as the user can demonstrate adequate justification for this approach.

Spectra obtained from reflectance standards are subject to variability due to the differences between the experimental conditions under which they were factory-calibrated and those under which they are subsequently put to use. Hence, the percentage reflectance values supplied with a set of calibration standards may not be useful in the attempt to establish an "absolute" calibration for a given instrument. As long as the standards do not change chemically or physically, and the same reference background is used as was used to obtain the certified values, subsequent measurements of the same standards under identical conditions (including precise sample positioning) give information on long-term stability of the photometric response.

Table 1. Control of Instrument Performance

Measurement Mode	Reflection	Transflection	Transmission
Verification of wavelength scale (except for filter apparatus)	Typical tolerances for agreement with standard values are ± 1.0 nm from approximately 700–2000 nm and ± 1.5 nm above 2000 nm to approximately 2500 nm (± 8 cm^{-1} below 5000 cm^{-1} and ± 4 cm^{-1} from 5000 cm^{-1} to approximately 14 000 cm^{-1}). For the reference material used, apply the tolerance for the nearest wavelength (or wavenumber) from the above for each peak used. For diode-array instruments, most often the pixel resolution (wavelength between pixels) can be as large as 10 nm. The pixel resolution must be adapted to match the spectral resolution. The peak-finding algorithms are critical to wavelength accuracy. Practically, ± 2 nm is appropriate for peak wavelength accuracy using such instrumentation. Alternatively, refer to manufacturer's specifications for acceptance.		

<p>Bench/mobile instrument</p>	<p>Measure talc <i>R</i> via a suitable medium or by fiber-optic probe. Talc <i>R</i> has suitable for calibration, characteristic peaks at 948, 1391, 2077, and 2312 nm. Alternatively, other suitable standards may also be used that ensure wavelength accuracy in the region of working methodology. For example, measure an internal polystyrene standard if built in, or measure a NIST standard or other traceable material (e.g., USP Near IR System Suitability RS) and assess 3 peaks across the wavelength range for calibration.</p>	<p>A suspension of 1.2 g of dry titanium dioxide <i>R</i> in about 4 mL of methylene chloride <i>R</i> is used directly through the cell or using a probe. Titanium dioxide has no absorption in the NIR range. Spectra are recorded with a maximum nominal instrument bandwidth of 10 nm at 2500 nm (16 cm^{-1} at 4000 cm^{-1}). Methylene chloride has characteristic sharp bands at 1155, 1366, 1417, 1690, 1838, 1894, 2068, and 2245 nm. Choose 3 peaks across the wavelength range for calibration. Other suitable standards may also be used, such as TS5 liquid mixed with titanium dioxide or some other reflective medium.</p>	<p>Methylene chloride <i>R</i> may be used and has characteristic sharp bands at 1155, 1366, 1417, 1690, 1838, 1894, 2068, and 2245 nm. Choose 3 peaks across the wavelength range for calibration. Other suitable standards may also be used, such as TS5 liquid.</p>
<p>Process instrument</p>	<p>If it is not practically possible to measure a traceable standard material at the point of sample measurement, use internal material such as polystyrene, fiberglass, or solvent and/or water vapor. Alternatively, adopt a second external fiber/probe. For FT instruments, the calibration of the wavenumber scale may be performed using a narrow, isolated water-vapor line, for example, the line at 7306.74 cm^{-1}, or 7299.45 cm^{-1}, or 7299.81 cm^{-1} or a narrow line from a certified reference material.</p>		
<p>Verification of wavelength repeatability (except for filter apparatus)</p>	<p>The standard deviation of the wavelength is consistent with the specifications of the instrument manufacturer, or otherwise scientifically justified.</p>		
<p>Bench/mobile instrument</p>	<p>Verify the wavelength repeatability using a suitable external or internal standard.</p>		
<p>Process instrument</p>	<p>Verify the wavelength repeatability using a suitable external or internal standard.</p>		
<p>Verification of photometric linearity and response stability⁽¹⁾</p>	<p>Measure 4 photometric standards across the working method absorbance range.</p>		

Bench/mobile instrument	Analyze 4 reference standards, for example in the range of 10%–90%, including 10%, 20%, 40%, and 80% with respective absorbance values of 1.0, 0.7, 0.4, and 0.1. Evaluate the observed absorbance values against the reference absorbance values, for example perform a linear regression. Acceptable tolerances are 1.00 ± 0.05 for the slope and 0.00 ± 0.05 for the intercept for the first verification of photometric linearity of an instrument. Subsequent verifications of photometric linearity can use the initial observed absorbance values as the reference values.	
Process instrument	If photometric reflectance standards cannot be measured at the point of sample measurement, use the manufacturer's internal photometric standards. Process instruments can use internal photometric standards for photometric linearity. Follow the manufacturer's verified tolerances in such cases.	Analyze 4 reference standards to cover the absorbance values over the working absorbance range of the modelled data. Evaluate the observed absorbance values against the reference absorbance values, for example perform a linear regression. Acceptable tolerances are 1.00 ± 0.05 for the slope and 0.00 ± 0.05 for the intercept for the first verification of photometric linearity of an instrument. Subsequent verifications of photometric linearity can use the initial observed absorbance values as the reference values.
Verification of photometric noise	Determine the photometric noise using a suitable reflectance standard, for example white reflective ceramic tiles or reflective thermoplastic resins (for example, PTFE). Follow the manufacturer's methodology and specifications.	
Bench/mobile instrument	Scan the reflectance low-flux (e.g., 10% reflectance) standard over a suitable wavelength range in accordance with the manufacturer's recommendation and calculate the photometric noise as peak-to-peak noise.	
Process instrument	As above, or if not practically possible, use manufacturer's internal standard for noise testing and specifications.	Scan the transmittance high-flux standard over a suitable wavelength/wavenumber range in accordance with the manufacturer's recommendation and calculate the photometric noise as peak-to-peak noise.

5.3.3 EXTERNAL CALIBRATION

Detailed functional validation employing external reference standards is recommended to demonstrate instrumental suitability of laboratory instruments, even for instruments that

possess an internal calibration approach. The use of external reference standards does not obviate the need for internal quality-control procedures; rather, it provides independent documentation of the fitness of the instrument for the specific analysis or purpose. For instruments installed in a process location or in a reactor where positioning of an external standard routinely is not possible, including those instruments that employ an internal calibration approach, the relative performance of an internal versus an external calibration approach should be evaluated periodically. The purpose of this test is to check for changes or drifts in components (e.g., the process lens or fiber-optic probe) that might not be included in the internal calibration method; this could affect the photometric calibration of the optical system, for example.

5.3.4 MULTI-INSTRUMENT CALIBRATION

There are many approaches for ensuring a transferable calibration across multiple instruments of the same type (same make and model) and different types. This is an area of growing interest because of the globalization of manufacturing and regulatory controls. Today, reference standards have been developed to cover this region, but many deficiencies still exist regarding wavelength uncertainty and photometric response curves. Therefore, verification of compendial procedures, method transfers, and spectrometer platform changes is compromised by the bias involved in differences between instruments. Understanding the risk associated with these variations should be a part of any quality-by-design initiative to use NIR spectroscopic procedures for product control.

The use of appropriate certified reference materials can be helpful in establishing instrumental variance and/or bias. As stated above, the value assignments included on the certification of such materials will contain significant contributions to the overall expanded uncertainty budget from the instrumental factors used in generating these values. For example, the optical geometry, polarization, and other aspects of a reflectance measurement will significantly affect the measured value, and therefore wherever possible these characteristics should be matched to the required measurement configuration. However, by definition, a reference material should have the essential characteristics of appropriate stability and homogeneity to at least allow comparative measurements to be made, albeit they may be biased by known or unknown factors.

6. APPLICATIONS

NIR spectroscopy has a wide variety of applications for chemical, physical, and process analysis.

For chemical analysis, the applications include:

- Identification of active substances, excipients, dosage forms, manufacturing intermediates, chemical absorbance materials, and packaging materials;
- Qualification of ingredients, intermediates, and drug products, including batch-to-batch spectral comparison and supplier change assessment;
- Quantification of active substances and excipients; determination of chemical values such as hydroxyl value; determination of absolute water content; determination of degree of hydroxylation; and control of solvent content.

For physical analysis, the applications include:

- Crystalline form and crystallinity, polymorphism, solvates, and particle size;

- Dissolution behavior, disintegration pattern, and hardness;
- Examination of film properties.

For process analysis, the applications include:

- Monitoring of unit operations such as synthesis, crystallization, blending, drying, granulation, and coating, for the purpose of process control.

Measurements in the NIR spectral region are influenced by many chemical and physical factors. The reproducibility and relevance of the results depend on the control of these factors. Usually, measurements are only valid for a defined system.

6.1 Qualitative Analysis: Identification and Characterization

6.1.1 ESTABLISHMENT OF A SPECTRAL REFERENCE LIBRARY

To establish a spectral reference library, record the spectra of a suitable number of representative samples of the substance; the samples should have known, traceable identities. These representative samples should exhibit the variation that is typical for the substance to be analyzed (e.g., variation in solid-state form and particle size). The set of spectra obtained represents the information that can be used for chemical and/or physical identification of the sample to be analyzed.

The collection of spectra in the library may be represented in different ways, which are defined by the mathematical technique used for identification. These may be:

- All individual spectra representing the substance;
- A mean spectrum of the measured batches for each chemical substance or physical form;
- If necessary, a description of the variability within the substance spectra.

The number of substances to be included in the library depends on the specific application. All spectra in the library will have the same spectral range and number of data points; technique of measurement; and data pretreatment. If subgroups (libraries) are created, the above criteria should be applied independently to each group. Origin spectral data for the preparation of the spectral library must be archived. Caution must be exercised when performing any mathematical transformation, as artifacts can be introduced or essential information can be lost. The suitability of the algorithm chosen should be demonstrated by successful method validation, and in all cases, the rationale for the use of transformation must be documented.

6.1.2 NIR REFERENCE SPECTRA

NIR references provide known, stable measurements to which other measurements can be compared; thus they are used to minimize instrumental and environmental variations that would affect the measurement. Direct comparison of representative spectra of the substance to be examined and a reference substance for qualitative chemical or physical identification purposes may not require use of a reference spectral library, where specificity permits.

6.1.3 DATA EVALUATION

Direct comparison is made between the representative spectrum of the substance to be examined and the individual or mean reference spectra of all substances in the database on the basis of their mathematical correlation or other suitable algorithms. A set of known reference mean spectra and the variability around this mean can be used with an algorithm for classification; alternatively, this can be achieved visually by overlaying spectral data if

specificity is inherent. Different calibration techniques are available, such as principal component analysis, cluster analysis, and soft independent application which has to be validated according to the following.

6.1.3.1 Validation of the model: Chemical or physical identification methods using direct spectral comparison must be validated in accordance with identification method validation procedures. The validation parameters for qualitative methods are robustness and specificity.

6.1.3.2 Relative comparison of spectra: A calibration is not required when comparing a set of spectra for limit analysis purposes, such as determining the maximum or minimum absorbance at which an analyte absorbs. Also, in-process control of a drying operation may use a qualitative approach around a specific absorbing wavelength. Appropriate spectral ranges and pretreatments (if used) must be shown to be fit for purpose.

6.1.3.3 Specificity: The relative discriminatory power and selectivity of a limit test must be demonstrated. The extent of specificity testing is dependent on the application and the risks being controlled. Variations in matrix concentrations within the operating range of the method must not affect the measurement.

6.2 Trend Analysis

6.2.1 RELATIVE COMPARISON OF SPECTRA

A calibration is not necessarily required when comparing a set of spectra for trend analysis purposes, for example, using the moving-block approach to estimate statistical parameters such as mean, median, and standard deviation. For example, blend-uniformity monitoring using NIR spectroscopy has adopted such data analysis approaches. Appropriate spectral ranges and algorithms must be used for trend analyses.

6.2.2 SPECIFICITY

The relative discriminatory power and selectivity for trend analysis must be demonstrated. The extent of specificity testing is dependent on the application and the risks being controlled. Variations in matrix concentrations within the operating range of the method must not affect the trend analysis.

6.3 Quantitative Analysis

6.3.1 SPECTRAL REFERENCE LIBRARY FOR A CALIBRATION MODEL

Calibration is the process of constructing a mathematical model to relate the response from an analytical instrument to the properties of the samples. Any calibration model that can be defined clearly in a mathematical expression and gives suitable results can be used. Record the spectra of a suitable number of representative samples with known or future-established values of the attribute of interest (for example, content of water) throughout the range to be measured. The number of samples for calibration will depend on the complexity of the sample matrix and interferences (e.g., temperature, particle size, and others). It is encouraged to take a risk management approach to identify critical interferences that may need to be assessed. All samples must give quantitative results within a calibration interval as defined by the intended purpose of the method. Multiple linear regression, principal component regression (PCR), and partial least squares regression (PLS) are commonly used. For PLS or PCR calibrations, the regression coefficients and/or the loadings should be plotted, and the regions of large coefficients or loadings should be compared with the spectrum of the analyte. Predicted

residual error sum of squares plots or similar plots are useful for facilitating the optimization of the number of PCR or PLS factors.

6.3.2 PRETREATMENT OF DATA

Wavelength selection, or exclusion of certain wavelength ranges, may enhance the efficiency of calibration models. Wavelength compression (wavelength averaging) techniques may be applied to the data.

6.3.3 MODEL VALIDATION PARAMETERS

Analytical performance characteristics to be considered for demonstrating the validation of NIR methods are similar to those required for any analytical procedure. Specific acceptance criteria for each validation parameter must be consistent with the intended use of the method. Validation parameters for quantitative methods are accuracy, linearity, precision (repeatability and intermediate precision), robustness, and specificity.

6.3.4 ONGOING MODEL EVALUATION

NIR models validated for use are subjected to ongoing performance evaluation and monitoring of validation parameters. If discrepancies are found, corrective action is necessary.

7. PROCEDURE VALIDATION

Validation of NIR methods will follow the same protocols described in the general chapter *Validation of Compendial Procedures* (1225) in terms of accuracy, precision, and other suitable parameters.

Detector linearity must be confirmed over the range of possible signal levels. Method precision must also encompass sample position. The sample presentation is a critical factor for both solids and liquids, and must be either tightly controlled or accounted for in the calibration model. Sample-position sensitivity can often be minimized by appropriate sample preparation or sample holder geometry, but will vary from instrument to instrument on the basis of excitation and optical configuration. In addition, many suitable chemometric algorithms for data pretreatment and calibration are available. Selection of an algorithm should be based on sound scientific judgment and suitability for the intended application.

7.1 Ongoing Method Evaluation

Validated NIR methods should be subject to ongoing performance evaluation, which may include monitoring accuracy, precision, and other suitable method parameters. If performance is unacceptable, corrective action is necessary. This involves conducting an investigation to identify the cause of change in method performance, and may indicate that the NIR method is not suitable for continued use. Improving the NIR method to meet measurement suitability criteria may require additional method development and documentation of validation experiments demonstrating that the improved method is suitable for the intended application. The extent of revalidation that is required depends on the cause of change in method performance and the nature of corrective action needed to establish suitable method performance. Appropriate change controls should be implemented to document ongoing method improvement activities.

Revalidation of a qualitative model may be necessary as a result of the following:

- Major changes in instrument hardware
- Addition of a new material to the spectral reference library
- Changes in the physical properties of the material
- Changes in the source of material supply
- Identification of previously unknown critical attribute(s) of material(s)

Revalidation of a quantitative model may be necessary as a result of the following:

- Changes in the composition of the test sample or finished product
- Changes in the manufacturing process
- Changes in the sources or grades of raw materials
- Changes in the reference analytical method
- Major changes in instrument hardware

7.2 Method Transfer

Controls and measures for demonstrating the suitability of NIR method performance following method transfer are similar to those required for any analytical procedure. Any exceptions to general principles for conducting method transfer for NIR methods should be justified on a case-by-case basis. The transfer of a NIR method is often performed by using a NIR calibration model on a second instrument that is similar to the primary instrument used to develop and validate the method. When a calibration model is transferred to another instrument, procedures and criteria must be applied to demonstrate that the calibration model meets suitable measurement criteria on the second instrument. The selection of an appropriate calibration-model transfer procedure should be based on sound scientific judgment.

USP Reference Standards < 11 >

USP Near IR System Suitability RS

8.APPENDIX: GLOSSARY

Absorbance: absorbance (A) is represented by the equation

$$A = -\log T = \log (1/T)$$

where T is the transmittance of the sample. Absorbance is also frequently given as

$$A = \log (1/R)$$

where R is the reflectance of the sample.

Background spectrum: this is used for generating a sample spectrum with minimal contributions from instrument response; it is also referred to as a "reference spectrum" or "background reference." The ratio of the sample spectrum to the background spectrum produces a transmittance or reflectance spectrum dominated by NIR spectral response associated with the sample. In reflection measurements, a highly reflective, diffuse standard reference material is used for the measurement of the background spectrum. For transmission measurement, the background spectrum may be measured with no sample present in the spectrometer or using a cell with the solvent blank or a cell filled with appropriate reference material.

Diffuse reflectance: ratio of the spectrum of radiated light (penetrating the sample surface, interacting with the sample, and passing back through the sample's surface) reaching the

detector to the background spectrum. This is the component of the overall reflectance that produces the absorption spectrum of the sample.

Fiber-optic probes: these consist of two components: optical fibers that may vary in length and number, and a terminus, which contains specially designed optics for examination of the sample matrix.

Installation qualification: the documented collection of activities necessary to establish that an instrument is delivered as designed and specified and is properly installed in the selected environment, which is suitable for the instrument's intended purpose.

Instrument bandwidth or resolution: a measure of the ability of a spectrometer to separate radiation of similar wavelengths.

Operational qualification: the process of demonstrating and documenting that an instrument performs according to specifications and that it can perform the intended task. This process is required following any significant change, such as instrument installation, relocation, or major repair.

Overall reflectance: the sum of diffuse and specular reflectance.

Performance qualification: the process of using one or more well-characterized and stable reference materials to verify consistent instrument performance. Performance qualification may employ the same or different standards for different performance characteristics.

Photometric linearity: also referred to as "photometric verification," it is the process of verifying the response of the photometric scale of an instrument.

Pseudo-absorbance: A , is represented by the equation

$$A = -\log R = \log (1/R)$$

where R is the diffuse reflectance of the sample.

Reference spectrum: see *Background spectrum*.

Reflectance: is described by the equation:

$$R = \frac{I}{I_R}$$

in which I is the intensity of radiation reflected from the surface of the sample and I_R is the intensity of radiation reflected from a background reference material and its incorporated losses due to solvent absorption, refraction, and scattering.

Root-mean-square (RMS): calculated using the equation

$$\text{RMS} = \sqrt{\sum_{i=1}^N \frac{(\hat{A} - A_i)^2}{N}}$$

where \hat{A} is the mean absorbance over the spectral segment, A_i is the absorbance for each data point, and N is the number of points per segment.

Specular (surface) reflectance: the reflectance from the front surface of the sample, where the angle of reflection matches the angle of incidence (mirror effect).

Standard error of calibration (SEC): a measure of the capability of a model to fit reference data. SEC is the standard deviation of the residuals obtained from comparing the known values for each of the calibration samples to the values that are calculated from the calibration. SEC should not be used as an assessment tool for the expected method accuracy (trueness and precision of prediction) of the predicted value of future samples. The method accuracy should generally be verified by calculating the *Standard error of prediction (SEP)* using an independent validation set of samples. An accepted method is to mark a part of the calibration set as the validation set. This set is not fully independent but can be used as an alternative for the determination of the accuracy.

Standard error of the laboratory (SEL): a calculation based on repeated readings of one or more samples to estimate the precision and/or accuracy of the reference laboratory method, depending on how the data were collected.

Standard error of prediction (SEP): a measure of model accuracy of an analytical method based on applying a given calibration model to the spectral data from a set of samples different from but similar to those used to calculate the calibration model. SEP is the standard deviation of the residuals obtained from comparing the values from the reference laboratory to those from the method under test for the specified samples. SEP provides a measure of the model accuracy to be expected when one measures future samples.

Surface reflectance: also known as "specular reflection," it is that portion of the radiation not interacting with the sample but simply reflecting back from the sample surface layer (i.e., the sample-air interface).

Transflection: a transmittance measurement technique in which the radiation traverses the sample twice. The second time occurs after the radiation is reflected from a surface behind the sample.

Transmittance: represented by the equation

$$T = \frac{I}{I_0} \text{ or } T = 10^{-A}$$

where I is the intensity of the radiation transmitted through the sample; I_0 is the intensity of the radiant energy incident on the sample and includes losses due to solvent absorption, refraction, and scattering; and A is the absorbance.

▲USP39

¹ Burgess C, Hammond J. Wavelength standards for the near-infrared spectral region. *Spectroscopy* 2007;22(4):40-48.

BRIEFING

〈 1790 〉 **Visual Inspection of Injections.** The General Chapters, Dosage Forms Expert Committee proposes this new general chapter to provide guidance on the inspection of injectable drug products for visible particles. The methods discussed are also applicable to detection of other visible defects that may affect container integrity or cosmetic appearance of the product.

(GCDF: D. Hunt.)

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Comment deadline: March 31, 2015

Add the following:

▲〈 1790 〉 VISUAL INSPECTION OF INJECTIONS

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1. SCOPE

1.1 Introduction

This chapter provides guidance on the inspection of injections for visible particles. The terms particle, particulates, and particulate matter are equivalent and do not have different meaning when used in this chapter. Individual visible particles are generally greater than 50 μm in diameter, and the threshold for routine, reliable detection ($\geq 70\%$ probability) is often near 150 μm for single spherical particles in clear solutions contained in clear glass vials [1]. The methods discussed in this chapter are also applicable to the detection of other visible defects. These include, but are not limited to, container integrity defects such as cracks, misplaced stoppers, or incomplete seals, any of which may compromise the sterility of the product. Additional container defects [2], as well as other product characteristics such as fill level, are also detected during visual inspection, and non-conforming units should be rejected using the

methods described in this chapter. Inspection for these other quality attributes often occurs at the same time as the inspection for particles. The primary focus of this chapter is a manual reference inspection method; however, semi-automated and automated methods are also discussed.

1.2 Related Chapters

The general chapter *Injections and Implanted Drug Products (Parenterals)—Product Quality Tests* 〈 1 〉 provides an overview of injectable dosage forms and the quality tests associated with them. Another general chapter, *Visible Particulates in Injections* 〈 790 〉, has been added to the *U.S. Pharmacopeia–National Formulary (USP–NF)* to provide a clear definition of routine inspection procedures for injectable products; the goal is to comply with the expectation that products be essentially free of visible particulate matter. Additionally, information on the detection of subvisible particulates is provided in general chapters *Subvisible Particulate Matter in Therapeutic Protein Injections* 〈 787 〉, *Particulate Matter in Injections* 〈 788 〉, and *Particulate Matter in Ophthalmic Solutions* 〈 789 〉. The general chapter *Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions* 〈 1788 〉 provides additional supporting information on measurement methods for subvisible particles.

1.3 Defect Prevention

Although this chapter focuses on detection and removal of product units that show evidence of visible particles, the need for preventing such contamination should not be overlooked. No inspection process, manual or automated, can guarantee complete removal of all visible particulate matter or other visible defects; thus, prevention of such defects is an important consideration. Good process and product design, along with environmental control, are necessary to ensure the reliable production of products with a low particle burden. To ensure the control of defects throughout the process, manufacturers should consider an inspection life-cycle approach [3] in critical areas. This approach begins with developing quality attributes based on incoming component specifications, followed by component-level acceptance testing. It extends to component preparation and product-filling procedures, followed by 100% in-process inspection of filled product, and concluding with final acceptance sampling and testing of the finished product. The approach must extend to purchased, ready-to-use components such as containers or closures, where there is no opportunity for subsequent particle removal after receipt and before filling. Stability and retention sample inspection, customer complaint handling, and in-house investigative procedures support this integrated process. The inspection life-cycle is composed of, and supported by, sub-cycles involving qualification, maintenance, personnel training, defect characterization, and the use of standards within each of the critical areas. The final element of the life-cycle is a feedback loop of trending and data review from each of these process areas, resulting in a mechanism that supports continuous process improvement.

2. INTRODUCTION

2.1 Inspection Process Capability

Visual inspection of injections is necessary to minimize the introduction of unintended particles to patients during the delivery of injectable medications. Such inspection also offers the opportunity to reject containers whose integrity has been compromised, such as those with cracks or incomplete seals, which pose a risk to the sterility of the product. The desire to detect these defects despite their very low frequency and the randomness of their occurrence has resulted in the current expectation that each finished unit will be inspected (100% inspection). Although zero defects is the goal and this should drive continuous process improvement, zero defects is not a feasible specification for visible particles given current packaging components and processing capability.

Understanding human performance is critical to establishing visual inspection criteria. The threshold for human vision is generally accepted to be 50 μm . Individual receptors in the eye have a resolution of 11 μm , but typical resolving power is reported as 85–100 μm [4]. The detection process is probabilistic: the likelihood of detection is a cumulative function of visible attributes such as particle size, shape, color, density, and reflectivity. Analysis of inspection results pooled from several studies [5] [6] shows that the probability of detection for a single 50- μm particle in a clear solution contained in a 10-mL vial utilizing diffuse illumination between 2000 and 3000 lux is only slightly greater than 0%. The detection probability increases to approximately 40% for a 100- μm particle, and typically exceeds 95% for particles that are 200 μm and larger. Thus, in a qualified visual inspection system, the vast majority of extraneous particles that might go undetected and be introduced into the pharmaceutical supply chain will be smaller than 200 μm . Changes to the container (e.g., increasing size and opacity), fill level, and particle characteristics beyond size (e.g., color, shape, and density) will all affect the probability of detection.

2.2 Patient Risk

A complete review of the medical literature is beyond the scope of this chapter, but the effect of extraneous particles on the patient must be considered. A number of reviews on this subject are available [6] [7] [8] [9] [10] [11] [12]. The clinical implications of extraneous particulate matter in injections are determined by many factors, including the size and number of particles, the composition of the material, the potential for microbiological contamination, the route of administration, the intended patient population, and the clinical condition of the patient. For example, an otherwise healthy individual receiving a subcutaneous or intramuscular injection containing sterile, extraneous inert particulates would likely experience no adverse effect or at worst would develop a small granuloma. On the other hand, a critically ill premature infant receiving a particle-laden infusion directly through an umbilical catheter might suffer considerable pathophysiologic sequelae [13] [14].

Garvin and Gunner were among the first to report a concern about the effects of particles in human patients [15] [16]. For obvious ethical reasons, there is a lack of controlled studies on the effect of particles in human patients. Some anecdotal information about human patient safety may be obtained by examining case reports of intravenous drug abusers [17] [18] [19]. In these cases, solid oral dosages are often ground up and injected as a slurry; pulmonary foreign body emboli and granulomas were observed in these patients [20]. Unfortunately, the clinical risks to human patients posed by small numbers of particles are difficult to infer from these observations due to the extreme number of foreign particles and the uncontrolled conditions in which they were administered.

Numerous animal studies have been conducted to determine the fate of intravenous particles with different sizes and composition [21] [22] [23] [24]. Most studies have focused on

subvisible particles with a diameter of less than 50 μm . In these studies, a massive infusion of particles has been accompanied by histologic evidence of injury to pulmonary capillary endothelial cells [25], microscopic thrombi in the pulmonary capillaries [26], pulmonary microscopic granulomata [27], and hepatic inflammatory effects [28]. Although useful for understanding the pathophysiologic response to particulate matter, the large number of particles used in these studies (e.g., 10^9 particles/kg per injection) provides little insight into the risk to humans posed by small numbers of macroscopic particles. Arterial embolization using materials such as polyvinyl alcohol (PVA), collagen-coated acrylic microspheres, and gelatin spheres also provides some insight into the potential human pathophysiologic implications of non-target embolization of extraneous-particle intravenous infusions. In these cases, massive particle loads moving from the arterial injection site into the venous circulation were also reported [29] [30] [31] [32] [33].

In a review of the hazards of particle injection, it has been found that the primary contributor of particulate matter in vial presentations is the rubber closure, a risk that is present with almost every injection. In addition, case reports have documented injury associated with infusion of significant quantities of precipitated admixtures or therapeutic use of particles for embolization [13] [14] [34]. Despite the administration of an estimated 15 billion doses of injectable medicines each year [35], no reports of adverse events associated with the injection of individual visible particles have been found.

Ultimately, the safety considerations related to particulate matter in injections must be assessed for each drug product, intended patient population, and method of administration. No single set of inspection criteria can adequately anticipate all of the potential risks to the patient. The methods outlined in chapter 〈 790 〉 should serve as essential requirements when assessing the adequacy of the visual inspection procedure, but supplemental acceptance criteria should be added when the patient population and intended use of the product warrant these additional measures.

2.3 History of Inspection Standards

The requirement for injections to be "true solutions" appeared in *USP IX* in 1915, and the first appearance of "solution clarity" for parenterals occurred in 1936 in *NF IV*. Since then, there have been numerous modifications to the compendia in this regard. A comprehensive history of compendial inspection standards is available in the *Pharmaceutical Forum* [1].

3. TYPICAL INSPECTION PROCESS FLOW

3.1 100% Inspection

Chapter 〈 790 〉 establishes the expectation that each unit of injectable product will be inspected as part of the routine manufacturing process. This inspection should take place at a point when defects are most easily detected, for example, prior to labeling or insertion into a device or combination product. Each unit may be examined manually with the unaided eye, or by using a conveyor to transport and present the containers to a human inspector (semi-automated inspection), or by means of electronic image analysis (automated inspection). Manual and semi-automated inspection should only be performed by trained, qualified inspectors. Inspection may also be enhanced by means of a device that holds more than a single unit at one time for examination. This inspection may be performed in-line with filling or

packaging or in a separate, off-line inspection department. The intent of this inspection is the detection and removal of any observed defect. When in doubt, units should be removed.

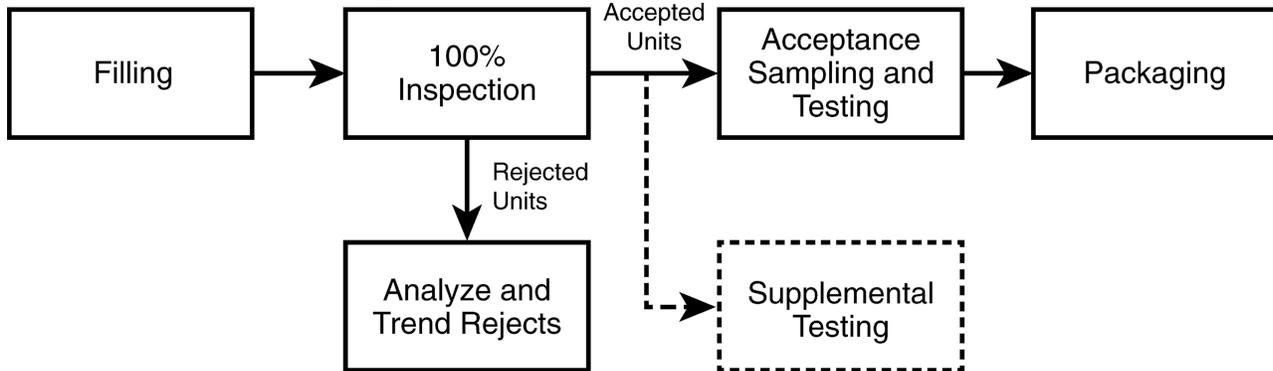


Figure 1. Typical Process Flow Chart.

Note—100% inspection refers to the complete inspection of the container/closure system and its contents. Inspection may be accomplished in a single operation or in multiple steps using a combination of technologies. See additional discussion in *Section 3.3 Remediation and Alternative Practices* and *Section 6 Inspection Methods and Technologies*.

Note—Supplemental testing is required when the nature of the product or container limits visual inspection of the contents (e.g., with a lyophilized cake or powder or with an amber glass or opaque container). See additional discussion in *Section 5.2 Unique Product and Container Considerations*.

During 100% inspection, limits on typical rejection rates should be established to identify atypical lots [36]. These limits may be established for categories of defects (e.g., critical, major, and minor) or for specific types of defects (e.g., particles). A review of historical performance is useful in establishing these limits, and the review may include products similar in appearance and manufacture. Periodic reassessment of these limits is recommended to account for expected process improvements and/or normal fluctuations in process baseline [37]. If a limit is exceeded, it should trigger an investigation. The outcome of the investigation will determine whether additional inspection is necessary.

3.2 Acceptance Sampling and Testing

After 100% inspection, a statistically valid sample is taken from the units accepted by the inspection process. This may be a random sample from across the batch or a representative sample (e.g., at fixed time intervals or a fixed number per tray). Typical sampling plans used for this purpose can be found in the ANSI/ASQ Z1.4 standard [38]. Defects may not be distributed equally over the lot, and therefore a sampling process that represents the whole lot is required. Equivalent plans may also be found in the ISO 2859 [39] or JIS Z9015 [40] standards. For batch release, the sampling plans listed as Normal II are typically used. Tightened sampling plans may be appropriate when an atypical result is observed or reinspection is performed. These plans specify a sample size for a range of batch sizes and require selection of an Acceptable Quality Level (AQL). The AQL is the defect rate at which 95% of the lots examined will be accepted and is a measure of falsely rejecting good batches. Critical defects (those that pose the greatest risk to the patient) should be assigned an AQL with a low value. Often, the accept number (the number of defective units allowed in the sample) for a critical defect is zero. Major and minor defects, which pose less risk to the patient, will have increasing (less stringent) AQL values and accept numbers greater than zero. *Table 1* shows the range of AQL

values typically used for visual inspection processes [41].

Table 1. Typical AQL Values for Visual Inspection Processes

Defect Category	AQL Range (%)
Critical	0.010–0.10
Major	0.10–0.65
Minor	1.0–4.0

[Note—When establishing a sampling plan, select the sample size required for the AQL associated with the critical defect category. Use this sample plan for major and minor defects, adjusting the accept numbers as needed for the larger sample size.]

The Unacceptable Quality Level (UQL) for the sampling plan used should also be known. The UQL is the defect rate at which 90% of the lots examined will be rejected and is a measure of the customer or patient risk. Sampled units should be manually inspected under controlled conditions by trained inspectors. Inspection condition should be aligned with the 100% inspection process.

Acceptance sampling should be performed after any type of 100% inspection process, including manual, semi-automated, and automated inspection processes. It provides a measure of the performance of the overall inspection process and the quality of a specific lot, compared with predefined acceptance criteria. Although automated systems are validated before use and are routinely challenged to ensure acceptable performance, the use of acceptance sampling detects unexpected defects that were not included in the development and training of the automated system by the manual inspection process.

If the acceptance criteria of the sampling plan are not met, an investigation should be conducted. Depending on the nature of the failure, this investigation should include examinations of the manufacturing process, the raw materials, and the packaging materials, as well as the inspection process. If, after investigation, the inspection process is deemed capable of detecting the defect(s) in question, the batch may be reinspected. After reinspection, a new sample of the accepted units is taken and compared against established acceptance criteria. It is a good practice to use a tightened sampling plan and acceptance criteria under these circumstances because of the atypical nature of this process step.

3.3 Remediation and Alternative Practices

3.3.1 REINSPECTION

As discussed in the preceding section, reinspection may be appropriate if the initial 100% inspection is not successful. This includes instances when the established 100% inspection failure rate(s) and/or the accept number(s) associated with the chosen AQL values have been exceeded. Reinspection should only be conducted using a prior-approved procedure that addresses key parameters such as the inspection conditions (e.g., same as primary inspection or modified to enhance detection of a specific defect type), the number of times reinspection may be performed (this should be limited, and repeated reinspection should not be permitted), and the acceptance criteria (e.g., same as primary inspection or tightened).

3.3.2 TWO-STAGE INSPECTION

In cases where an assignable cause—such as formation of air bubbles or a specific container or closure variation—results in a high false-rejection rate (rejection of acceptable units), the use

of a second inspection step may be considered. This is more common with automated inspection systems, where there is less ability to tolerate normal variation in product or container. Under these circumstances, the inspection system is adjusted to ensure acceptance of good units. Those not accepted are considered of uncertain disposition until inspected by another means (e.g., manual inspection following automated inspection). Inspection conditions may be adjusted to provide greater sensitivity in this second inspection step (e.g., additional inspection time) to ensure a high probability that true defective units will be rejected. The second inspection of these units by the same method (e.g., automated inspection after automated inspection) is generally not recommended, because the same limitation in inspection method is present for both inspections. However, it may be suitable when the root cause is air bubbles in the solution; a study has been performed to establish an appropriate holding time to allow the bubbles to dissipate before performing the second inspection. It is recommended that each inspection stream (those accepted by the first stage and those accepted by the second stage) be sampled separately and evaluated against the sampling plan acceptance criteria before they are confirmed as accepted and recombined into a single batch.

4. INSPECTION LIFE-CYCLE

4.1 Common Sources of Added Particulates

Particles may originate from many sources. They are discussed here, as well as in other chapters in the *USP-NF* (e.g., chapter *Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections* (1787)). Those that are foreign to the manufacturing process are considered to be exogenous or "extrinsic" in origin; these include hair, non-process-related fibers, starch, minerals, insect parts, and similar inorganic and organic materials. Extrinsic material is generally a one-time occurrence and should result in the rejection of the affected container in which it is seen; however, elevated levels in the lot may implicate a broader contribution from the same source. These particles may carry an increased risk of microbiological or extractable contamination, because less is known about their path prior to deposition in the product container.

Other particles are considered "intrinsic", or from within. These may come from processing equipment or primary packaging materials that were either added during processing or not removed during container preparation. These primary product-contact materials may include stainless steel, seals, gaskets, packaging glass and rubber, fluid transport tubing, and silicone lubricant. Such particles still pose the risk of a foreign body, but generally come from sterile or sanitized materials and more is known about their interactions when in contact with the product. These process-related intrinsic particles should have controls established based on the use of a life-cycle approach as outlined in *Section 1.3*. Another group of particles considered intrinsic is related to the stability of the product. These product stability-related particles come from container/closure interaction, unintended changes to the drug formulation (degradation), or temperature sensitivity over time. Stability-related intrinsic particles should be identified and addressed as early in the product development process as possible.

A third category of particles is the "inherent" particles, which are known to be or intended to be associated with specific product formulations. The physical form or nature of the inherent particles varies from product to product and includes solutions, suspensions, emulsions, and other drug delivery systems that are designed as particle assemblies (agglomerates, aggregates). For protein-based products in which the aggregates may form longer chains or

protein strands, the protein species may become visibly detectable as haze or individual particles during changes over the shelf-life. Product formulation-related suspensions or particulate formation should be studied in the development phase and in samples placed on stability to determine the normal characteristics and time-based changes that can occur. Use of automated particle counting or image analysis in the subvisible and visible ranges for particle sizes $\geq 2 \mu\text{m}$ may be required to fully characterize inherent formulation-related particles. Inherent particles should have a defined allowable profile over the shelf-life of the product. Where applicable, the inherent particle profile may be covered in product-submission documentation or product-specific monographs.

An evaluation of the potential impact of particles identified from any of these sources may be enhanced by incorporating a clinical risk assessment. This assessment may include factors such as the intended patient population, route of administration, source of the particles, and implications for product sterility. For intrinsic or inherent particulate matter sources, a risk assessment may be useful in developing product-specific control strategies. Given the probabilistic nature of particle detection, it is important to assess the possible implications of particles identified through the product life-cycle to better ensure the product's safe use.

4.2 Prevention of Particulates

The manufacturing process is designed to keep the final container and its contents clean within the control parameters established for process-related intrinsic particulates. Once the container is filled, the stability of the product needs to be maintained throughout its shelf life. Changes that occur as the product ages during its normal shelf life must be characterized. Avoidance of intrinsic particle sources that may affect final product stability depends on careful consideration of the entire product system. If these intrinsically sourced changes occur, and they affect stability, particles ranging from sub-visible to visible may develop. Typically, these particles result from change mechanisms that slowly affect the on-shelf product.

4.2.1 ROBUST DESIGN DURING DEVELOPMENT

To anticipate potential sources of instability that yield intrinsic particles, the product design is evaluated from many perspectives, beginning with a literature review of similar formulae/packages. Points to consider include the reported sensitivities of the active, the formulation type, and the final container/closure system needed for delivery. Knowledge of how glass containers are fabricated, controlled, sterilized, and tested is important as this may affect the tendency to form glass lamellae [42] [43]. Obtaining further information on residual extracts, possible leachates, metals, or solubility-edge conditions is important as these factors may promote formation of solid material in the desired solution. Several additional key factors for successful product design are the product concentration, solution pH, critical micelle concentration, oligomerization content/potential, package effects (large surface area, product volume, head space, light/oxygen transmission), and compatibility of the formulation with the package. Some key formulation design factors include the formula components chosen and their purity; the solubilities of the active ingredient(s) and excipients, and consideration of potential salt forms. Finally, to maximize product stability, consider the final product preparation for delivery, product dilutions, and shelf stability of the commercial product or its therapeutic preparations.

To examine the appropriateness of the product design for maintaining product stability, there are two levels of evaluation. Both levels examine retained containers for visible changes using

methods described in this chapter, but neither level dwells on low percentage defects. For the first level of stability study, bench trials consisting of visual inspection of trial containers in the formulation lab will show general compatibility of the chosen components over time with regard to clarity, color, and particle formation. Careful product assembly in clean containers, with consideration of the container type, headspace, and sealing, will yield a beneficial first-pass trial of stability over several months' time. Detection of extrinsic particles at this stage of development is generally not significant, as the particles do not reflect on the formulation under development.

The second, more refined level of stability study involves conducting visual inspections of the injection in defined, ICH-relevant trials requiring periodic inspection of the same containers over time. Detection of minor or subtle differences in these containers is not the goal at this stage of development. Catastrophic change and the occurrence of intrinsic product-related visible particles should be the focus. Typically, a set of containers is carefully prepared to exclude extrinsic particles and is then inspected to cull out any units with visible defects. Next, a numbered set of containers appropriate for the batch size is placed on trial and visually inspected periodically; a typical sample size is 80 or 100 units. Additional sets of containers stored at selected extremes of ICH temperatures can be followed to aid discovery of solubility-edge phenomena. When unwanted changes are detected—such as particle formation, solution color change, solution haze, and package changes—the process of isolation, characterization, and identification can commence. Identification of the material making up the changes aids in determination of the cause, as well as development of improvements for future use.

4.2.2 COMMON SOURCES OF INTRINSIC PARTICULATES

Process-related intrinsic particles originating from product contact materials tend to be stable and unchanging (e.g., glass, rubber, or metal). In contrast, product instability results from change mechanisms within the final product. It is very important to understand that these changes only have to be slight in certain cases, far below the detection limit of most release or stability assays, to result in visible changes to the product. The threshold levels for the formation of visible change for certain substances may be only 10–100 ppm (0.001%–0.01%). However, if all of this insoluble material were contained in a single visible particle, it would likely cause rejection of the container. Physical instability may be promoted by content below the threshold of acceptable chemical purity.

4.2.3 FORMULATION COMPONENTS

The active ingredient may also contribute to the formation of intrinsic particles. The determination of whether the particulate is inherent or intrinsic to the process is based upon toxicology studies and clinical studies. However, these studies may not be sufficiently long term or sensitive to reveal a particularly insoluble component of the drug at low levels that grow with time. It is also possible for substances to be incorporated into the bulk drug from all points of contact in the synthetic process. These are miscible in the formulation, yet may precipitate from solution over time as a visible haze or particulate matter. For example, significant haze and particles have manifested in aqueous formulations due to extraction of plasticizers from filtration media during bulk drug production [6]. Metal content in the active ingredient has contributed to organometallic salt formation and has also been observed as precipitated inorganic salts, blooming long after product release. The active ingredient and related degradation products may also be relatively insoluble and may grow to form visible particles. Monomers or single molecules may join together through chemical processes to form dimers,

trimers, and oligomers (a limited assemblage of monomers, short of polymerization). Such changes are not unexpected [44]. In high-concentration and/or saturated formulations, and especially for micellar drug associations, the solubility of related forms is significant when the aging formulations contain progressively higher concentrations of these substances. Larger molecules may have a greater effect on solution integrity due to their inherent insolubility, especially if the active drug is in a micellar formulation.

Polymorphs are unique crystalline forms of identical chemical entities. Although uncommon in solutions that have been mixed homogeneously and filtered, small seed crystals of a relatively stable polymorph may form over time, especially at nucleation sites such as container-surface defects. More common than formation of polymorphs is formation of a modified crystal lattice containing an integral liquid, typically water or solvent. The lattice may form slowly, promoted by evaporation, nucleation, and temperature extremes [45] [46].

4.2.4 PACKAGING COMPONENTS

Extractables and leachables are terms commonly used to describe the potential for containers to contribute unwanted agents to the product. Extractables represent all of the materials that could be contributed, and leachables represent the practical contribution upon contact between container and drug formulation [47]. These substances can also contribute to the formation of subvisible and visible particles.

Formulation attack of the container is most dramatic in glass container systems. Glass containers undergo corrosion that is 25 times greater at pH 8 than at pH 4 [48]. A formulation pH above 7, especially with high ionic strength solutions, promotes attack of the inner glass surface, resulting in particle generation.

Silicone oil is added to prefilled glass syringe systems to enhance lubricity for closure insertion and/or syringe movement. Silicone may also come from tubing used for fluid transfer and a variety of polymeric fittings and seals that are used in the processing equipment. All of these components must be compatible with the formulation to minimize leachates. Although silicones are processed to be sterile and are widely used, their use must still be controlled. Silicone can cause container sidewall droplets and a variety of visible semi-solid forms. No more than the minimum quantity should be used during processing. Silicone and other hydrophobic substances have the capacity to coalesce and agglomerate with other particles, reaching a visible size.

4.3 Particulate Removal by Component Washing

4.3.1 GLASS CONTAINERS

Each step of the glass-container washing and rinsing process should be evaluated for particle-reduction capability. The washer validation studies should demonstrate a reduction in naturally occurring particles or should use seeded containers to demonstrate such reduction capability. The use of statistical sampling plans with light obscuration and/or membrane microscopic particle-counting methods can provide a means to demonstrate reduction of both subvisible and visible particles during washing cycle development and validation. During process development, validation, and routine use, container-washing procedures should include periodic visual operational checks. This routine verification ensures that effective draining of all containers is occurring during all washing and rinsing steps. Review the wash-water recirculating filter maintenance procedures to ensure that particle overloading or breakthrough is being prevented.

Glass breakage that occurs during the component washing process should be evaluated for

possible glass particle generation that could affect surrounding containers. Effective written container-clearance procedures following these occurrences should specify the number of containers to be removed from the affected portion of the line. Removing units that could potentially contain glass particles aids in minimizing particle transfer to the downstream process.

4.3.2 RUBBER STOPPERS OR PLUNGERS

Each step of the rubber-component washing and rinsing process should be evaluated for particle-reduction opportunities. Utilize statistical sampling plans to collect meaningful test units. Light obscuration or other automated particle counting and membrane microscopic particle-counting methods may be used to demonstrate reduction of both subvisible and visible particles during washing validation. During process development and validation and in routine use, container-washing procedures should include visual checks to ensure that stoppers are not routinely sticking together. Such sticking surfaces reduce cleaning efficacy and entrap particles. Periodic assessment of component cleanliness and supplier washing capabilities should be included as part of the supplier qualification program when using purchased, ready-to-sterilize, or ready-to-use components.

Evaluate any current siliconization process used—whether in-house or by the supplier—to minimize excess silicone levels while maintaining machinability of the stoppers. Light obscuration or other automated particle-counting method may be used to compare overall particle level reduction (background silicone oil droplets) during process development or validation. The level of residual silicone oil will affect the particulate quality of the final filled product, observed as dispersed droplets and particle-forming matrices.

4.3.3 GLASS DEPYROGENATION

Processes that use racks or trays for transporting and holding samples, as are typically used in batch ovens, should be monitored for metal particle generation. The racks or trays should have a formal maintenance program associated with their routine use. Trays should be inspected for wear and scoring, which can be sources of particulates. Periodic cleaning, polishing, and/or resurfacing may be warranted to effectively control particles. Tunnels used for depyrogenation should also have a routine maintenance program for periodic cleaning, inspection, and replacement of parts that may wear and generate particles. Routine process observation for glass breakage allows for clearance of any potentially affected surrounding containers and minimizes the occurrence of glass particles being carried downstream to filling.

4.3.4 EQUIPMENT PREPARATION

It is important to minimize redeposition of particles on product contact surfaces after cleaning. Cleaned and sanitized equipment should be protected by HEPA-filtered, unidirectional airflow until transferred to, and installed on, the filling line. For cleaned equipment that needs to be wrapped or bagged prior to sterilization, utilize low-shedding, non-cellulose (synthetic) wrapping materials. Cellulose fibers are one of the most common particles found in the injections-manufacturing environment and injectable products.

4.3.5 FILLING LINE

The transfer of open containers should be conducted in Grade A (ISO 5, Class 100), unidirectional air flow to minimize particle contamination. The air in critical zones should be

monitored continuously during operation to confirm compliance.

Routine checks to detect particles and potential particle-generation locations should be explained in the procedures. Effective, written container-clearance procedures to be used after glass breakage should specify the number of containers to remove from the affected portion of the line. Note that improper set-up and adjustment of the filler can lead to "needle strikes," where the filling needles make contact with the container being filled. This can generate either stainless steel or glass particles.

Filling pump design and the pump's compatibility with the filling solution are important considerations. Metal-on-metal piston pumps have a greater potential for generating metal particles, compared with other types of piston pumps. Pump maintenance is essential and includes a requirement to resurface the cylinders and pistons periodically. Peristaltic-action pumps must be monitored for generation of silicone tubing particles, especially with aggressive, near-saturated solutions or suspensions. Friction in the peristaltic roller area can break down the tubing, resulting in the generation of particles.

Stopper bowl surfaces should have a formal maintenance program, and stopper handling or replenishment by operators should be specifically designed to minimize particle transfer to the stoppers.

Proper operator positioning and avoidance of open containers is important in good, aseptic filling practices to avoid microbial contamination. These same principles help reduce particle transfer to the open containers.

Careful selection of cleaning and gowning materials will help reduce contamination from extrinsic particles and fibers. These clean-room materials should be selected for their superior non-shedding and low-particle properties.

4.4 Trending

Data obtained from the inspection process are used for batch release. These data should also be analyzed for adverse trends on a periodic basis, typically at least once per year. High-volume products may generate sufficient data to allow quarterly analysis, whereas a longer period of time may be necessary to accumulate data for products that are produced infrequently. Data from component inspection, production 100% inspection, and the AQL inspections should be evaluated based upon sound statistical principles to determine whether the current action levels are accurately reflecting the current process capability. Alert levels may be introduced and/or adjusted accordingly if the statistical analyses indicate that lower defect levels are being observed consistently.

When establishing new action or alert levels, a preliminary value may be used until sufficient production experience is obtained. Consideration should be given to planned improvements in the manufacturing and inspection processes. If significant improvements are planned, the reduction of the action/alert level should not be instituted until the impact of the improvement is measured over sufficient time to establish the validity of the new value.

5. INTERPRETATION OF INSPECTION RESULTS

5.1 Defect Classification

Defects are commonly grouped into classifications based on patient and compliance risk [2]. The most common system uses three groups: critical, major, and minor. Critical defects are

those that will cause serious adverse reaction or death of the patient if the product is used. This classification includes any nonconformity that compromises the integrity of the container and thereby risks microbiological contamination of the sterile product. Major defects carry the risk of a temporary impairment or medically reversible reaction, or involve a remote probability of a serious adverse reaction. This classification is also assigned to any defect which causes impairment to the use of the product. These may result in a malfunction that makes the product unusable. Minor defects do not impact product performance or compliance; they are often cosmetic in nature, affecting only product appearance or pharmaceutical elegance. Visible particles from multiple sources can be present in drug products before 100% inspection. Particulate matter is defined as "mobile undissolved particles, other than gas bubbles, unintentionally present in the solutions" [49]. For visible particles, particle motion aids in detection. Stationary particles are difficult to detect. Upon 100% inspection, visible extrinsic and intrinsic particles should be reliably removed. The test method allows inherent particles to be accepted if the product appearance specification allows the inherent particle types. The size of particles reliably detected ($\geq 70\%$ probability of detection) is often between 100 and 300 μm . This is dependent on the container characteristics (size, shape, transparency), inspection conditions (lighting and duration), and particle characteristics (size, shape, color, and density).

5.1.1 EXTRINSIC, INTRINSIC, OR INHERENT PARTICLES

Extrinsic particles are foreign to the manufacturing process and should be a rare occurrence. Intrinsic material is generally associated with the primary packaging or processing equipment. These process-related intrinsic particles should be monitored and trended for control purposes. Other intrinsic materials are more often stability indicating and may indeed change due to aging, concentration change, degradation, acceleration of reaction, and component interaction. The intrinsic category should be recognized as different from inherent particle types. A good example would be the inherent slight haze imparted by the light-scattering properties of a protein formulation versus the intrinsic variable haze, droplets, and tearing imparted by excess siliconization. Solution properties such as a slight haze or faint coloration of high-concentration formulae and protein formulations are typical examples of an inherent characteristic of the product fluid, with particle detection being enhanced with backlighting or bottom lighting if the product has significant turbidity. In biologics, floating protein particles are considered inherent when their presence may be measured, characterized, and determined to be part of the clinical profile. Inherent particles may be accepted if the drug product has a control strategy showing that this particulate category is part of the product clinical profile. The manufacturer may allow inherent particles if the product appearance specification also allows their presence or if the product is an emulsion or suspension. For suspension products, a test dissolving the suspension that provides for extrinsic particle detection is also acceptable.

5.2 Unique Product and Container Considerations

5.2.1 LYOPHILIZED PRODUCT

Lyophilized products receive 100% inspection after the freeze-drying step has been completed and each unit has been sealed. However, the solid, lyophilized cake can mask the presence of visible particles because they cannot be seen within the solid matrix. The cake surface is visible during inspection but accounts for only a small fraction of the cake volume. Because of these challenges in evaluating acceptability, a small sample of units is reconstituted and inspected for

visible particles in addition to the 100% inspection of the cakes for visible particles. Care must be taken during reconstitution of these samples to avoid contamination that can lead to false-positive results. Sample preparation should be done in a clean environment with appropriate particle-control measures. Reconstituted samples should be inspected using the same conditions as those for visible particles. The destructive nature of this test limits the size of the sample; however, the resultant fluid allows visible particles to be detected. Typical sampling plans for this type of test can be found in the special sampling plans S-3 and S-4 in ANSI/AQS Z1.4 [38]. Once inspection of these reconstituted samples has been performed, they may be used for other required testing, such as that for subvisible particles, potency, impurities, or other specified tests. If particles are detected in this relatively small sample, additional units may be reconstituted as part of an investigation and to assess the compliance of the entire batch.

5.2.2 POWDER PRODUCT

Sterile powders are difficult to inspect for particles due to powder flow and the occlusion of white or light-colored particles by the drug product itself. Sterile powders should be reconstituted and inspected for visible foreign particles using an approach similar to that for lyophilized products, as discussed above.

5.2.3 AMBER CONTAINERS

Inspecting amber containers is challenging because selected elements have been added to mask UV light penetration into the Type I glass container. Light transmission is blocked below 500 nm, and thus increased light intensity (e.g., 8,000–10,000 lux) may be required to observe visible particles during inspection. Directional lighting from behind the container may also be beneficial. At the extreme, filled solution in practically opaque containers may be audited via sampling and transfer to clear, clean containers.

5.2.4 TRANSLUCENT PLASTIC CONTAINERS

Plastic or translucent containers are chosen for break resistance or other properties that glass cannot offer, such as injection molding into shapes that minimize hold-up volume or for use in a combination product. Rigid plastic containers may have optical properties that require significantly more light (e.g., 8,000–10,000 lux) to illuminate any visible particles against black and white backgrounds. Directional lighting from behind the container may also be beneficial.

5.2.5 LARGE-VOLUME CONTAINERS

Large-volume containers (> 100 mL) may require additional time to complete a thorough inspection. Due to the semi-transparent film characteristics of PVC film used in flexible bags, additional light intensity may also be used to enhance the visibility of extrinsic and intrinsic particles. Directional lighting from behind the container may also be beneficial.

5.2.6 COMBINATION PRODUCTS

When inspecting the unlabeled primary drug container for a combination product, the inspection considerations should be the same as those specified for a conventional drug product in a vial or syringe. This inspection should be performed before assembly into the device. Where there are critical attributes that are only visible after assembly (such as alignment with a fill-level

window), a second inspection after assembly may also be required.

6. INSPECTION METHODS AND TECHNOLOGIES

6.1 Manual Visual Inspection (MVI)

Manual visual inspection (MVI) is the reference inspection method described in all of the major pharmacopeias [49] [50]. It consists of viewing filled and sealed containers under controlled conditions. This process may be aided by the use of a tool to allow consistent examination of more than one container at a time. The quality decision, to either accept or reject the container, is made by a trained person. Inspection is a probabilistic process, and detection rates less than 100% are to be expected, especially for smaller or low-contrast defects.

6.1.1 CRITICAL PROCESS PARAMETERS IN MVI

Light intensity: The results of the manual inspection process are influenced by the intensity of the light in the inspection zone. In general, increasing the intensity of light that illuminates the container being inspected will improve inspection performance; chapter 〈 790 〉 recommends light levels NLT 2000–3750 lux at the point of inspection for routine inspection of glass containers. Increased light levels are recommended for plastic containers or those made from amber glass. Under these circumstances, light levels as high as 10,000 lux may prove beneficial. The final inspection condition will depend on measured performance.

Light should be diffuse and even in the inspection zone, and it is a good practice to clearly identify this zone within the inspection station where the intensity meets the required levels. Fluorescent lamps have often been used as the light source for inspection. When fluorescent lamps are used, high-frequency ballasts are recommended to reduce visible flicker (and associated inspector fatigue). Incandescent lamps have also been used successfully for this purpose, but they generate significant heat during use. Light emitting diodes (LED) offer an energy efficient, stable source of light without the added heat of incandescent lamps. Light intensity in each inspection station should be measured periodically to ensure continued compliance with the specified range. The frequency of monitoring should be based on historical experience with the type of light source in use. A lower light-intensity action limit should be established to trigger corrective action before inspection is performed below the specified value or range.

Background and contrast: Contrast between the defect of interest and the surrounding background is required for detection, and increased contrast improves detection. The use of both black and white backgrounds is described in chapter 〈 790 〉, as well as other global pharmacopeias. The use of both backgrounds provides good contrast for a wide range of particulate and container defects, which can be light or dark in appearance.

Inspection rate: Sufficient time must be provided to allow for thorough inspection of each container; chapter 〈 790 〉 specifies a reference time of 10 seconds per container (5 seconds each against the black and white backgrounds). Larger or more complex containers may require additional time for inspecting all attributes. Increased time may facilitate detection of defects near the threshold of detection, but studies by Wolfe et al. [51] [52] suggest that there are diminishing gains with increasing inspection time. Time spent per container may be controlled through the use of a pacing device such as a light or tone, or these may be used during

training only, much as a musician uses a metronome during practice to learn the tempo of a musical piece for later performance. Recording the time spent inspecting each batch and then calculating a nominal inspection rate is a good way to confirm that the rate of inspection was within established limits.

Container handling and movement: When observing objects, the human eye is very sensitive to movement. Good techniques for manual inspection include a careful swirl or inversion of the liquid product within the container. This rinses any particles from the upper inner surfaces of the container and the closure and puts them into motion. A technique that minimizes the introduction of air bubbles is important, as air bubbles can appear as particles and interfere with detection. A tool that holds multiple containers for consistent presentation can be useful when performing inspection. Holding many containers by hand at once should be avoided, as it is difficult to obtain a complete view of all container surfaces and contents. Container motion is also helpful for identifying small container defects such as cracks or chips.

Magnification: Some inspection processes use a large magnifier to increase image size and thus increase the probability of detecting and rejecting containers with defects near the threshold of detection. Although magnification can be useful for critical examination of a portion of the container, it does not often lead to increased overall detection rates for defects of interest. This may be due in part to the added eye strain that often results from use of magnification, which is not recommended as part of the reference inspection method described in chapter 〈 790 〉 or in other global pharmacopeias [49] [50]. Although not recommended for use during routine inspections, magnification can be helpful for critical examination of a small number of units, as may be needed during an investigation.

6.1.2 INSPECTOR FATIGUE AND ERGONOMIC CONSIDERATIONS

Inspecting for extended periods of time can cause inspector fatigue and a decrease in inspection performance. Based on industry experience [41], it is recommended that inspectors be given a break from performing inspection at least every hour. This break should allow time to rest the eyes and mind, and may be achieved with a short rest (e.g., 5 min) or a longer meal break. This need for regular breaks may also be met through rotation to a non-inspection function, such as material handling or documentation.

Inspection stations should be designed and operated in a manner that minimizes the inspector's risk of repetitive-motion injury. Adjustable chairs and careful positioning of light sources as well as incoming and inspected product can reduce the risk of such injury. These adjustments can also reduce inspector fatigue and discomfort, both of which can be distracting and thus can decrease performance.

6.2 Semi-Automated Visual Inspection

Semi-automated visual inspection combines automated material handling of the containers to be inspected with human vision and judgment to make the accept-or-reject decision. These systems often use a conveyor equipped with rollers to transport the containers in front of the inspector inside an inspection booth or station. For inspection of liquids, the booth can be equipped with a high-speed spin station to set particles in motion. The rollers are also used to slowly rotate the containers in front of the inspector as they traverse the inspection zone. These systems offer a means to control the presentation of the vials and can offer additional lighting options, such as Tyndall lighting, which may enhance the appearance of some defects

such as cracks or small particles. Mirrors may also be used to provide a clear view of the top and bottom of each container. Rejected units may be removed from the rollers by hand, and some systems are equipped with a remote rejection system that can be triggered by the inspector. Care should be taken in the qualification and operation of these systems to ensure full rotation of vials in the inspection zone; this allows examination of all surfaces. In addition, studies should be conducted to ensure the detection of heavy particles, which may not be lifted from the bottom of the container, and to ensure that the rate of inspection produces an acceptable detection rate for defects of interest.

With semi-automated visual inspection, performance is similar to that with MVI. Some increase in throughput may be achieved because the inspector spends all of the available time viewing the containers, rather than splitting the time between inspection and material handling.

6.2.1 CRITICAL PROCESS PARAMETERS FOR SEMI-AUTOMATED INSPECTION

Light intensity must be controlled, as with MVI. The rate of inspection is controlled by the speed of the roller/conveyor. Spin speed for liquid products and rotation rate for all containers should be established during validation/qualification and maintained within the validated range for routine inspection. The background color is controlled by the color of the rollers selected and the color of the background seen through the spaces between the rollers.

6.3 Automated Visual Inspection (AVI)

AVI combines automated material handling of the containers with electronic sensing of product appearance. Containers that do not meet preprogrammed acceptance criteria are automatically rejected by the machine. Early machines performed inspection for particles and fill level, but manual or semi-automated inspection was required for the container and closure system. Newer models have the capability to inspect all attributes of the containers, along with the contents. As with MVI, machines often spin the containers to set particles in motion and make them easier to detect. Multiple cameras are used to image various regions on the container in great detail. Each camera is coupled with unique lighting to highlight specific defects in the region of interest. Light-field and dark-field lighting techniques offer the same benefits as white and black backgrounds as discussed above, offering contrast for a full range of light- and dark-colored defects. A defect found by any camera is tracked through the machine to allow accurate ejection by the reject system. The machine also offers detailed reporting of defects observed in a specific production lot.

AVI offers advantages in the areas of throughput and consistency, compared with MVI [5]. AVI may also offer enhanced sensitivity for some defects, compared with MVI, but may suffer from higher false rejection rates due to the inability to tolerate normal variation in containers or product. This is especially true for molded glass containers.

6.3.1 LIGHT-OBSCURATION METHODS

Some systems use an optical sensor to detect the shadow of particles in solution products. This method requires particles to be in motion, typically using a high-speed spin and rapid braking of the container to achieve this motion. Spin conditions must be optimized to provide sensitivity for heavier particles while minimizing false rejections due to bubbles. Some biological products experience shear-induced agglomeration, so care should be taken with regard to agitation of these products.

Light obscuration methods are optimized for sensitivity to moving particles, and can thus be

made less sensitive to minor container imperfections. This technique can be used with both tubing and molded containers. Results are generally robust in detecting particles that are 100 μm in diameter and larger.

These systems can also detect fill height by detecting the shadow of the solution meniscus. Generally, this process is not sensitive enough to ensure compliance with fill-weight specifications, but it can provide a secondary check of gross fill. Sensitivity is a function of the container shape, with greater sensitivity achieved in small-diameter containers.

6.3.2 IMAGING METHODS

Continuing advances in camera technology now allow the rapid capture of high-resolution images for inspection. When coupled with high-speed processors that have ever-increasing computational capability, a powerful inspection tool can result. Images are divided into inspection windows, and an array of tools such as image subtraction, pixel counting, intensity analysis, and others is used to assess the images against programmed quality attributes. Significant amounts of time are required to train inspectors and test the performance of such systems against a range of known defects, as well as acceptable containers. Imaging systems can detect particles and fill level, as well as other container and closure attributes. Inspection in this manner can provide 100% inspection of all visual attributes. These systems can offer high sensitivity, but may also have high false-rejection rates if container and product attributes are not tightly controlled.

6.3.3 OTHER TECHNOLOGIES

Container integrity [53] can be assessed using non-optical methods such as high-voltage [54] or vacuum decay. Optical spectroscopy [55] can also be used if there is a modified headspace such as vacuum or inert gas. Generally, these container-integrity inspection methods offer greater sensitivity than visual detection, with a significant reduction in false rejection of acceptable product.

X-ray imaging has also been explored as a means to detect particles within freeze-dried cakes, powders, or suspensions [56].

These technologies may be used alone or in combination with other inspection methods to provide a comprehensive assessment of product quality before labeling and packaging.

7. QUALIFICATION AND VALIDATION OF INSPECTION PROCESSES

7.1 Standards

The use of standards for visual inspection has been described by Melchore and Berdovich [57]. Development of inspection standards begins with identification or characterization of the defect types that will be represented in the test set(s). This information typically comes from the manufacturing area, where naturally occurring defective units can be identified from rejected product. The defects are categorized as critical, major, or minor. These defects must be further characterized to allow for 1) selection from naturally occurring particulate and physical or cosmetic production rejects removed from product lots, and/or 2) re-creation of equivalent defect types in a controlled laboratory environment. Characterization information on defects should include, where appropriate, the range of sizes typically observed, the specific location on the container, the volume, and the angle. If feasible, a photograph of the defect should be

included. All information that could support consistent re-creation of the defect standards should be included in the characterization description.

7.2 Preparing Defect Standards

Visual inspection standards may be identified from known production rejects, or may be created manually with characterized particulate material. A single particle per seeded container should be used when determining detection thresholds.

7.3 Particle Types

The primary packaging materials that directly contact the product and the potential environmental contaminants can be divided into specific particle groups such as glass, stainless steel, rubber stopper, plastic, and fibers (synthetic or natural). Naturally occurring particles from rejects should be no smaller than the visible particle (measured in situ) in the container. Measurement can be accomplished with a wide field microscope or loupe with a calibrated reticle. Physically prepared particles can be sieved initially to target a specific size, and then the individual particles are measured using optical microscopy. These materials, or production defects, are preferred for inspector training and qualification as well as machine validation as they better represent actual inspection performance. Spherical standard particles may be utilized as surrogates for naturally occurring particulates; however, these are best used for routine machine calibration rather than validation or inspector qualification, as they do not move or look like actual production defects.

7.4 Rejection Probability Determination

Once a well-defined defect standard is available, it is assigned a detection frequency by conducting a documented, manual human inspection qualification that is accomplished by repeated manual inspection. This repeated inspection is the basis for qualifying the defect standard. This approach has been described by Knapp and Kushner [58] [59]. The Knapp methodology recognizes that the detection of particles is probabilistic, and repeated inspections with strict controls on lighting and inspection pacing/sequencing generate the statistical confidence to assign a reject probability to each standard unit. A manual, visual inspection probability of detection (POD) of ≥ 0.7 or a detection rate (DR) of $\geq 70\%$ is required to assign the container to the reject zone for subsequent calculation of the reject zone efficiency (RZE). Secure probabilistic data for particulate standards can be achieved with 30–50 inspections for each container. This is best achieved with multiple inspectors. Inspection reject probability is calculated for the defect as follows: $POD = (\text{Number of times rejected}) / (\text{Number of times inspected})$.

7.5 Test Sets

These qualified defect standard units are then assembled into test sets which may be used to specifically challenge the particle detection technique of human inspectors, used as part of a defect test set (including container/closure defects) for human qualification, or for comparison during automated equipment qualification and validation. When possible, the test set should be prepared with duplicate product units per particle type and size to ensure that backup units are available in the event that a standard container is broken or the particle is trapped or lost

within the container. When using test sets, it is a good practice to verify the presence of particles before and after use, as particles may become lodged between the container and the closure. When a freely moving particle cannot be verified, the unit should not be used and the data should be excluded from subsequent calculations. When this happens, it may be possible to free the particle with the use of an ultrasonic bath. If this is not possible, the unit should be replaced. The number of defective units in each test set should be limited to approximately 10% to prevent rejection bias [51]. The accept containers will be identified as having a predetermined manual, visual inspection POD < 0.3 or a DR of < 30%. Any particle standards found to fall within the acceptable "grey zone" display, indicating a manual inspection rejection probability $\geq 30\%$ and < 70%, may be included as an "acceptable unit" in a test set, if desired. It is important to prepare a written procedure for the creation and maintenance of standards. This procedure should define the qualification criteria, appropriate storage conditions, periodic examination and requalification, expiration, and sample custody during use. Test sets should be approved by the quality unit. The container in which the specific particle set is stored must be clearly labeled with the test set identification information.

7.6 Types of Test Sets

The particle detection threshold can be determined for a specific inspection method and product/package combination. It is a standard curve of detection probabilities at various particle types and sizes in an approximate range of 100–500 μm (with recommended increments of 100 μm). Fibers are typically observed in sizes > 500 μm . The typical size range of particles used in threshold studies incorporates a variety of particle types and densities that are typically found in the manufacturing environment.

Threshold studies are conducted to determine the sensitivity of manual inspection methods, using a range of particle sizes, in a blinded study that yields the particle-size detection capabilities of a defined group or of an individual inspector. The threshold studies indicate that the method of inspection is valid and appropriate if particle detection is reproducible in detecting particles within the range of 150–250 μm (500–2000 μm for fibers). Threshold studies are also useful as an assessment tool when evaluating or qualifying visual inspection staff on a specific method with fixed testing parameters. Detection threshold studies are typically the first step in evaluating the performance of any new inspection method.

Depending on product and/or presentation, rejects in the test set should represent all defects anticipated for a given container type or product family. For particles, use a bracketed range of types (densities) and sizes from near the visual detection threshold range to the largest routinely observed in the pool of rejects. For an individual manual test set, it is important that all containers and stoppers are of the same type, and the samples are blinded. UV ink (invisible to the inspectors) may be used to mark all containers. Alternatively, bar codes or other coded labels may be used. Manual test sets can be used initially to qualify or periodically to re-qualify human inspectors. These test sets may also be used for direct comparison to semi-automated or automated inspection methods.

If significantly different formulations (e.g., clear solution, suspension, lyophilized) or packages (e.g., clear vials, amber vials, ampoules, syringes) are produced at the same facility, separate test sets should be prepared for each combination.

7.7 Training and Qualification of Human Inspectors

Before training, potential inspectors should be tested for visual acuity [60] and color

perception. Near-vision performance should be the equivalent of 20/20 with no impairment of color vision. Both the Snellen and Jaeger charts are useful for verifying visual acuity; they test far and near vision, respectively. Training should include a phased approach with a specified number of training hours expected for each segment. Initially, train the potential inspectors with defect photographs or a video library and clear written descriptions. Utilize subject matter experts to mentor and provide hands-on training with defect standards for the specified method. Reinforce mental or silent counting and follow the paced sequence to achieve consistent inspection timing. Stress the importance of strict adherence to the inspection process (procedure, sequence, and timing). Address inspector fatigue in the qualification process by testing under worst case conditions (i.e., at the end of a typical inspection shift). Train all inspectors (QC, QA, and Production) with common procedures used for 100% inspections and AQL inspections. All inspection practices should be standardized and consistently executed across all inspection groups. Qualification should be performed for each product type and package that the inspector will encounter. A bracketed or matrix approach can be used to simplify qualification of products with similar physical or visual characteristics such as container type and size, formulation type, product viscosity, color, and others. It is common to initially train and qualify personnel on clear solutions in clear containers (if produced at the facility) and then expand their expertise to inspections of more difficult formulations or presentations.

7.8 Inspector Qualification Requirements

The qualification of all inspection personnel utilizes a manual test set to be inspected under normal operating conditions and inspection critical parameters, including inspection timing and sequence, physical environment, and inspection duration. Three successful inspections of the test set are recommended to demonstrate consistent performance for initial qualification of new inspectors. Suggested acceptance criteria for each defect class are 100% detection of critical defects, $\geq 80\%$ detection of major defects, and $\geq 70\%$ detection of minor defects. A limit is also needed for false rejection, with a recommended target of less than 5% falsely rejected good units.

7.9 Requalification

Inspectors should be requalified at least annually. Requalification includes vision testing and testing with at least one product/test set configuration. A single successful inspection of the test set is sufficient for requalification. Requalification may also be necessary in the event that poor performance is observed during routine inspection or if the inspector has been away from the inspection operation for an extended period of time (e.g., 3 months). If an inspector fails the requalification test, a retraining process should be initiated to identify the root cause and allow the inspector to receive additional instruction. After this process has been completed, the inspector may attempt to meet the acceptance criteria one additional time. If the inspector fails, he or she may attempt to qualify again after a specified time period.

8. CONCLUSIONS AND RECOMMENDATIONS

Visual inspection for particles and other visible defects continues to be an important part of the manufacturing process for injections. Chapter { 790 } provides a useful reference method and

acceptance criteria for visible particulates in injections. Successful execution of visual inspection requires an understanding of the inspection process and careful control of inspection conditions. Inspectors must be trained to ensure consistent, high-quality performance. Alternative inspection methods, either semi-automated or fully automated, may be used in place of manual inspection methods. Where machine methods are used, the equipment must be validated to demonstrate equivalent or better performance when compared to manual inspection. The use of test sets that contain standard defects is an important element in inspector training and qualification, as well as machine validation. Good product development will lead to a stable product with a lower risk of particle formation. Identification of the type or types of particles found during product development and routine manufacturing is an important aid in source identification and reduction. Inspection results should be trended to further aid in continuous process improvement with the ultimate goal of defect prevention.

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BRIEFING

1-Naphthylacetic Acid. It is proposed to add this new reagent used in the test for *Organic Impurities* in the monograph for *Naphazoline Hydrochloride*, published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C126907

Comment deadline: March 31, 2015

Add the following:

▲1-Naphthylacetic Acid

(1-Naphthaleneacetic Acid, α -Naphthaleneacetic Acid Free Acid), $C_{12}H_{10}O_2$ —**186.21** [86-87-3]—Use a suitable grade with a content of NLT 95%. ▲*USP39*

BRIEFING

3-Chloropropane-1,2-diol. It is proposed to add this new reagent used in the *Limit of 3-Chloropropane-1,2-diol* test in the monograph for *Iohexol*, published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C103376

Comment deadline: March 31, 2015

Add the following:

▲3-Chloropropane-1,2-diol

((±)-3-Chloro-1,2-propanediol), $C_3H_7ClO_2$ —**110.54** [96-24-2]—Use a suitable grade with a content of NLT 97.5%. [Note—A suitable grade is available as catalog number 107271 from www.sigma-aldrich.com.] ▲*USP39*

BRIEFING

Cupric Nitrate Hydrate, *USP 37* page 1390. It is proposed to correct the CAS number of this

reagent.

Additionally, minor editorial changes have been made to update the reagent to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C153189

Comment deadline: March 31, 2015

Change to read:

Cupric Nitrate Hydrate, $\text{Cu}(\text{NO}_3)_2 \cdot 2.5\text{H}_2\text{O}$ —**232.59** [~~3252-23-8~~

▲19004-19-4▲*USP39*

]; $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ —**241.60** [10031-43-3]—Use ACS reagent grade.

[Note—This reagent is available containing either 2.5 or 3 molecules of water of hydration.]

BRIEFING

Desmosterol. It is proposed to add this new reagent used in the test for *Limit of Related Sterols and Other Organic Impurities* in the monograph for *Cholesterol*, published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C136255

Comment deadline: March 31, 2015

Add the following:

▲Desmosterol

(3β -Hydroxy-5,24-cholestadiene; 24-Dehydrocholesterol; 5,24-Cholestadien- 3β -ol), $\text{C}_{27}\text{H}_{44}\text{O}$ —**384.64** [313-04-2]—Use a suitable grade with a content of NLT 94%. [Note—A suitable grade is available as catalog number 700060 from www.avantilipids.com or as catalog number 190190 from www.mpbio.com.] ▲*USP39*

BRIEFING

Lathosterol. It is proposed to add this new reagent used in the test for *Limit of Related Sterols and Other Organic Impurities* in the monograph for *Cholesterol*, published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C136255

Comment deadline: March 31, 2015

Add the following:

▲Lathosterol

(5α -Cholest-7-en- 3β -ol), $\text{C}_{27}\text{H}_{46}\text{O}$ —**386.65** [80-99-9]—Use a suitable grade with a content of NLT 95%. [Note—A suitable grade is available as catalog number 700069 from www.avantilipids.com or as catalog number C3652 from www.sigma-aldrich.com.] ▲*USP39*

BRIEFING

Pregnenolone Isobutyrate. It is proposed to add this new reagent used in the Assay and the

test for *Limit of Related Sterols and Other Organic Impurities* in the monograph for *Cholesterol*, published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C136255

Comment deadline: March 31, 2015

Add the following:

▲Pregnenolone Isobutyrate

(5-Pregnen-3-ol-20-one Isobutyrate), $C_{25}H_{38}O_3$ —**386.57**—Use a suitable grade with a content of NLT 99%. [Note—A suitable grade is available as catalog number EPP2920000 from www.lgcstandards.com or catalog number P2920000 from <http://crs.edqm.eu>.] ▲*USP39*

BRIEFING

Sodium Pentacyanoammineferrate(II), *USP 38* page 1869. It is proposed to update the specification of this reagent to reflect the products currently available.

Additionally, minor editorial changes have been made to update the reagent to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C153700

Comment deadline: March 31, 2015

Change to read:

Sodium (tri) Pentacyanoamino Ferrate [~~Trisodium Aminepentacyanoferrate (3-)~~], $Na_3[Fe(CN)_5NH_3]$

▲Sodium Pentacyanoammineferrate(II)

[~~Trisodium Aminepentacyanoferrate (3-)~~], $C_5H_3FeN_6Na_3$ ▲*USP39*

—**271.93** [14099-05-9]—Yellow to tan powder. Soluble in water.

Solubility—Dissolve 500 mg in 50 mL of water, and allow to stand for 1 h. The solution is clear and free from foreign matter.

Sensitivity

1,1-Dimethylhydrazine standard solution—Place 500 mL of water in a 1-L volumetric flask, and add from a buret 1.27 mL of anhydrous 1,1-dimethylhydrazine. Dilute with water to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, and dilute with water to volume. Each mL of this solution contains the equivalent of 100 µg of 1,1-dimethylhydrazine.

Buffer solution—Transfer 4.8 g of citric acid monohydrate to a 1-L volumetric flask, dissolve in water, add 14.6 g of sodium phosphate, swirl to dissolve, and dilute with water to volume.

Test preparation—Dissolve 100 mg of sodium pentacyanoammineferrate(II) in 100 mL of water.

Procedure—Into each of five 25-mL volumetric flasks pipet 0, 0.25, 0.50, 1.0, and 1.5 mL, respectively, of 1,1-Dimethylhydrazine standard solution; to each add 15 mL of Buffer solution, and swirl to mix. To each flask, add by pipet 2 mL of Test preparation, mix, dilute with Buffer solution to volume, and allow to stand for 1 h. Using a suitable spectrophotometer, 1-cm cells, and the solution containing no 1,1-Dimethylhydrazine

standard solution as the blank, determine the absorbances of the remaining solutions at 500 nm. Plot the observed absorbance as the ordinate versus the concentration of standard as the abscissa on coordinate paper, and draw the curve of best fit. The plot is linear and the absorbance of the 150- μ g solution is NLT 0.65.

▲

Note—A suitable grade is available as catalog number 14099-05-9 from www.bocsci.com.]

▲USP39

BRIEFING

Triethylammonium Acetate, 1 M. It is proposed to add this new reagent used in the *Assay* and the test for *Organic Impurities* in the revision to the monographs for *Miconazole Nitrate* and *Miconazole Nitrate Topical Powder*.

(HDQ: M. Marques.)

Correspondence Number—C131426

Comment deadline: March 31, 2015

Add the following:

▲Triethylammonium Acetate, 1 M,

$C_8H_{19}NO_2$ —**161.2** [5204-74-0]—Use a suitable grade.

[Note—A suitable grade is available as catalog number 625718 from www.calbiochem.com.]

▲USP39

BRIEFING

Edetate Disodium, Twentieth-Molar (0.05 M), *USP 38* page 1895. It is proposed to clarify the instructions on how to dry the chelometric standard.

Additionally, minor editorial changes have been made to update the volumetric solution to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C151771

Comment deadline: March 31, 2015

Change to read:

Edetate Disodium, Twentieth-Molar (0.05 M)

$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, **372.24**

18.61 g in 1000 mL

Dissolve 18.6 g of edetate disodium in water to make 1000 mL, and standardize the solution as follows.

Accurately weigh about 200 mg of chelometric standard calcium carbonate, previously dried at 110° for 2 hours and cooled in a desiccator, or dried according to the label instructions,

▲according to the label instructions or, if this information is not available, at 110° for 2 h,

▲USP39

transfer to a 400-mL beaker, add 10 mL of water, and swirl to form a slurry. Cover the beaker

with a watch glass, and introduce 2 mL of diluted hydrochloric acid from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass with water, and dilute with water to about 100 mL. While stirring the solution, preferably with a magnetic stirrer, add about 30 mL of the edetate disodium solution from a 50-mL buret. Add 15 mL of sodium hydroxide TS and 300 mg of hydroxy naphthol blue, and continue the titration with the edetate disodium solution to a blue endpoint.

$$M = \frac{(g \text{ CaCO}_3)(1000)}{100.09 \times \text{mL EDTA}}$$

BRIEFING

Potassium Arsenite, Tenth-Normal (0.1 N), *USP 37* page 1458. It is proposed to clarify the instructions on how to dry the primary standard arsenic trioxide.

Additionally, minor editorial changes have been made to update the volumetric solution to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C151772

Comment deadline: March 31, 2015

Change to read:

Potassium Arsenite, Tenth-Normal (0.1 N)

KAsO₂, **146.02**

7.301 g in 1000 mL

Dissolve 4.9455 g of arsenic trioxide primary standard, ~~previously dried at 105° for 1 hour,~~
 ▲dried according to the label instructions or, if this information is not available, dried at 105°
 for 1 h, ▲*USP39*

in 75 mL of 1 N potassium hydroxide. Add 40 g of potassium bicarbonate, dissolved in about 200 mL of water, and dilute with water to 1000.0 mL.

BRIEFING

Potassium Permanganate, Tenth-Normal (0.1 N), *USP 38* page 1899. It is proposed to specify the use of sodium oxalate primary standard in the standardization of this volumetric solution.

Additionally, minor editorial changes have been made to update the volumetric solution to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C151773

Comment deadline: March 31, 2015

Change to read:

Potassium Permanganate, Tenth-Normal (0.1 N)

KMnO₄, **158.03**

3.161 g in 1000 mL

Dissolve about 3.3 g of potassium permanganate in 1000 mL of water in a flask, and boil the solution for about 15 min. Insert the stopper in the flask, allow it to stand for at least 2 days, and filter through a fine-porosity, sintered-glass crucible. If necessary, the bottom of the sintered-glass crucible may be lined with a pledget of glass wool. Standardize the solution as follows.

Accurately weigh about 200 mg of

▲primary standard▲*USP39*

sodium oxalate, dried according to the instructions on its label, and dissolve it in 250 mL of water. Add 7 mL of sulfuric acid, heat to about 70°, and then slowly add the permanganate solution from a buret, with constant stirring, until a pale pink color, which persists for 15 s, is produced. The temperature at the conclusion of the titration should be NLT 60°. Calculate the normality. Each 6.700 mg of sodium oxalate is equivalent to 1 mL of 0.1 N potassium permanganate.

Since potassium permanganate is reduced on contact with organic substances such as rubber, the solution must be handled in an apparatus made entirely of glass or other suitably inert material. It should be frequently restandardized. Store in glass-stoppered, amber-colored bottles.

$$N = \frac{\text{g Na}_2\text{C}_2\text{O}_4}{\text{mL KMnO}_4 \text{ solution} \times 0.06700}$$

BRIEFING

Sodium Thiosulfate, Tenth-Normal (0.1 N), *USP 38* page 1900. It is proposed to clarify the instructions on how to dry the primary standard potassium dichromate.

Additionally, minor editorial changes have been made to update the volumetric solution to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C151770

Comment deadline: March 31, 2015

Change to read:

Sodium Thiosulfate, Tenth-Normal (0.1 N)

Na₂S₂O₃·5H₂O, **248.19**

24.82 g in 1000 mL

Dissolve about 26 g of sodium thiosulfate and 200 mg of sodium carbonate in 1000 mL of recently boiled and cooled water. Standardize the solution as follows.

Accurately weigh about 210 mg of primary standard potassium dichromate, previously

pulverized and dried at 120° for 4 h, or dried according to the instructions on its label,

▲according to the label instructions or, if this information is not available, dried at 120° for 4 h,

▲USP39

and dissolve in 100 mL of water in a glass-stoppered, 500-mL flask. Swirl to dissolve the solid, remove the stopper, and quickly add 3 g of potassium iodide, 2 g of sodium bicarbonate, and 5 mL of hydrochloric acid. Insert the stopper gently in the flask, swirl to mix, and allow to stand in the dark for exactly 10 min. Rinse the stopper and the inner walls of the flask with water, and titrate the liberated iodine with the sodium thiosulfate solution until the solution is yellowish green in color. Add 3 mL of starch TS, and continue the titration until the blue color is discharged. Perform a blank determination.

Restandardize the solution as frequently as supported by laboratory stability data. In the absence of such data, restandardize the solution weekly.

$$N = \frac{\text{mg K}_2\text{Cr}_2\text{O}_7}{49.04 \times \text{mL Na}_2\text{S}_2\text{O}_3}$$

BRIEFING

L65, *USP 38* page 1903. It is proposed to update the description of this phase to reflect the column that originated it and the website of a possible supplier.

Additionally, minor editorial changes have been made to update the packing to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C152904

Comment deadline: March 31, 2015

Change to read:

L65—Strongly acidic cation-exchange resin consisting of 8%

▲2%▲USP39

sulfonated cross-linked styrene-divinylbenzene copolymer with a sulfonic acid group in the hydrogen form, 45–250 μm in diameter. [Note—A suitable grade is available as AG 50W-X2 resin hydrogen form from www.discover.bio-rad.com

▲www.bio-rad.com.▲USP39

]

BRIEFING

L78, *USP 38* page 1904. It is proposed to update the description of this phase to better reflect the HPLC column that originates it.

Additionally, minor editorial changes have been made to update the packing to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C153253

Comment deadline: March 31, 2015**Change to read:**

L78—A silane ligand that consists of both reversed-phase (an alkyl chain longer than C8) and anion-exchange (primary, secondary, tertiary, or quaternary

▲or tertiary ▲USP39

amino groups) functional groups chemically bonded to porous or nonporous silica or ceramic microparticles, 1.0–50 µm in diameter, or a monolithic rod. [Note—Available as Acclaim Mixed-Mode WAX-1 from Thermo Fisher (www.thermofisher.com).]

BRIEFINGL88. It is proposed to add this new column used in the *Limit of Specified Impurities* in the monographs for *Palonosetron Hydrochloride* and *Palonosetron Injection* published elsewhere in this issue of *PF*.

(HDQ: A. Wise.)

Correspondence Number—C125958

Comment deadline: March 31, 2015**Add the following:**

▲L88

—Glycopeptide vancomycin linked through multiple covalent bonds to 100 ▲ spherical silica.

▲USP39

CONTAINERS FOR DISPENSING CAPSULES AND TABLETS**BRIEFING****Container Specifications for Capsules and Tablets, USP 38** page 7209.

(HDQ.)

Correspondence Number—C96679; C113423; C120793; C127211; C131646; C148875

The following table is provided as a reminder for the pharmacist engaged in the typical dispensing situation who already is acquainted with the *Packaging and Storage* requirements set forth in the individual monographs. It lists the capsules and tablets that are official in the *United States Pharmacopeia* and indicates the relevant tight (T), well-closed (W), and light-resistant (LR) specifications applicable to containers in which the drug that is repackaged should be dispensed.

This table is not intended to replace, nor should it be interpreted as replacing, the definitive requirements stated in the individual monographs.

Container Specifications for Capsules and Tablets

Monograph Title	Container Specification
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Add the following:

▲Aripiprazole Orally Disintegrating Tablets

T ▲*USP39***Add the following:**

▲Candesartan Cilexetil Tablets

T, LR ▲*USP39***Add the following:**

▲Candesartan Cilexetil and Hydrochlorothiazide Tablets

T, LR ▲*USP39***Change to read:**Orphenadrine Citrate Extended-Release Tablets ~~T~~▲W, ▲*USP39*

LR

Add the following:

▲Perindopril Erbumine Tablets

T ▲*USP39***BRIEFING****Description and Relative Solubility of USP and NF Articles, USP 38 page 1917.**

(HDQ.)

Correspondence Number—C143602; C151721; C140651

Add the following:

▲**Ethylparaben Sodium:** White or almost-white, hygroscopic, crystalline powder. Freely soluble in water; soluble in anhydrous alcohol; practically insoluble in methylene chloride. *NF category:* Antimicrobial preservative. ▲*USP39*

Change to read:

▲**Magnesium Aluminometasilicate:** White powder or granules with an amorphous structure. Very slightly soluble in acids; ~~and in alkalis;~~

▲▲*USP39*

practically insoluble

▲or insoluble ▲*USP39*in water, ~~and~~▲▲*USP39*

in alcohol,

▲and in alkalis. ▲*USP39***Change to read:**

▲**Magnesium Aluminosilicate:** White powder or granules with an amorphous structure. Very slightly soluble in acids; ~~and in alkalis;~~

▲▲*USP39*

practically insoluble

▲or insoluble▲*USP39*
 in water, and
 ▲▲*USP39*
 in alcohol,
 ▲and in alkalis.▲*USP39*

Add the following:

▲**Palonosetron Hydrochloride:** White to off-white, crystalline powder. Freely soluble in water; soluble in propylene glycol; slightly soluble in ethanol and in isopropyl alcohol. ▲*USP39*

BRIEFING

Excipients, USP and NF Excipients, Listed by Functional Category, USP 38 page 6493. It is proposed to add Ethylparaben Sodium to the *Antimicrobial Preservative* category to complement the proposed new monograph for *Ethylparaben Sodium*, which also appears in this issue of *PF*.

(EXC: G. Holloway.) Correspondence Number—C151720

In the following reference table, the grouping of excipients by functional category is intended to summarize commonly identified purposes that these excipients serve in drug product formulations. The association of a functional category with a particular dosage form in this table is not absolute and does not limit the use of an excipient to a single type of dosage form or delivery system.

Change to read:

Antimicrobial Preservative

▲Ethylparaben Sodium▲*NF34*

BRIEFING

Cholesterol, NF 33 page 6612 and *PF 39(3)* [May–June 2013]. In the *PF 39(3)* proposal, *Identification* tests *A* and *B* were replaced with IR identification and a test for specific rotation, respectively. *Identification* test *C* was also added. An *Assay* and the *Labeling* section were added as well in the *PF 39(3)* proposal, together with the proposed new USP Cholesterol RS. On the basis of further comments and data received, it is proposed to make the following revisions:

1. Revise the *Definition* by adding an upper limit of 102.0% for the cholesterol content and by including antioxidants. Correspondingly, add a *Labeling* requirement to indicate added antioxidants.
2. In the newly added *Assay* procedure based on a GC method of analysis performed with the Agilent DB-1 brand of G2 column, replace the internal standard, pregnenolone acetate, with a different internal standard, pregnenolone isobutyrate. The typical retention times for pregnenolone isobutyrate and cholesterol are 6.2 and 7.6 min, respectively.
3. Add a test for *Limit of Related Sterols and Other Organic Impurities*, which is based

on the same procedure used for the Assay. The typical retention times for pregnenolone isobutyrate, cholesterol, desmosterol, lathosterol, and 24-dehydrolathosterol are 6.2, 7.6, 8.1, 8.3, and 8.8 min, respectively.

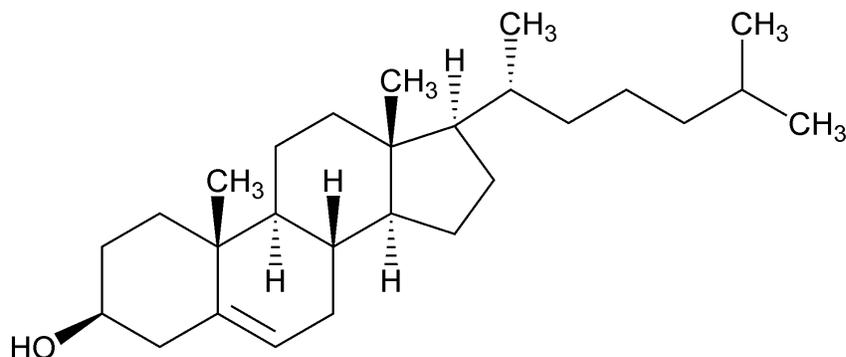
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC: H. Wang.)

Correspondence Number—C136254; C136255

Comment deadline: March 31, 2015

Cholesterol



$C_{27}H_{46}O$ 386.65

Cholest-5-en-3-ol, (3 β)-;

Cholest-5-en-3 β -ol [57-88-5].

DEFINITION

Change to read:

Cholesterol is a steroid alcohol ~~used as an emulsifying agent.~~

▲containing NLT 95.0% and NMT 102.0% of cholest-5-en-3 β -ol ($C_{27}H_{46}O$), calculated on the dried basis. It may contain suitable stabilizers. ▲NF34

IDENTIFICATION

Change to read:

- A.

▲Infrared Absorption < 197A > or < 197K > ▲NF34

~~**Sample solution:** 10 mg in 1 mL of chloroform~~

~~**Analysis:** To the *Sample solution* add 1 mL of sulfuric acid.~~

~~**Acceptance criteria:** The chloroform acquires a blood-red color, and the sulfuric acid shows a green fluorescence.~~

Change to read:

- B.

Sample: ~~5 mg~~

Analysis: ~~Dissolve the Sample in 2 mL of chloroform, add 1 mL of acetic anhydride, and follow with 1 drop of sulfuric acid.~~

Acceptance criteria: ~~A pink color is produced, and it rapidly changes to red, then to blue, and finally to a brilliant green.~~

▲It meets the requirements of the test for *Optical Rotation* 〈 781S 〉, *Specific Rotation*. ▲NF34

Add the following:

▲● C. It meets the requirements of the test for *Melting Range or Temperature* 〈 741 〉. ▲NF34

ASSAY

Add the following:

▲● **Procedure**

Standard solution: 1.0 mg/mL of USP Cholesterol RS and 1.0 mg/mL of ~~pregnenolone~~ acetatepregnenolone isobutyrate (internal standard) in heptane

Sample solution: 1.0 mg/mL of Cholesterol and 1.0 mg/mL of ~~pregnenolone~~ acetatepregnenolone isobutyrate (internal standard) in heptane

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m capillary column bonded with a 0.25-μm layer of phase G2

Temperatures

Detector: 300°

Injection port: 285°

Column: 275°

Carrier gas: Helium

Flow rate: 2.0 mL/min

Injection volume: 1.0 μL

Injection type: Split ratio, 25:1

Liner: Cup splitter liner (4 mm × 6.3 × 78.5) with deactivated wool

System suitability

Sample: *Standard solution*

[Note—The relative retention times for ~~pregnenolone acetate and cholesterol are 1.0 and 1.6, respectively.~~The relative retention times for pregnenolone isobutyrate and cholesterol are 1.0 and 1.2, respectively.]

System suitability requirements

Resolution: ~~NLT 20 between pregnenolone acetate and cholesterol~~NLT 10 between pregnenolone isobutyrate and cholesterol

Relative standard deviation: NMT 2.0% for the peak response ratio of cholesterol to the internal standard

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of cholesterol in the portion of sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U peak response ratio of cholesterol to the internal standard (peak response of cholesterol/peak response of the internal standard) from the *Sample solution*

R_S peak response ratio of cholesterol to the internal standard (peak response of cholesterol/peak response of the internal standard) from the *Standard solution*

C_S concentration of USP Cholesterol RS in the *Standard solution* (mg/mL)

C_U concentration of Cholesterol in the *Sample solution* (mg/mL)

Acceptance criteria: NLT 95.0%–102.0% on the dried basis ▲NF34

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

Add the following:

▲• Limit of Related Sterols and Other Organic Impurities

Internal standard solution: 0.02 mg/mL of pregnenolone isobutyrate (internal standard) in heptane

System suitability solution: 0.02 mg/mL of USP Cholesterol RS, 0.04 mg/mL of desmosterol, and 0.02 mg/mL lathosterol in *Internal standard solution*

Sample solution: 2.0 mg/mL of Cholesterol in *Internal standard solution*

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Sample: *System suitability solution*

[Note—See *Table 1* for the relative retention times.]

Table 1

Name	Relative Retention Time
Pregnenolone isobutyrate (internal standard)	1.00
Cholesterol	1.23
Desmosterol (cholesta-5,24-dien-3 β -ol)	1.31
Lathosterol (5 α -cholest-7-en-3 β -ol)	1.34

System suitability requirements

Resolution: NLT 2.0 between desmosterol and lathosterol

Relative standard deviation: NMT 5.0% for peak response ratio of desmosterol to the internal standard

Analysis

Samples: *System suitability solution* and *Sample solution*

An impurity, 24-dehydrolathosterol (5 α -cholesta-7,24-dien-3 β -ol), may be observed with a relative retention time with reference to pregnenolone isobutyrate (internal standard): 1.42.

Calculate the percentage of desmosterol or lathosterol in the portion of Cholesterol taken:

$$\text{Result} = (R_{U1}/R_{S1}) \times (C_{S1}/C_U) \times 100$$

R_{U1} peak response ratio of desmosterol or lathosterol to the internal standard (peak response of desmosterol or lathosterol/peak response of the internal standard) from the *Sample solution*

R_{S1} peak response ratio of desmosterol or lathosterol to the internal standard (peak response of desmosterol or lathosterol/peak response of the internal standard) from the *System suitability solution*

C_{S1} concentration of desmosterol or lathosterol in the *System suitability solution* (mg/mL)

C_U concentration of Cholesterol in the *Sample solution* (mg/mL)

Calculate the percentage of 24-dehydrolathosterol or any other unspecified organic impurity in the portion of Cholesterol taken:

$$\text{Result} = (R_{U2}/R_{S2}) \times (C_{S2}/C_U) \times 100$$

R_{U2} peak response ratio of 24-dehydrolathosterol or any other unspecified impurity to the internal standard (peak response of 24-dehydrolathosterol or any other unspecified impurity/peak response of the internal standard) from the *Sample solution*

R_{S2} peak response ratio of cholesterol to the internal standard (peak response of cholesterol/peak response of the internal standard) from the *System suitability solution*

C_{S2} concentration of USP Cholesterol RS in the *Standard solution* (mg/mL)

C_U concentration of Cholesterol in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05% for any unspecified impurities and any peaks due to solvent.

Table 2

Name	Acceptance Criteria, NMT (%)
Desmosterol	≤ 3
Lathosterol	≤ 2
Total impurities including related sterols	≤ 5

▲NF34

SPECIFIC TESTS

- **Melting Range or Temperature** 〈 741 〉: 147°–150°

- **Optical Rotation, Specific Rotation** 〈 781S 〉

Sample solution: 20 mg/mL, undried, in dioxane

Acceptance criteria: –34° to –38°

- **Acidity**

Sample: 1.0 g

Analysis: Dissolve the *Sample* in 10 mL of ether in a small flask, add 10.0 mL of 0.10 N sodium hydroxide, and shake for about 1 min. Heat gently to expel the ether, then boil for 5 min. Cool, dilute with 10 mL of water, add phenolphthalein TS, and titrate with 0.10 N sulfuric acid until the pink color just disappears, stirring the solution vigorously throughout

the titration. Perform a blank determination (see *Titrimetry* 〈 541 〉, *Residual Titrations*).

Acceptance criteria: The difference between the number of mL of 0.10 N sulfuric acid consumed in the blank and the number of mL consumed in the *Sample* is NMT 0.3 mL.

- **Loss on Drying** 〈 731 〉

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 0.3%

- **Solubility in Alcohol**

Sample: 500 mg

Analysis: Dissolve the *Sample* in 50 mL of warm alcohol in a stoppered flask or cylinder, and allow to stand at room temperature for 2 h.

Acceptance criteria: No deposit or turbidity is formed.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers.

Add the following:

- ▲● **Labeling:** Label it to indicate whether cholesterol is derived from animal, synthetic, or vegetable sources. For animal-derived sources, indicate the species and tissue used (for example, bovine brain and spinal cord, wool fat, or chicken eggs). Indicate the names and amounts of any added stabilizers. ▲NF34

Add the following:

- ▲● **USP Reference Standards** 〈 11 〉

USP Cholesterol RS

▲NF34

BRIEFING

Ethyl Oleate, *NF 33* page 6656. As part of the USP monograph modernization effort, and on the basis of comments and data received, it is proposed to make the following changes:

1. Update the monograph *Definition* and chemical names.
2. Add an *Identification* section, then under the section add the following tests: *A. Presence of Ester* that is based on the tests for *Saponification Value* and *Acid Value*, and *B. Chromatographic Identity* which is based on GC peak agreement using USP Ethyl Oleate RS in the newly proposed *Assay*.
3. Add a capillary GC *Assay*. The proposed capillary GC procedure in the *Assay* is based on analysis performed with the Agilent CP-Wax 52 CB brand of G16 column. The typical retention times for ethyl palmitate, ethyl stearate, ethyl oleate, and ethyl linoleate are about 6.6, 9.7, 10.1, and 10.9 min, respectively.
4. Add a test for *Total Ash* under *Impurities* section.
5. Delete the test for *Specific Gravity* 〈 841 〉.
6. Delete the test for *Viscosity—Capillary Methods* 〈 911 〉 or *Viscosity—Rotational Methods* 〈 912 〉.
7. As the test for *Acid Value* is moved to the *Identification* section, it is deleted from the *Specific Tests*.
8. As the test for *Saponification Value* is moved to the *Identification* section, it is

deleted from the *Specific Tests*.

9. Delete the test for *Refractive Index* 〈 831 〉.
10. Add a test for *Peroxide Value*.
11. Add a test for *Water Determination*.
12. Add a section for *Labeling* requirements.
13. Add a section of *USP Reference Standards*, and add USP Ethyl Palmitate RS, USP Ethyl Stearate RS, USP Ethyl Oleate RS, and USP Ethyl Linoleate RS to the section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

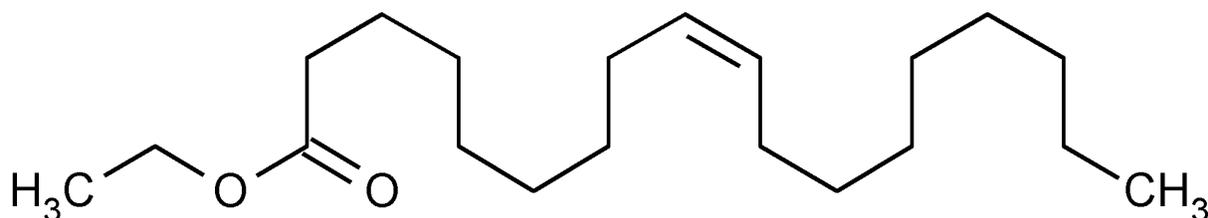
(EXC: H. Wang.)

Correspondence Number—C138301

Comment deadline: March 31, 2015

Ethyl Oleate

Change to read:



$C_{20}H_{38}O_2$ 310.51

9-Octadecenoic acid, (Z)-, ethyl ester;

Ethyl oleate

▲Ethyl (Z)-9-octadecenoate;

Ethyl *cis*-9-octadecenoate

▲*NF34*

[111-62-6].

DEFINITION

Change to read:

Ethyl Oleate consists of esters of ethyl alcohol and high molecular weight fatty acids, principally oleic acid.

▲It contains NLT 65.0% of Ethyl (Z)-9-octadecenoate [$C_{20}H_{38}O_2$]. It may contain suitable stabilizers. ▲*NF34*

IDENTIFICATION

Add the following:

▲● A. Presence of Ester

Analysis 1: Proceed as directed in *Fats and Fixed Oils, Saponification Value* 〈 401 〉.

Acceptance criteria: 177–188

Analysis 2: Proceed as directed in *Fats and Fixed Oils, Acid Value* (401).

Acceptance criteria: NMT 0.5▲NF34

Add the following:

▲● **B. Chromatographic Identity**

Analysis: Proceed as directed in the Assay.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to the ethyl oleate peak of the *System suitability solution*.▲NF34

ASSAY

Add the following:

▲● **Procedure**

System suitability solution: 5 mg/mL of USP Ethyl Oleate RS, 1.2 mg/mL of USP Ethyl Palmitate RS, 1.2 mg/mL of USP Ethyl Linoleate RS, and 0.5 mg/mL of USP Ethyl Stearate RS in *n*-hexane

Sample solution: 5 mg/mL of Ethyl Oleate in *n*-hexane

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 25-m capillary column bonded with a 0.2-μm layer of phase G16

Temperatures

Detector: 270°

Injection port: 250°

Column: See temperature program shown in *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
178	—	178	2
178	3.3	240	2.5

Carrier gas: Hydrogen

Flow rate: 0.7 mL/min

Injection volume: 1.0 μL

Injection type: Split injection; split ratio is 200:1

Run time: 23–24 min

System suitability

Sample: *System suitability solution*

[Note—See *Table 2* for the relative retention times.]

Table 2

Component	Relative Retention Time
Ethyl palmitate	0.65
Ethyl stearate	0.96
Ethyl oleate	1.00
Ethyl linoleate	1.08

System suitability requirements

Resolution: NLT 2.0 between the ethyl stearate and ethyl oleate peaks

Analysis

Samples: *System suitability solution* and *Sample solution*

Identify each ethyl ester (ethyl palmitate, ethyl stearate, ethyl oleate, or ethyl linoleate) peak in the *Sample solution* based on that in the *System suitability solution*.

Calculate the percentage of each ethyl ester (ethyl palmitate, ethyl stearate, ethyl oleate, or ethyl linoleate) in the portion of Ethyl Oleate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of each ethyl ester (ethyl palmitate, ethyl stearate, ethyl oleate, or ethyl linoleate) from the *Sample solution*

r_T sum of all the peak responses excluding peak responses due to solvent from the *Sample solution*

Acceptance criteria

Disregard peaks that are less than 0.05% for any unspecified impurities, and any peaks due to solvent.

Ethyl Oleate exhibits the composition profiles shown in *Table 3* below.

Table 3

Component	Percentage (%)
Ethyl palmitate	≤ 16.0
Ethyl stearate	≤ 6.0
Ethyl oleate	≥ 65.0
Ethyl linoleate	≤ 18.0

▲NF34

IMPURITIES

Add the following:

▲● Total Ash

Sample: 2.0 g

Analysis: Heat a silica or platinum crucible to redness for 30 min, allow to cool in a desiccator, and weigh. Transfer the *Sample* to the crucible. Dry at 100°–105° for 1 h and ignite to constant weight in a muffle furnace at 600 ± 25°, allowing the crucible to cool in a desiccator after each ignition. Flames should not be produced at any time during the procedure. If after prolonged ignition the ash still contains black particles, take up with hot water, pass through an ashless filter paper, and ignite the residue and the filter paper.

Combine the filtrate with the ash, carefully evaporate to dryness, and ignite to constant weight.

Acceptance criteria: NMT 0.1% ▲NF34

SPECIFIC TESTS

Delete the following:

▲ ~~Specific Gravity $\langle 841 \rangle$: 0.866–0.874 at 20°~~ ▲NF34

Delete the following:

▲ ~~Viscosity—Capillary Methods $\langle 911 \rangle$ or Viscosity—Rotational Methods $\langle 912 \rangle$: NLT 5.15 centipoises~~ ▲NF34

Delete the following:

▲ ~~Fats and Fixed Oils, Acid Value $\langle 401 \rangle$: NMT 0.5~~ ▲NF34

● Fats and Fixed Oils, Iodine Value $\langle 401 \rangle$: 75–85

Delete the following:

▲ ~~Fats and Fixed Oils, Saponification Value $\langle 401 \rangle$: 177–188~~ ▲NF34

Delete the following:

▲ ~~Refractive Index $\langle 831 \rangle$: 1.443–1.450~~ ▲NF34

Add the following:

▲ Fats and Fixed Oils, Peroxide Value $\langle 401 \rangle$: NMT 10.0 ▲NF34

Add the following:

▲ Water Determination $\langle 921 \rangle$: NMT 1.0% ▲NF34

ADDITIONAL REQUIREMENTS

● **Packaging and Storage:** Preserve in tight, light-resistant containers.

Add the following:

▲ **Labeling:** Label it to indicate whether oleic acid is derived from vegetable, animal, or synthetic sources. Indicate the names and amounts of any added stabilizers. ▲NF34

Add the following:

▲ **USP Reference Standards $\langle 11 \rangle$**

USP Ethyl Linoleate RS

USP Ethyl Oleate RS

USP Ethyl Palmitate RS

USP Ethyl Stearate RS

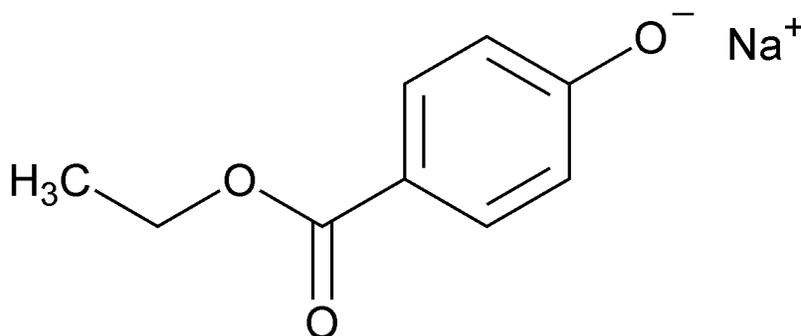
▲NF34

BRIEFING

Ethylparaben Sodium. Because there is no existing *NF* monograph for this excipient, a new monograph is being proposed. This monograph is based on the corresponding monograph for *Ethylparaben Sodium* in the *European Pharmacopoeia 8.0*. The proposed liquid chromatographic method in the *Assay* is based on analyses performed with the YMC-Pack Pro C18 brand of L1 column. The *p*-hydroxybenzoic acid, methylparaben, and ethylparaben peaks elute at approximately 1.5, 2.2, and 2.9 min, respectively. Interested parties are encouraged to comment on the proposal.

(EXC: G. Holloway.)

Correspondence Number—C140969

Comment deadline: March 31, 2015**Add the following:****▲Ethylparaben Sodium**C₉H₉NaO₃ 188.2

Benzoic acid, 4-hydroxy-, methyl ester, sodium salt;
Ethyl *p*-hydroxybenzoate, sodium salt;
Sodium 4-ethoxycarbonylphenolate [35285-68-8].

DEFINITION

Ethylparaben Sodium contains NLT 95.0% and NMT 102.0% of ethylparaben sodium (C₉H₉NaO₃), calculated on the anhydrous basis.

IDENTIFICATION• **A.****Standard:** 0.5 g of USP Ethylparaben RS**Sample:** 0.5 g of Ethylparaben Sodium

Analysis: Dissolve the *Sample* in 5 mL of water, acidify with hydrochloric acid, and filter the resulting precipitate. Wash the precipitate with water, and dry under vacuum at 80° for 2 h.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the *Sample*

exhibits maxima only at the same wavelengths as those of a similar preparation of the *Standard*.

- **B.**

Sample solution: Ignite 0.3 g of Ethylparaben Sodium, cool, and dissolve the residue in about 3 mL of 3 N hydrochloric acid.

Acceptance criteria: A platinum wire dipped in the *Sample solution* imparts an intense, persistent yellow color to a nonluminous flame.

ASSAY

- **Procedure**

Mobile phase: Methanol and a 6.8-g/L solution of potassium dihydrogen phosphate (65:35)

System suitability solution: 5.0 µg/mL each of *p*-hydroxybenzoic acid, USP

Methylparaben RS, and USP Ethylparaben RS in *Mobile phase*

Standard solution: Dissolve 50.0 mg of USP Ethylparaben RS in 2.5 mL of methanol, and dilute with *Mobile phase* to 50.0 mL. Dilute 10.0 mL of this solution with *Mobile phase* to 100.0 mL.

Sample solution: Dissolve 50.0 mg of Ethylparaben Sodium in 2.5 mL of methanol, and dilute with *Mobile phase* to 50.0 mL. Dilute 10.0 mL of this solution with *Mobile phase* to 100.0 mL.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 272 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.3 mL/min

Injection volume: 10 µL

Run time: About 4 times the retention time of the ethylparaben peak

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The retention time of ethylparaben is about 2.9 min; the relative retention times for *p*-hydroxybenzoic acid, methylparaben, and ethylparaben are about 0.5, 0.8, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between methylparaben and ethylparaben peaks, *System suitability solution*

Relative standard deviation: NMT 0.85% for six injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ethylparaben sodium in the portion of Ethylparaben Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times P$$

r_U = peak area of ethylparaben from the *Sample solution*

r_S = peak area of ethylparaben from the *Standard solution*

C_S = concentration of USP Ethylparaben RS in the *Standard solution*

C_U = concentration of Ethylparaben Sodium in the *Sample solution*

$M_{i\bar{r}}$ molecular weight of ethylparaben sodium, 188.2

$M_{i\bar{z}}$ molecular weight of ethylparaben, 166.17

P = labeled purity of USP Ethylparaben RS expressed as a percentage

Acceptance criteria: 95.0%–102.0% on the anhydrous basis

IMPURITIES

• Related Compounds

Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: Dilute 1.0 mL of the *Sample solution* with *Mobile phase* to 20.0 mL. Dilute 1.0 mL of this solution with *Mobile phase* to 10.0 mL.

System suitability

Sample: *System suitability solution*

[Note— The retention time of ethylparaben is about 2.9 min; the relative retention times for *p*-hydroxybenzoic acid, methylparaben, and ethylparaben are about 0.5, 0.8, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between methylparaben and ethylparaben peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria

***p*-Hydroxybenzoic acid:** The peak area in the *Sample solution*, multiplied by 1.4 to correct for the calculation of content, is NMT 6 times the area of the principal peak in the *Standard solution*; NMT 3.0%.

Unspecified impurities: The peak area of each impurity in the *Sample solution* is NMT the area of the principal peak in the *Standard solution*; NMT 0.5%.

Total impurities: The total peak area for all unspecified impurities in the *Sample solution* is NMT twice the area of the principal peak in the *Standard solution*; NMT 1.0%.

• Chloride and Sulfate, Chloride 〈 221 〉

Sample: 0.2 g

Control solution: 0.10 mL of 0.020 N hydrochloric acid

Acceptance criteria: 0.035%; the *Sample* shows no more chloride than the *Control solution*

• Chloride and Sulfate, Sulfate 〈 221 〉

Sample: 1.0 g

Control solution: 0.31 mL of 0.020 N sulfuric acid

Acceptance criteria: 0.030%; the *Sample* shows no more sulfate than the *Control solution*

SPECIFIC TESTS

• pH 〈 791 〉

Sample solution: 1 mg/mL

Acceptance criteria: 9.5–10.5

• Water Determination, Method I 〈 921 〉: NMT 5.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** { 11 }
 - USP Ethylparaben RS
 - USP Methylparaben RS

▲NF34

BRIEFING

Sesame Oil, *NF 33* page 6858. Based on a request to accommodate certain grades used in injectable dosage forms that are not currently included in the monograph, it is proposed to make the following changes:

1. Revise the specification of *Specific Gravity* by decreasing the lower limit from 0.916 to 0.912.
2. In the *Labeling* section, add a statement to accommodate a special grade that is used in injectable dosage forms. The specific grade must meet the *Other Requirements* that will be added under the section of *Additional Requirements*.
3. Add the subsection for *Other Requirements* that are applied to the special grade of sesame oil, which is used in injectable dosage forms.

The comment period for the above revision ends March 31, 2015. In the absence of significant adverse comments, it is proposed to implement this revision via an *Interim Revision Announcement* to *USP 38-NF33*, with an official date of July 1, 2015.

Interested parties are encouraged to submit comments to Hong Wang, Ph.D., senior scientific liaison to the Excipient Expert Committees (301-816-8351 or hw@usp.org)

As part of the USP monograph modernization effort, the following revisions are proposed:

1. Add a specification for *Alkaline Impurities*.
2. Add a specification for *Peroxide Value*.
3. Add the specification for *Water Determination*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC: H. Wang.)

Correspondence Number—C149850; C152089

Comment deadline: March 31, 2015

Sesame Oil

DEFINITION

Sesame Oil is the refined fixed oil obtained from the seed of one or more cultivated varieties of *Sesamum indicum* L. (Fam. Pedaliaceae). It may contain suitable antioxidants.

IDENTIFICATION

- **A. Identity by Triglyceride Profile**

Analysis: Proceed as directed in the test for *Triglyceride Composition*

Acceptance criteria: The peak responses for the eight major triglycerides—LLL, OLL, PLL,

OOL, POL, OOO, SOL, and POO—elute between 0 and about 40 min, in the order specified, and at relative retention times of about 0.55, 0.65, 0.69, 0.77, 0.82, 0.93, 0.97, and 1.0, respectively, as obtained in the chromatogram of the *Sample solution* in the test for *Triglyceride Composition*.

ASSAY

• Triglyceride Composition

[Note—The fatty acid radicals are designated as linoleic (L), oleic (O), palmitic (P), and stearic (S), and the common abbreviations for triglycerides used are as follows: trilinolein (LLL), 1,2-dilinoleoyl-3-oleoyl-rac-glycerol (OLL), 1,2-dilinoleoyl-3-palmitoyl-rac-glycerol (PLL), 1,2-dioleoyl-3-linoleoyl-rac-glycerol (OOL), 1-palmitoyl-2-oleoyl-3-linoleoyl-rac-glycerol (POL), triolein (OOO), 1-linoleoyl-2-oleoyl-3-stearoyl-rac-glycerol (SOL), and 1,2-dioleoyl-3-palmitoyl-rac-glycerol (POO).]

Mobile phase: Acetonitrile and methylene chloride (60:40)

System suitability solution: 3.0 mg/mL of USP Sesame Oil Related Compound A RS and USP Sesame Oil Related Compound B RS in *Mobile phase*. [Note—USP Sesame Oil Related Compound A RS is OLL, and USP Sesame Oil Related Compound B RS is PLL.]

Sample solution: 20 mg/mL of Sesame Oil in *Mobile phase*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Refractive index

Columns: Two 4.6-mm × 25-cm in series; packings L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for OLL and PLL are about 0.93 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.8 between OLL and PLL

Relative standard deviation: NMT 1.5% determined from peak areas; NMT 2.2% determined from the peak area ratio of OLL to PLL

Analysis

[Note—The relative retention times for the eight major triglyceride peaks are listed in *Table 1*.]

Sample: *Sample solution*

Calculate the percentage of each of these triglycerides in the portion of sample taken:

$$\text{Result} = (A/B) \times 100$$

A = peak area for each individual triglyceride

B = sum of the areas of all the peaks, excluding the solvent peak

Table 1

Triglyceride	Relative Retention Time	Composition (%)

LLL	0.55	7.0–19.0
OLL	0.65	13.0–30.0
PLL	0.69	5.0–9.0
OOL	0.77	14.0–25.0
POL	0.82	8.0–16.0
OOO	0.93	5.0–14.0
SOL	0.97	2.0–8.0
POO	1.0	2.0–8.0

IMPURITIES

Delete the following:

-

• Heavy Metals, Method II 〈 231 〉

: NMT 10 µg/g (Official 1-Dec-2015)

Add the following:

▲• Alkaline Impurities

Sample: 10 mL of Sesame Oil

Analysis: Mix 10 mL of freshly opened acetone and 0.3 mL of water, and add 0.05 mL of bromophenol blue TS. Add the *Sample*, shake, and allow to stand. Titrate with 0.01 N hydrochloric acid VS to change the color of the upper layer to yellow.

Acceptance criteria: NMT 0.1 mL of 0.01 N hydrochloric acid is required. ▲NF34

SPECIFIC TESTS

Change to read:

• Specific Gravity 〈 841 〉 : 0.916

• 0.912 (IRA 1-Jul-2015)

-0.921

• Fats and Fixed Oils, Acid Value (Free Fatty Acids) 〈 401 〉

Sample: 10 g

Acceptance criteria: NMT 2.0 mL of 0.020 N sodium hydroxide is required for neutralization.

• Fats and Fixed Oils, Iodine Value 〈 401 〉 : 103–116

• Fats and Fixed Oils, Saponification Value 〈 401 〉 : 188–195

• Fats and Fixed Oils, Solidification Temperature of Fatty Acids 〈 401 〉 : 20°–25°

Add the following:

▲• Fats and Fixed Oils, Peroxide Value 〈 401 〉 : NMT 10.0 ▲NF34

• Fats and Fixed Oils, Unsaponifiable Matter 〈 401 〉 : NMT 1.5%

• Cottonseed Oil

Sample: 5 mL

Analysis: Mix the *Sample* in a test tube with 5 mL of a mixture of equal volumes of amyl alcohol and a 10-mg/mL solution of sulfur in carbon disulfide. Warm the mixture carefully until the carbon disulfide is expelled, and immerse the tube to one-third of its depth in a boiling saturated solution of sodium chloride.

Acceptance criteria: No reddish color develops within 15 min.

Add the following:

▲● **Water Determination, Method Ic** 〈 921 〉: NMT 0.1% ▲*NF34*

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and prevent exposure to excessive heat.

Change to read:

- **Labeling:** Label it to indicate the name and quantity of any added antioxidant.
- Where Sesame Oil is intended for use in the manufacture of injectable dosage forms, it is so labeled. ●(IRA 1-Jul-2015)

Add the following:

- **Other Requirements:** For Sesame Oil intended for use in injectable dosage forms, which is specified in the labeling, the requirements must be met for *Unsaponifiable Matter, Acid Value, Peroxide Value, and Water, Method Ic*, under *Specific Tests* in the chapter *Injections and Implanted Drug Products* 〈 1 〉, *Vehicles and Added Substances, Nonaqueous Vehicles*. ●(IRA 1-Jul-2015)

- **USP Reference Standards** 〈 11 〉

USP Sesame Oil Related Compound A RS
USP Sesame Oil Related Compound B RS

BRIEFING

Sodium Starch Glycolate, NF 33 page 6875. The *Identification* by infrared absorption is a non-harmonized attribute; therefore, the note "Disregard any peaks at about 845, 1285, and 1305 cm^{-1} , which are attributed to the presence of citrate" is being added through the regular revision process for *USP-NF*, instead of the revision process for the Pharmacopeial Discussion Group (PDG).

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC: J. Liu.)

Correspondence Number—C149843

Comment deadline: March 31, 2015

Sodium Starch Glycolate

Portions of this monograph that are national *USP-NF* text, and are not part of the harmonized

text, are marked with symbols (◆◆) to specify this fact.

Starch carboxymethyl ether, sodium salt.

DEFINITION

Sodium Starch Glycolate is the sodium salt of a carboxymethyl ether of starch or of a cross-linked carboxymethyl ether of starch. It may contain NMT 7.0% of Sodium Chloride. The pH and assay requirements for Type A and Type B are set forth in the accompanying table.

Type	pH		% Sodium, Combined as Sodium Starch Glycolate	
	Min.	Max.	Min.	Max.
A	5.5	7.5	2.8	4.2
B	3.0	5.0	2.0	3.4

IDENTIFICATION

Change to read:

- ◆◆ **A. Infrared Absorption** (197K)

▲[Note—Disregard any peaks at about 845, 1285, and 1305 cm^{-1} , which are attributed to the presence of citrate.]▲*NF34*

◆

- ◆ **B.** An acidified solution of it is colored blue to violet by the addition of iodine and potassium iodide TS 1.

- ◆ **C.**

Potassium pyroantimonate solution: Dissolve 2 g of potassium pyroantimonate in 85 mL of hot water. Cool quickly, and add 10 mL of a solution of potassium hydroxide (3 in 20). Allow to stand for 24 h, filter, and dilute with water to 100 mL.

Analysis: To a 2-mL portion of the *Sample solution* prepared for the test for *Limit of Iron*, add 2 mL of 15% potassium carbonate, and heat to boiling. No precipitate is formed. Add 4 mL of *Potassium pyroantimonate solution*, and heat to boiling. Allow to cool in ice water and, if necessary, rub the inside of the test tube with a glass rod.

Acceptance criteria: A dense precipitate is formed.

- ◆◆ **D.** Sodium Starch Glycolate imparts an intense yellow color to a nonluminous flame.◆◆

ASSAY

- ◆ **Procedure**

Sample: 1 g

Analysis: Transfer the *Sample* to a conical flask, add 20 mL of 80% alcohol, stir for 10 min, and filter. Repeat the extraction until the chloride has been completely extracted, as shown by a test with silver nitrate. Dry the insoluble portion at 105° to constant weight, and transfer an accurately weighed portion (700 mg) of the dried 80% alcohol-insoluble portion to a suitable flask. Add 80 mL of glacial acetic acid, and heat the mixture under reflux on a boiling water bath for 2 h. Cool to room temperature, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically.

Calculate the percentage of sodium combined in the form of sodium starch glycolate:

$$\text{Result} = 100 \times 22.99 \times V \times (N/W)$$

V = volume of perchloric acid consumed (mL)

N = normality of the perchloric acid

W = weight of the dried alcohol-insoluble residue taken for the Assay (mg)

Acceptance criteria: 2.8%–4.2% for Type A; 2.0%–3.4% for Type B

OTHER COMPONENTS

• Limit of Sodium Chloride

Sample: 500 mg of Sodium Starch Glycolate

Titrimetric system

(See *Titrimetry* 〈 541 〉.)

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Potentiometric

Electrodes

Indicator: Suitable silver-based

Reference: Double junction electrode containing a 10% potassium nitrate filling solution in the outer jacket, and a standard filling solution in the inner jacket

Analysis: Transfer the *Sample* to a beaker, and suspend in 100 mL of water. Add 1 mL of nitric acid. Titrate with the *Titrant*. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride.

Acceptance criteria: NMT 7.0%

• Limit of Sodium Glycolate

[Note—Conduct this test without exposure to daylight. Use low-actinic glassware.]

Solution A: 0.1 mg/mL of 2,7-dihydroxynaphthalene in sulfuric acid; allow to stand until decolorized, and use within 2 days.

Standard solution: Transfer 310 mg of glycolic acid, previously dried over phosphorus pentoxide in a desiccator at room temperature overnight, to a 500-mL volumetric flask. Dissolve in and dilute with water to volume. Transfer 5.0 mL of this solution to a 100-mL beaker, add 4 mL of 6 N acetic acid, and allow to stand for about 30 min. Add 50 mL of acetone and 1 g of sodium chloride, mix, and pass through fast filter paper moistened with acetone into a 100-mL volumetric flask. Rinse the beaker and the filter paper with acetone. Combine the filtrate and washings, dilute with acetone to volume, and mix. Allow to stand for 24 h without shaking. Use the clear supernatant as the *Standard solution*.

Sample solution: Transfer 200 mg to a 100-mL beaker. Add 4 mL of 6 N acetic acid and 5 mL of water. Stir until dissolution is complete (about 10 min). Add 50 mL of acetone and 1 g of sodium chloride, mix, and pass through fast filter paper moistened with acetone into a 100-mL volumetric flask. Rinse the beaker and filter paper with acetone. Combine the filtrate and washings, dilute with acetone to volume, and mix. Allow to stand for 24 h without shaking. Use the clear supernatant as the *Sample solution*.

Analysis: Treat the *Sample solution* and the *Standard solution* as follows. Heat 2.0 mL of the solution on a water bath for 20 min to remove the acetone. Cool to room temperature. Add 20.0 mL of *Solution A* to the solution under test, mix, and heat on a water bath for 20 min. Cool under running water, and quantitatively transfer to a 25-mL volumetric flask.

Maintain the flask under running water, and dilute with sulfuric acid to volume. Within 10 min, determine the absorbance of the solution at 540 nm with a suitable spectrophotometer, using water as the blank.

Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (2.0%).

IMPURITIES

Delete the following:

- ✦

- **Heavy Metals, Method II** 〈 231 〉

: 20 ppm ✦ • (Official 1-Dec-2015)

- **Limit of Iron**

Standard solution: Dissolve 863.4 mg of ferric ammonium sulfate [$\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$] in water, add 25 mL of 2 N sulfuric acid, dilute with water to 500.0 mL, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 5 mL of this solution into a 100-mL volumetric flask, dilute with water to volume, and mix. This solution contains the equivalent of 1.0 $\mu\text{g}/\text{mL}$ of iron.

Sample solution: Place 2.5 g in a silica or platinum crucible, and add 2 mL of 10 N sulfuric acid. Heat on a water bath, then cautiously raise the temperature progressively over an open flame. Ignite, preferably in a muffle furnace, at $600 \pm 25^\circ$. Continue heating until all black particles have disappeared. Cool, add a few drops of 2 N sulfuric acid, and heat and ignite as above. Add a few drops of 2 M ammonium carbonate, evaporate to dryness, and ignite as above. Cool, dissolve the residue in 50 mL of water, and mix.

[Note—Reserve a portion of this solution for *Identification* test C.]

Analysis: Treat the *Sample solution* and the *Standard solution* as follows. Transfer 10 mL of the solution to a suitable beaker, add 2 mL of citric acid solution (1 in 5) and 0.1 mL of thioglycolic acid, and mix. Render the solution alkaline, using litmus paper as an external indicator, by the addition of ammonium hydroxide. Dilute with water to 20 mL, and mix. Allow the solutions to stand for 5 min.

Acceptance criteria: The color of the solution from the *Sample solution* is a shade of pink no deeper than that of the solution from the *Standard solution* (0.002%).

SPECIFIC TESTS

- **Microbial Enumeration Tests** 〈 61 〉 and **Tests for Specified Microorganisms** 〈 62 〉: It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.
- **pH** 〈 791 〉: Disperse 1 g in 30 mL of water. The pH of the resulting suspension is either 5.5–7.5 for Type A or 3.0–5.0 for Type B.
- **Loss on Drying** 〈 731 〉
 - Analysis:** Dry at 130° for 90 min.
 - Acceptance criteria:** NMT 10.0%

ADDITIONAL REQUIREMENTS

- ◆ **Packaging and Storage:** Preserve in well-closed containers, preferably protected from wide variations in temperature and humidity, which may cause caking.◆
- ◆ **Labeling:** Label it to indicate the botanical source of the starch from which it was derived, the cross-linking agent (if used), the pH range, and whether it is Type A or Type B.◆
- **USP Reference Standards** 〈 11 〉
 - USP Sodium Starch Glycolate Type A RS
 - USP Sodium Starch Glycolate Type B RS

BRIEFING

Amoxapine, *USP 38* page 2217. On the basis of comments and supporting data received, it is proposed to modernize the monograph as follows:

1. Revise the limits in the *Definition* and *Assay* from "NLT 98.5% and NMT 101.0%" to "NLT 98.0% and NMT 102.0%," which is typical for liquid chromatographic procedures.
2. Replace the existing liquid chromatographic procedure used in the *Assay* with a more specific liquid chromatographic procedure. USP has received comments indicating that the existing procedure does not separate amoxapine and amoxapine related compound G. The isocratic liquid chromatographic procedure in this proposal is based on analyses performed with the Waters XBridge brand of L1 column. The typical retention time for amoxapine is about 6 min.
3. Replace the nonspecific TLC test in *Organic Impurities* with a specific gradient liquid chromatographic procedure. The isocratic liquid chromatographic procedure proposed in *PF 37(5)* [Sept.–Oct. 2011] was reported to not be specific for amoxapine related compound G. The procedure currently proposed is based on validated analyses performed with the Ascentis Express C18 brand of L1 column with a dwell volume of 0.30 mL. The typical retention time for amoxapine is about 4 min.
4. Update the *USP Reference Standards* section to support the proposed *Assay* and *Organic Impurities* procedures.

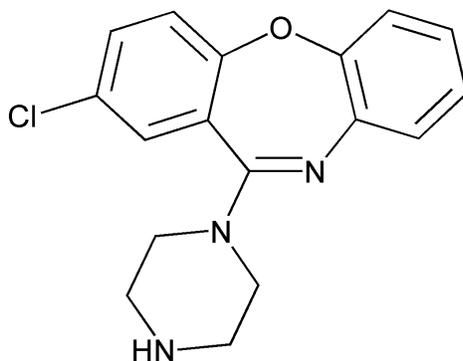
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: H. Joyce.)

Correspondence Number—C112218

Comment deadline: March 31, 2015

Amoxapine



$C_{17}H_{16}ClN_3O$ 313.78

Dibenz[*b,f*][1,4]oxazepine, 2-chloro-11-(1-piperazinyl)-;
2-Chloro-11-(1-piperazinyl)dibenz[*b,f*][1,4]oxazepine [14028-44-5].

DEFINITION

Change to read:

Amoxapine contains ~~NLT 98.5% and NMT 101.0%~~

▲NLT 98.0% and NMT 102.0%▲*USP39*

of amoxapine ($C_{17}H_{16}ClN_3O$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

Change to read:

- **Procedure**

Solution A: ~~11.3 g of tetramethylammonium chloride in 100 mL of water~~

Solution B: ~~Phosphoric acid (1 in 5)~~

Buffer: ~~1.4 g/L of monobasic sodium phosphate~~

Mobile phase: ~~Dilute a mixture of 360 mL of acetonitrile, 20 mL of *Solution A*, and 2 mL of *Solution B* with *Buffer* to 1 L.~~

System suitability solution: ~~0.1 mg/mL of USP Amoxapine RS and 0.1 mg/mL of USP Loxapine Succinate RS in *Mobile phase*. Sonication may be used to aid in dissolution.~~

Standard solution: ~~0.1 mg/mL of USP Amoxapine RS in *Mobile phase*. Sonication may be used to aid in dissolution.~~

Sample solution: ~~0.1 mg/mL of Amoxapine in *Mobile phase*. Sonication may be used to aid in dissolution.~~

Chromatographic system

~~(See *Chromatography* (621), *System Suitability*.)~~

Mode: LC

~~**Detector:** UV 254 nm~~~~**Column:** 4.6 mm × 15 cm; 5-µm packing L1~~~~**Flow rate:** 1.0 mL/min~~~~**Injection volume:** 10 µL~~~~**System suitability**~~~~**Samples:** *System suitability solution* and *Standard solution*~~~~**Suitability requirements**~~~~[Note—The relative retention times for amoxapine and loxapine are 1.0 and 1.2, respectively.]~~~~**Resolution:** NLT 2.5 between amoxapine and loxapine, *System suitability solution*~~~~**Tailing factor:** NMT 1.8, *Standard solution*~~~~**Relative standard deviation:** NMT 0.73%, *Standard solution*~~~~**Analysis**~~~~**Samples:** *Standard solution* and *Sample solution*~~Calculate the percentage of amoxapine ($C_{17}H_{16}ClN_3O$) in the portion of Amoxapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 ~~r_U = peak response of amoxapine from the *Sample solution*~~ ~~r_S = peak response of Amoxapine from the *Standard solution*~~ ~~C_S = concentration of USP Amoxapine RS in the *Standard solution* (mg/mL)~~ ~~C_U = concentration of Amoxapine in the *Sample solution* (mg/mL)~~~~**Acceptance criteria:** 98.5%–101.0% on the dried basis~~~~**▲Buffer:** 3.9 g/L of ammonium acetate in water adjusted with acetic acid or diluted ammonia solution to a pH of 7.3~~~~**Mobile phase:** Acetonitrile and *Buffer* (30:70)~~~~**Diluent:** Acetonitrile and *Buffer* (70:30)~~~~**System suitability solution:** 0.1 mg/mL each of USP Amoxapine RS and USP Amoxapine Related Compound G RS in *Diluent*~~~~**Standard solution:** 0.1 mg/mL of USP Amoxapine RS in *Diluent*~~~~**Sample solution:** 0.1 mg/mL of Amoxapine in *Diluent*~~~~**Chromatographic system**~~~~(See *Chromatography* 〈 621 〉, *System Suitability*.)~~~~**Mode:** LC~~~~**Detector:** UV 254 nm~~~~**Column:** 4.6-mm × 7.5-cm; 2.5-µm or 2.7-µm packing L1~~~~**Column temperature:** 35°~~~~**Flow rate:** 1.2 mL/min~~~~**Injection volume:** 10 µL~~~~**System suitability**~~~~**Samples:** *System suitability solution* and *Standard solution*~~~~[Note—The relative retention times for amoxapine and amoxapine related compound G are 1.0 and 1.3, respectively.]~~~~**Suitability requirements**~~~~**Resolution:** NLT 1.5 between amoxapine and amoxapine related compound G,~~

System suitability solution

Tailing factor: 0.8–1.8, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of amoxapine ($C_{17}H_{16}ClN_3O$) in the portion of Amoxapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of amoxapine from the *Sample solution*

r_S

= peak response of amoxapine from the *Standard solution*

C_S

= concentration of USP Amoxapine RS in the *Standard solution* (mg/mL)

C_U

= concentration of Amoxapine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ▲*USP39*

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

Change to read:

- **Organic Impurities**

Standard solution A: 0.5 mg/mL of USP Amoxapine RS in chloroform

Standard solution B: 0.25 mg/mL of USP Amoxapine RS in chloroform, from *Standard solution A*

Sample solution: 50 mg/mL of Amoxapine in chloroform

Developing solvent system: Chloroform, methanol, and ammonium hydroxide (18: 2: 0.1)

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.2-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, examine it under short-wavelength UV light, and compare the intensities of any

~~secondary spots observed in the chromatogram of the *Sample solution* with those of the principal spots in the chromatogram of the *Standard solutions*.~~

~~**Acceptance criteria:** No secondary spot from the chromatogram of the *Sample solution* is larger or more intense than the principal spot of *Standard solution B* (0.5%), and the sum of the intensities of the secondary spots of the *Sample solution* corresponds to NMT 1.0%.~~

▲Solution A: 3.9 g/L of ammonium acetate in water adjusted with acetic acid or diluted ammonia solution to a pH of 7.3

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
5	70	30
7.5	60	40
15	60	40
20	20	80
25	20	80
30	70	30
35	70	30

Diluent: *Solution A* and *Solution B* (30:70)

System suitability solution: 1 mg/mL of USP Amoxapine RS and 1.5 µg/mL of USP Amoxapine Related Compound G RS in *Diluent*

Standard solution: 1 µg/mL of USP Amoxapine RS, and 1.5 µg/mL each of USP Amoxapine Related Compound G RS and USP Amoxapine Related Compound D RS in *Diluent*

Sample solution: 1000 µg/mL of Amoxapine in *Diluent*

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Peak-to-valley ratio: NLT 3 between amoxapine and amoxapine related compound G, *System suitability solution*

Relative standard deviation: NMT 5.0% each for amoxapine, amoxapine related compound G, and amoxapine related compound D, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of amoxapine related compound G and amoxapine related compound D in the portion of Amoxapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of amoxapine related compound G or amoxapine related compound D from

the *Sample solution*

r_s

= peak response of amoxapine related compound G or amoxapine related compound D from the *Standard solution*

C_s

= concentration of USP Amoxapine Related Compound G RS or USP Amoxapine Related Compound D RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= concentration of Amoxapine in the *Sample solution* ($\mu\text{g/mL}$)

Calculate the percentage of any other impurity in the portion of Amoxapine taken:

$$\text{Result} = (r_U/r_s) \times (C_s/C_U) \times 100$$

r_U

= peak response of any other impurity from the *Sample solution*

r_s

= peak response of amoxapine from the *Standard solution*

C_s

= concentration of USP Amoxapine RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= concentration of Amoxapine in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 2*. Disregard peaks that are less than 0.02% of the amoxapine peak.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorophenoxyaniline urea analog ^a	0.57	0.10
Amoxapine	1.0	—
Amoxapine related compound G	1.4	0.15
Amoxapine related compound D	1.7	0.15
Chlorophenoxyaniline ^b	2.9	0.10
Chlorophenoxyaniline carbamate ^c	3.8	0.10
<i>N</i> -Carbamoyl amoxapine ^d	4.3	0.10

Amoxapine dimer ^e	5.0	0.10
Any individual unspecified impurity	—	0.10
Total impurities	—	0.50
a <i>N</i> -[2-(4-Chlorophenoxy)phenyl]piperazine-1-carboxamide.		

b 2-(4-Chlorophenoxy)aniline.

c Ethyl [2-(4-Chlorophenoxy)phenyl]carbamate.

d 4-(2-Chlorodibenzo[*b,f*][1,4]oxazepin-11-yl)-*N*-[2-(4-chlorophenoxy)phenyl]piperazine-1-carboxamide.

e 1,4-Bis(2-chlorodibenzo[*b,f*][1,4]oxazepine-11-yl)piperazine.

▲*USP39*

SPECIFIC TESTS

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Amoxapine RS

~~USP Loxapine Succinate RS~~

▲USP Amoxapine Related Compound D RS

2-Chlorodibenzo[*b,f*]-[1,4]-oxazepin-11-one.

C₁₃H₈ClNO₂ 245.66

USP Amoxapine Related Compound G RS

3-Chloro-11-(piperazin-1-yl)dibenzo[*b,f*][1,4]oxazepine.

C₁₇H₁₆ClN₃O 313.78▲*USP39*

BRIEFING

Aripiprazole Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph based on validated methods is being proposed.

1. The liquid chromatographic procedures in the *Assay* and in the *Dissolution* test are based on analyses performed with the YMC ODS-A303 brand of L1 column. The typical retention times for aripiprazole in the *Assay* and the *Dissolution, Analytical Procedure 1* test are about 10 and 5.6 min, respectively. The YMC ODS-AM column may also be suitable.
2. The liquid chromatographic procedure in the test for *Organic Impurities* is based on

analyses performed with the Develosil ODS-HG brand of L1 column. The typical retention time for aripiprazole is about 21.5 min. A YMC ODS-A column may also be suitable.

(SM4: H. Joyce.)

Correspondence Number—C133425

Comment deadline: March 31, 2015

Add the following:

▲Aripiprazole Tablets

DEFINITION

Aripiprazole Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$).

IDENTIFICATION

• **A. Infrared Absorption** 〈 197K 〉

Standard: Add 30 mL of ethyl acetate to 30 mg of USP Aripiprazole RS. Shake for 10 min, centrifuge for NLT 5 min, and pass the supernatant through a suitable membrane filter. To the filtrate, add 15 mL of water, shake for 5 min, and centrifuge for NLT 10 min. Transfer 20 mL of the upper layer to a container and add anhydrous magnesium sulfate, as needed. Shake well, pass through a suitable membrane filter, and evaporate the ethyl acetate on a water bath under reduced pressure. Use the residue. [Note—A centrifuge speed of 2000 rpm may be suitable.]

Sample: Grind a suitable number of Tablets and transfer a suitable portion of the ground Tablets, equivalent to 30 mg of aripiprazole, to an appropriate container. Add 30 mL of ethyl acetate, shake for 10 min, centrifuge for NLT 5 min, and pass the supernatant through a suitable membrane filter. To the filtrate, add 15 mL of water, shake for 5 min, and centrifuge for NLT 10 min. Transfer 20 mL of the upper layer to a container and add a suitable amount of anhydrous magnesium sulfate. Shake well, pass through a suitable membrane filter, and evaporate the ethyl acetate on a water bath under reduced pressure. Use the residue. [Note—A centrifuge speed of 2000 rpm may be suitable.]

Analysis

Samples: *Standard and Sample*

Acceptance criteria: Meet the requirements

- **B.** The retention time of the aripiprazole peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Solution A: 2.84 g/L of sodium sulfate in water

Mobile phase: Acetonitrile, methanol, *Solution A*, and glacial acetic acid (33:11:56:1)

Internal standard solution: 0.33 mg/mL of USP Propylparaben RS in *Mobile phase*

Standard stock solution: 1 mg/mL of USP Aripiprazole RS in *Mobile phase*

Standard solution: 0.2 mg/mL of USP Aripiprazole RS prepared as follows. Transfer 10.0 mL of *Standard stock solution* and 10.0 mL of *Internal standard solution* to a 50-mL

volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.2 mg/mL of aripiprazole from Tablets prepared as follows.

Powder NLT 20 Tablets and transfer a suitable portion of the powder to an appropriate volumetric flask. Add 40% of the final flask volume of *Mobile phase* and 20% of the final flask volume of *Internal standard solution*. Shake for 10 min, and dilute with *Mobile phase* to volume. Centrifuge, if necessary, and pass the supernatant through a suitable filter with a pore size of NMT 0.5 μm , discard the first 1 mL of filtrate, and use the subsequent filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

Run time: NLT 2 times the retention time of aripiprazole

System suitability

Sample: *Standard solution*

[Note—The relative retention times for aripiprazole and propylparaben are about 1.0 and 1.5, respectively.]

Suitability requirements

Resolution: NLT 8 between aripiprazole and propylparaben

Tailing factor: NMT 1.7 for aripiprazole and for propylparaben

Relative standard deviation: NMT 2.0% for the peak response ratio of aripiprazole to propylparaben

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aripiprazole ($\text{C}_{23}\text{H}_{27}\text{Cl}_2\text{N}_3\text{O}_2$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{U}}$ peak response ratio of aripiprazole to propylparaben from the *Sample solution*

$R_{\bar{S}}$ peak response ratio of aripiprazole to propylparaben from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)

$C_{\bar{U}}$ nominal concentration of aripiprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• Dissolution { 711 }

Medium: pH 1.2 hydrochloric acid buffer (Transfer 250 mL of 14.9 g/L of potassium chloride in water to a 1-L volumetric flask, add 425 mL of 0.2 N hydrochloride acid solution, and dilute with water to volume. Degas the resulting solution or pass the resulting solution through a filter under vacuum.), degassed; 900 mL

Apparatus 2: 60 rpm

Time: 30 min

Procedure: Determine the percentage of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) dissolved by using either the *Spectrometric procedure* or the *Chromatographic procedure* described below.

Spectrometric procedure

Standard stock solution: 1 mg/mL of USP Aripiprazole RS in alcohol

Standard solution: ($L/900$) mg/mL of USP Aripiprazole RS from *Standard stock solution* in *Medium* where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter, discarding the first 5 mL of filtrate.

Instrumental conditions

Mode: UV

Analytical wavelengths: 249 and 325 nm

Cell length: 1 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U absorbance at 249 nm minus the absorbance at 325 nm of the *Sample solution*

A_S absorbance at 249 nm minus the absorbance at 325 nm of the *Standard solution*

C_S concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Chromatographic procedure

Solution A: 0.02 M sodium sulfate

Solution B: 13.9 g/L of glacial acetic acid and 23.9 g/L of sodium acetate trihydrate in water

Mobile phase: Acetonitrile, methanol, *Solution A*, and glacial acetic acid (40:10:50:1)

Diluent: *Solution B* and methanol (50:50)

Internal standard solution: 0.35 μ g/mL of USP Propylparaben RS in *Diluent*

Standard stock solution A: 1 mg/mL of USP Aripiprazole RS in *Mobile phase*

Standard stock solution B: 0.002 mg/mL of USP Aripiprazole RS from *Standard stock solution A* in *Medium* passed through a suitable filter with a pore size of NMT 0.5 μ m, discarding the first 6 mL of filtrate

Standard solution: 0.001 mg/mL of USP Aripiprazole RS from *Standard stock solution B* prepared by combining 5 mL of *Standard stock solution B* and 5 mL of *Internal standard solution*

Sample stock solution: Pass a portion of the solution under test through a suitable filter with a pore size of NMT 0.5 μ m, discarding NLT the first 6 mL of filtrate.

Sample solution: Combine 2 mL of *Sample stock solution* with 2 mL of *Internal standard solution*.

Chromatographic system: Proceed as directed in the *Assay* except as follows.

Injection volume: 100 μ L

Run time: NLT 2 times the retention time of aripiprazole

System suitability

Sample: *Standard solution*

[Note—The relative retention times for aripiprazole and propylparaben are about 1.0 and 1.8, respectively.]

Suitability requirements

Resolution: NLT 10 between aripiprazole and propylparaben

Relative standard deviation: NMT 1.5% for the peak response ratios of aripiprazole to propylparaben

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) dissolved:

$$\text{Result} = (R_U/R_S) \times C_S \times V \times (1/L) \times 100$$

R_U peak response ratio of aripiprazole to propylparaben from the *Sample solution*

R_S peak response ratio of aripiprazole to propylparaben from the *Standard solution*

C_S concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) is dissolved.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

- **Organic Impurities**

Protect solutions from light.

Buffer: 9.6 g/L of dibasic ammonium citrate, 1.6 g/L of citric acid, and 2.9 g/L of sodium lauryl sulfate in water. Adjust with 0.05 M dibasic ammonia citrate solution or 0.05 M citric acid solution to a pH of 4.7, if needed.

Mobile phase: Acetonitrile and *Buffer* (45:55)

Diluent: Acetonitrile, water, and glacial acetic acid (40:60:1)

System suitability solution: 0.5 mg/mL of USP Aripiprazole RS, and 0.0005 mg/mL each of USP Aripiprazole Related Compound F RS and USP Aripiprazole Related Compound G RS in *Diluent*

Sample solution: Nominally 0.5 mg/mL of aripiprazole from Tablets prepared as follows.

Powder NLT 20 Tablets, transfer a suitable portion of the powder equivalent to NLT 4 mg of aripiprazole to an appropriate container, and add a suitable volume of *Diluent*. Shake for 10 min and centrifuge, if necessary. Pass the supernatant through a suitable filter with a pore size of NMT 0.5 μ m, discard the first 1 mL of filtrate, and use the subsequent filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 2 times the retention time of aripiprazole

System suitability

Sample: *System suitability solution*

[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 3 between aripiprazole related compound G and aripiprazole

Signal-to-noise ratio: NLT 10 for aripiprazole related compound F and aripiprazole related compound G

Analysis

Sample: *Sample solution*

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of each degradation product from the *Sample solution*

r_T sum of the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aripiprazole related compound F	0.54	0.20
Aripiprazole related compound G	0.81	0.20
Aripiprazole	1.0	—
Any individual unspecified degradation product	—	0.10
Total degradation products	—	1.0

ADDITIONAL REQUIREMENTS

● **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.

● **USP Reference Standards** { 11 }

USP Aripiprazole RS

USP Aripiprazole Related Compound F RS

4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazin-1-oxide.

C₂₃ H₂₇ Cl₂ N₃ O₃ 464.38

USP Aripiprazole Related Compound G RS

7-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy}quinolin-2(1H)-one.

C₂₃ H₂₅ Cl₂ N₃ O₂ 446.37

USP Propylparaben RS

▲USP39

BRIEFING

Aripiprazole Orally Disintegrating Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph based on validated methods is being proposed.

1. The isocratic liquid chromatographic procedure in the *Assay* is based on analyses performed with the Xterra RP18 brand of L1 column. The typical retention time for aripiprazole is about 8.6 min.
2. The isocratic liquid chromatographic procedure in the *Dissolution* test is based on analyses performed with the YMC-Pack ODS-AM brand of L1 column. The typical retention time for aripiprazole is about 4 min.
3. The gradient liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with the YMC-Pack Pro C18 brand of L1 column with a dwell volume of 1.0 mL. The typical retention time for aripiprazole is about 28.4 min.

(SM4: H. Joyce.)

Correspondence Number—C131646

Comment deadline: March 31, 2015

Add the following:

▲Aripiprazole Orally Disintegrating Tablets

DEFINITION

Aripiprazole Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$).

IDENTIFICATION

- **A. Infrared Absorption** 〈197A〉
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

● **Procedure**

Solution A: 2.84 g/L of sodium sulfate in water

Buffer: 3.48 g/L of dibasic potassium phosphate adjusted with phosphoric acid to a pH of 8.2

Mobile phase: Acetonitrile and *Buffer* (50:50)

Diluent A: Acetonitrile, methanol, *Solution A*, and glacial acetic acid (33:11:56:1)

Diluent B: Acetonitrile and 0.1 M hydrochloric acid (20:80)

System suitability solution: 0.01 mg/mL each of USP Aripiprazole RS and USP Aripiprazole Related Compound G RS in *Diluent A*. Sonication and shaking may be used to aid in dissolution.

Standard solution: 0.25 mg/mL of USP Aripiprazole RS in *Diluent B*. Sonication may be used to aid in dissolution.

Sample solution: Nominally 0.2–0.3 mg/mL of aripiprazole from NLT 5 Orally Disintegrating Tablets prepared as follows. Transfer NLT 5 Orally Disintegrating Tablets to a suitable volumetric flask and dilute with *Diluent B* to NMT 75% of the final flask volume. Sonicate for 5 min and shake for 15 min. Dilute with *Diluent B* to volume. Pass the resulting solution through a suitable filter and use the filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6-mm × 10-cm; 3.5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

Run time: NLT 1.4 times the retention time of aripiprazole

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times of aripiprazole related compound G and aripiprazole are 0.74 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between aripiprazole related compound G and aripiprazole, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aripiprazole (C₂₃H₂₇Cl₂N₃O₂) in the portion of Orally Disintegrating Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of aripiprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Disintegration** { 701 } : NMT 60 s

- **Dissolution** { 711 }

Medium: pH 4.0 sodium acetate trihydrate buffer (3.0 g/L of sodium acetate prepared as follows. Transfer a suitable quantity of sodium acetate to a suitable container containing 90% of the final container volume of water. Adjust with glacial acetic acid to a pH of 4.0. Add water to the final volume.), degassed; 1000 mL

Apparatus 2: 75 rpm

Time: 30 min

Mobile phase: Acetonitrile and 0.025 M hydrochloric acid (40:60)

Standard solution: (L/1000) mg/mL of USP Aripiprazole RS in *Mobile phase* where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter, discarding the first few mL.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: NLT 1.5 times the retention time of aripiprazole

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 1000 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) is dissolved.

- **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

IMPURITIES

- **Organic Impurities**

Solution A: Water and trifluoroacetic acid (100: 0.05)

Solution B: Acetonitrile and trifluoroacetic acid (100: 0.05)

Solution C: 2.84 g/L of sodium sulfate in water

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20	70	30
40	42	58
50	10	90
55	10	90
56	90	10
60	90	10

[Note—The gradient was established on an HPLC system with a dwell volume of

approximately 1.0 mL.]

Diluent: Acetonitrile, methanol, *Solution C*, and glacial acetic acid (33:11:56:1)

System suitability solution: 250 µg/mL of USP Aripiprazole RS, and 0.5 µg/mL each of USP Aripiprazole Related Compound F RS and USP Aripiprazole Related Compound G RS in *Diluent*. Sonication may be used to aid in dissolution.

Sample solution: Nominally 0.2–0.3 mg/mL of aripiprazole from NLT 5 Orally Disintegrating Tablets prepared as follows. Transfer NLT 5 Orally Disintegrating Tablets to a suitable volumetric flask. Add about 70% of the total volume of *Diluent*. Sonicate for 10 min and shake for 10 min. Dilute with *Diluent* to volume. Pass the resulting solution through a suitable filter and use the filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254

Column: 4.6-mm × 15-cm; 3-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 4.0 between aripiprazole related compound G and aripiprazole; NLT 1.5 between aripiprazole and aripiprazole related compound F

Analysis

Sample: *Sample solution*

Calculate the total peak response for individual impurities and aripiprazole in the *Sample solution*:

$$\text{Result} = \sum[r_i \times (1/F)] + r_U$$

r_i = peak response of each degradation product from the *Sample solution*

F = relative response factor (see *Table 2*)

r_U = peak response of aripiprazole from the *Sample solution*

Calculate the percentage of each degradation product in the portion of Orally Disintegrating Tablets taken:

$$\text{Result} = (r_i/r_T) \times (1/F) \times 100$$

r_i = peak response of each degradation product from the *Sample solution*

r_T = sum of the peak responses of individual impurities and aripiprazole from the *Sample solution*

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Aripiprazole related compound G	0.96	0.77	0.3
Aripiprazole	1.0	—	—
Aripiprazole related compound F	1.03	1.0	0.3
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.0

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.

- **USP Reference Standards** { 11 }

USP Aripiprazole RS

USP Aripiprazole Related Compound F RS

4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazin-1-oxide.

C₂₃ H₂₇ Cl₂ N₃ O₃ 464.38

USP Aripiprazole Related Compound G RS

7-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy}quinolin-2(1H)-one.

C₂₃ H₂₅ Cl₂ N₃ O₂ 446.37

▲USP39

BRIEFING

Ascorbic Acid Tablets, USP 38 page 2287. It is proposed to make the following changes in the monograph:

1. Revise the *Definition* to add other sources of ascorbic acid.
2. Add a cross-reference to the new general chapter *Vitamin C Assay* { 580 } where the new HPLC procedure for analysis of vitamin C is provided.
3. Add additional performance testing for Tablets recommended to be disintegrated in the mouth before swallowing.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(DS: N. Davydova.)

Correspondence Number—153939

Comment deadline: March 31, 2015

Ascorbic Acid Tablets

DEFINITION

Change to read:

Ascorbic Acid Tablets contain ~~NLT 90.0% and NMT 110.0%~~ of the labeled amount of ascorbic acid (C₆H₈O₆)

▲ascorbic acid in the form of ascorbic acid (C₆H₈O₆), sodium ascorbate (C₆H₇NaO₆), calcium

ascorbate dihydrate ($C_{12}H_{14}CaO_{12} \cdot 2H_2O$), or their mixture in an amount equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ascorbic acid ($C_6H_8O_6$). ▲*USP39*

IDENTIFICATION

• A.

Sample solution: Triturate a quantity of finely powdered Tablets with diluted alcohol to make a solution of ascorbic acid with a concentration of 20 mg/mL, and filter.

Analysis: Add alkaline cupric tartrate TS to a portion of the *Sample solution*.

Acceptance criteria: The *Sample solution* reduces alkaline cupric tartrate TS slowly at room temperature but more readily upon heating.

• B.

Sample solution: Use the *Sample solution* from Identification test A.

Analysis: To 2 mL of the *Sample solution* add 4 drops of methylene blue TS, and warm to 40°.

Acceptance criteria: The deep blue color of methylene blue becomes appreciably lighter or is completely discharged within 3 min.

• C.

Sample solution: Use the *Sample solution* from Identification test A.

Analysis: To 1 mL of the *Sample solution* add 15 mL of trichloroacetic acid solution (1 in 20) and 200 mg of activated charcoal, shake the mixture vigorously for 1 min, and pass through a small fluted filter, returning the filtrate if necessary, until clear. To 5 mL of the filtrate add 1 drop of pyrrole, agitate gently until dissolved, and then heat in a bath at 50°.

Acceptance criteria: A blue color develops.

ASSAY

Change to read:

▲[

Note—Where more than one assay procedure is given in the monograph, the requirements may be met by following any one of the specified procedures, the procedure used being stated in the labeling only if *Procedure 1* is not used.] ▲*USP39*

Change to read:

• Procedure

▲**Procedure 1** ▲*USP39*

Sample stock solution: Transfer NLT 20 Tablets to a 1000-mL volumetric flask containing 250 mL of metaphosphoric–acetic acids TS. Insert the stopper in the flask, and shake by mechanical means for 30 min or until the Tablets have disintegrated completely. Dilute with water to volume.

Sample solution: Transfer a portion of the *Sample stock solution* to a centrifuge tube, and centrifuge until a clear supernatant is obtained. Quantitatively dilute the clear supernatant with water, if necessary, to obtain a solution containing 0.5 mg/mL of ascorbic acid.

Blank: A mixture of 5.5 mL of metaphosphoric–acetic acids TS and 15 mL of water

Titrimetric system

(See *Titrimetry* 〈 541 〉.)

Mode: Direct titration

Titrant: Standard dichlorophenol–indophenol VS

Endpoint detection: Visual, a rose-pink color that persists for at least 5 s

Analysis: Transfer a volume of the *Sample solution*, equivalent to 2 mg of ascorbic acid, to a 50-mL conical flask. Add 5 mL of metaphosphoric–acetic acids TS, and titrate with *Titrant*. Correct for the volume of the *Titrant* consumed by the *Blank*.

Calculate the percentage of the labeled amount of ascorbic acid ($C_6H_8O_6$) in the portion of Tablets taken:

$$\text{Result} = \{[(V_S - V_B) \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample solution* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

F = concentration of the *Titrant* in terms of the equivalent of ascorbic acid (mg/mL)

W = nominal weight of ascorbic acid taken for *Analysis* (mg)

Acceptance criteria: 90.0%–110.0%

Add the following:

▲● **Procedure 2**

(See *Vitamin C Assay* 〈 580 〉, *Method II—Chromatographic Method.*)

Acceptance criteria: 90.0%–110.0%▲*USP39*

PERFORMANCE TESTS

Change to read:

● **Dissolution** 〈 711 〉

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Sample solution: Withdraw a portion of the solution under test, pass through a suitable filter, and use the pooled sample as the test specimen.

Analysis: Proceed as directed in the *Assay*,

▲*Procedure 1* or *Procedure 2*,▲*USP39*

conducting the procedure without delay and making any necessary modifications.

Calculate the percentage of the labeled amount of ascorbic acid ($C_6H_8O_6$) dissolved:

▲*For Procedure 1* ▲*USP39*

$$\text{Result} = (V_S - V_B) \times F \times [(V_M/a)/L] \times 100$$

V_S = *Titrant* volume consumed by the *Sample solution* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

F = concentration of the *Titrant* in terms of the equivalent of ascorbic acid (mg/mL)

V_M = volume of *Medium*, 900 mL

a = volume of the aliquot taken for *Analysis*

L = label claim of ascorbic acid (mg/Tablet)

▲For Procedure 2

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U

= peak area of ascorbic acid from the *Sample solution*

r_S

= peak area of ascorbic acid from the *Standard solution*

C_S

= concentration of USP Ascorbic Acid RS in the *Standard solution* (mg/mL)

V

= volume of *Medium*, 900 mL

L

= label claim (mg/Tablet)

▲USP39

Tolerances: NLT 75% (Q) of the labeled amount of ascorbic acid ($C_6H_8O_6$) is dissolved.

Add the following:

▲• **Disintegration** 〈 701 〉

[Note—Meet this additional test if the label recommends to disintegrate the Tablets in the mouth before swallowing.]

Medium: Water

Time: NMT 5 min

Acceptance criteria: Meet the requirements ▲USP39

- **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Add the following:

- ▲• **Labeling:** The label states the quantity of ascorbic acid in mg/Tablet, and the chemical form of ascorbic acid present in the Tablets. The labeling states with which assay procedure the product complies only if *Procedure 1* is not used. The label also states whether it is to be disintegrated in the mouth. ▲USP39

Add the following:

▲• **USP Reference Standards** 〈 11 〉

USP Ascorbic Acid RS

▲USP39

BRIEFING

Aspirin Capsules, *USP 38* page 2290. The United States Pharmacopeia (USP) is engaged in the ongoing challenge of obtaining information needed to create and sustain up-to-date

drug product quality monographs for drugs marketed under the FDA over-the-counter (OTC) drug monograph system (21 CFR Part 330). The following revisions of the *Assay*, *Identification*, and *Organic Impurities* tests are being proposed:

1. *Identification* test *A*, a non-specific wet chemical test, has been replaced with a high-performance liquid chromatographic procedure from the *Assay* that requires retention time agreement.
2. The *Assay*, which used an outdated column-partition chromatographic procedure, has been replaced with a high-performance liquid chromatographic procedure from the general chapter *Drug Product Assay Tests—Organic Chemical Medicines* 〈 321 〉, *Procedure 1*. This procedure was validated using the Waters Acquity UPLC HSS T3 brand of L1 column. The retention time of aspirin is about 7.5 min.
3. The *Organic Impurities—Limit of Free Salicylic Acid* test used an outdated column-partition chromatographic procedure. It is being proposed to replace this test with a high-performance liquid chromatographic procedure for *Salicylic Acid in Aspirin-Containing Drug Products* from the general chapter *Drug Product Impurities Tests* 〈 327 〉. This procedure requires the same chromatographic conditions as specified in the *Assay* and was validated using the same chromatographic column. The retention time of salicylic acid is about 8.6 min.
4. Additional Reference Standards have been added to the *USP Reference Standards* 〈 11 〉 section to support the revisions of the *Assay* and *Limit of Free Salicylic Acid* test.

The general chapters *Drug Product Assay Test—Organic Chemical Medicines* 〈 321 〉 and *Drug Product Impurities Tests* 〈 327 〉, published in this issue of *PF*, are procedures that were developed and validated for over-the-counter medicines. Further information can be found in the Stimuli article [Medicines Marketed under the Food and Drug Administration Over-the-Counter \(FDA OTC\) Drug Monograph System Regulations: Strategy for Developing Compendial Quality Standards](#) also published in this issue of *PF*. This article describes in detail the rationale for development of general chapters to organize procedures for the assay and specified impurities tests that can then be referenced in the individual product monographs. USP plans to continue with this initiative to revise or establish standards for OTC drug products.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: A. Potts.)

Correspondence Number—C150230

Comment deadline: March 31, 2015

Aspirin Capsules

DEFINITION

Aspirin Capsules contain NLT 93.0% and NMT 107.0% of the labeled amount of aspirin (C₉H₈O₄).

[Note—Capsules that are enteric-coated or the contents of which are enteric-coated meet the requirements for *Aspirin Delayed-Release Capsules*.]

IDENTIFICATION

Delete the following:

▲● A. Procedure

Sample: 100 mg of Capsule contents

Analysis: Combine *Sample* with 10 mL of water, heat for several minutes, cool, and add 1 drop of *ferric chloride TS*.

Acceptance criteria: A violet-red color is produced. ▲*USP39*

Add the following:

▲● **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP39*

● **B. Infrared Absorption** (197K)

Sample: Equivalent to 500 mg of aspirin, from Capsule contents

Analysis: Shake *Sample* with 10 mL of alcohol for several min. Centrifuge the mixture. Pour off the clear supernatant and evaporate it to dryness. Dry the residue in a vacuum at 60° for 1 h.

Acceptance criteria: Meet the requirements

ASSAY

Delete the following:

▲● Procedure

[Note—Use chloroform recently saturated with water.]

Diluent: A solution (1 in 100) of glacial acetic acid in chloroform

Standard stock solution: Transfer 50 mg of USP Aspirin RS to a 50 mL volumetric flask, add 0.5 mL of glacial acetic acid, and add chloroform to volume.

Standard solution: 50 µg/mL of USP Aspirin RS from *Standard stock solution* diluted with *Diluent*

Sample solution: Nominally 50 µg/mL of aspirin from Capsules prepared as follows. Remove, as completely as possible, the contents of NLT 20 Capsules. Mix the combined contents, and transfer a quantity of the powder, equivalent to 50 mg of aspirin, to a 50 mL volumetric flask containing 1 mL of a solution (1 in 50) of hydrochloric acid in methanol, and add chloroform to volume. Transfer 5.0 mL of this solution to the column, wash with 5 mL and then with 25 mL of chloroform, and discard the washings. Elute into a 100 mL volumetric flask with 10 mL of *Diluent*. Dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *Column Partition Chromatography*.)

Mode: LC

Column: Pack a chromatographic tube with a mixture of 3 g of *Solid Support* and 2 mL of freshly prepared sodium bicarbonate solution (1 in 12).

Analytical wavelength: 280 nm

Cell: 1-cm

Blank: Chloroform

Analysis

Samples: *Standard solution, Sample solution, and Blank*

Calculate the percentage of aspirin ($C_9H_8O_4$) in the portion of Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_S absorbances of the *Sample solution*

A_U absorbances of the *Standard solution*

C_S concentration of USP Aspirin RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U nominal concentration of aspirin in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 93.0%–107.0%▲*USP39*

Add the following:

▲● **Drug Product Assay Tests—Organic Chemical Medicines, Procedure 1** 〈 321 〉

Acceptance criteria: 93.0%–107.0%▲*USP39*

PERFORMANCE TESTS

● **Dissolution** 〈 711 〉

0.05 M acetate buffer: Mix 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of 4.50 ± 0.05 .

Medium: 0.05 M acetate buffer; 500 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: USP Aspirin RS having a known concentration in *Medium*. Prepare the *Standard solution* at the time of use. An amount of alcohol not to exceed 1% of the total volume of the *Standard solution* may be used to bring the Reference Standard into solution prior to dilution with *Medium*.

Sample solution: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium*, if necessary, and filter.

Instrumental conditions

Mode: UV

Analytical wavelength: 265 ± 2 nm

Analysis

Samples: *Standard solution and Sample solution*

Determine the labeled amount of aspirin ($C_9H_8O_4$) dissolved at the wavelength of the isosbestic point of aspirin and salicylic acid of the *Sample solution* in comparison with a *Standard solution* having a known concentration.

Tolerances: NLT 80% (Q) of the labeled amount of aspirin ($C_9H_8O_4$) is dissolved.

● **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

IMPURITIES**Delete the following:****▲Organic Impurities**● **Procedure: Limit of Free Salicylic Acid**

Ferric chloride-urea reagent: Dissolve by swirling, without the aid of heat, 60 g of urea in a mixture of 8 mL of ferric chloride solution (6 of 10) and 42 mL of 0.05 N hydrochloric acid. Adjust the resulting solution, if necessary, with 6 N hydrochloric acid to a pH of 3.2.

Standard solution: 15 µg/mL salicylic acid prepared as follows. Transfer 75.0 mg of salicylic acid, previously dried over silica gel for 3 h, to a 100 mL volumetric flask, and add chloroform to volume. Transfer 10.0 mL of this solution to a second 100 mL volumetric flask, and dilute with chloroform to volume. Transfer 10.0 mL of this last solution to a 50 mL volumetric flask containing 10 mL of methanol, 2 drops of hydrochloric acid, and 10 mL of a solution (1 in 10) of glacial acetic acid in ether, and dilute with chloroform to volume.

Sample solution: Nominally 2 mg of aspirin prepared from Capsules as follows. Weigh a portion of the contents of the Capsules, as determined by the Assay, equivalent to 100 mg of aspirin, mix with 10 mL of chloroform by stirring for 3 min, and then transfer to the chromatographic column with the aid of a few mL of chloroform. Pass 50 mL of chloroform through the column, rinse the tip of the chromatographic tube with chloroform, and discard the eluate. Prepare as a receiver a 50 mL volumetric flask containing 10 mL of methanol and 2 drops of hydrochloric acid, and elute any salicylic acid from the column by passing 10 mL of a solution (1 in 10) of glacial acetic acid in ether that has been recently saturated with water, followed by 30 mL of chloroform. Dilute the eluate with chloroform to volume.

Instrumental conditions

(See *Chromatography* ~~⟨ 621 ⟩~~, *Column Partition Chromatography*.)

Mode: LC

Column: Pack a chromatographic tube with two segments of packing material. The lower segment is a mixture of 1 g of *Solid Support* and 0.5 mL of 5 M phosphoric acid, and the upper segment is a mixture of 3 g of *Solid Support* and 2 mL of freshly prepared *Ferric chloride-urea reagent*.

Analytical wavelength: 306 nm

Cell: 1 cm

Blank: A solvent mixture of the same composition as that used for the *Standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Acceptance criteria: The absorbance of the *Sample solution* does not exceed that of the *Standard solution* (NMT 0.75%, calculated on the labeled aspirin content).

▲USP39

Add the following:

▲● **Limit of Free Salicylic Acid:** Proceed as directed for *Salicylic Acid in Aspirin-containing Drug Products* in *Drug Product Impurities Tests* ~~⟨ 327 ⟩~~.

Acceptance criteria: NMT 0.75%▲USP39

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

Change to read:

- **USP Reference Standards** { 11 }

USP Aspirin RS

▲USP Caffeine RS

USP Dextromethorphan Hydrobromide RS USP Diphenhydramine Hydrochloride RS USP Doxylamine Succinate RS USP Pseudoephedrine Hydrochloride RS

USP Salicylic Acid RS ▲USP39

BRIEFING

Benzocaine Lozenges, USP 38 page 2389. As part of the USP monograph modernization initiative, it is proposed to make the following changes:

1. Replace *Identification* test A, a wet chemistry test, with a test based on the UV spectrum agreement of the major peak in the chromatogram of the sample and standard in the *Assay*.
2. Add acceptance criteria for both the total labeled amount of benzocaine and free benzocaine in the *Assay* for clarity.
3. Add the test for *Organic Impurities* to the monograph. The proposed HPLC procedure in the test for *Organic Impurities* is based on analyses performed with the Waters Symmetry Shield RP-8 brand of L7 column. The typical retention time for benzocaine is about 6.1 min.
4. Add acceptance criteria for specified and unspecified degradation products. Aminobenzoic acid and ethyl 4-nitrobenzoate are potential degradation products of benzocaine. Acceptance criteria of NMT 0.2% are proposed for aminobenzoic acid and ethyl 4-nitrobenzoate. For unspecified degradation products, acceptance criteria of NMT 0.1% based on ICH guidelines are proposed. Manufacturers are encouraged to submit their approved specifications to USP if they are different from those proposed in this revision.
5. Add a storage condition in the *Packaging and Storage* section based on the product package label.
6. Add two USP Reference Standards to the *USP Reference Standards* section to support the proposed revision for the procedure in the test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: G. Hsu, D. Vicchio.)

Correspondence Number—C136869

Comment deadline: March 31, 2015

Benzocaine Lozenges

DEFINITION

Benzocaine Lozenges contain NLT 85.0% and NMT 120.0% of the labeled amount of benzocaine ($C_9H_{11}NO_2$).

IDENTIFICATION**Delete the following:****▲● A.**

Analysis: Dissolve an equivalent of 20 mg of benzocaine from powdered Lozenges in 10 mL of water with the aid of a few drops of 3 N hydrochloric acid. Filter, if necessary, to obtain a clear solution. Add 5 drops of a solution of sodium nitrite (1 in 10), followed by 2 mL of a solution of 100 mg of 2-naphthol in 5 mL of 1 N sodium hydroxide.

Acceptance criteria: An orange-red precipitate is formed. ▲USP39

Add the following:

▲● **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP39

● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**Change to read:**● **Procedure**

Buffer: 1.0 M monobasic potassium phosphate, adjusted with phosphoric acid to a pH of 3.0

Mobile phase: Acetonitrile, water, and *Buffer* (250:700:50)

Diluent A: 0.1 N hydrochloric acid

Diluent B: Acetonitrile and water (1:1)

Standard solution A: 0.01 mg/mL of USP Benzocaine RS in *Diluent A*

Standard solution B: 0.01 mg/mL of USP Benzocaine RS in *Diluent B*

Sample stock solution A: Transfer the equivalent of 40 mg of benzocaine from powdered Lozenges (NLT 20) to a 200-mL volumetric flask. Add 150 mL of *Diluent A*, and stir for NLT 2 h. Dilute with *Diluent A* to volume.

Sample stock solution B: Transfer the equivalent of 40 mg of benzocaine from powdered Lozenges (NLT 20) to a 200-mL volumetric flask. Add 150 mL of *Diluent B*, and stir for NLT 30 min. Dilute with *Diluent B* to volume.

Sample solution A: Nominally 0.01 mg/mL of benzocaine in *Diluent A* from *Sample stock solution A*

Sample solution B: Nominally 0.01 mg/mL of benzocaine in *Diluent B* from *Sample stock solution B*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm.

▲For *Identification* test A, use a diode array detector in the range of 200–400 nm.▲USP39

Column: 4.6-mm × 25-cm; packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Sample solution A*, and *Sample solution B*

Calculate the percentage of the total labeled amount of benzocaine (C₉H₁₁NO₂) in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from *Sample solution A*

r_S peak response from *Standard solution A*

C_S concentration of USP Benzocaine RS in *Standard solution A* (mg/mL)

C_U nominal concentration of benzocaine in *Sample solution A* (mg/mL)

Calculate the percentage of free benzocaine (C₉H₁₁NO₂) in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from *Sample solution B*

r_S peak response from *Standard solution B*

C_S concentration of USP Benzocaine RS in *Standard solution B* (mg/mL)

C_U nominal concentration of benzocaine in *Sample solution B* (mg/mL)

Acceptance criteria

▲**Total labeled amount of benzocaine:** 85.0%–120.0%

Free benzocaine: 85.0%–120.0%▲USP39

IMPURITIES

Add the following:

▲• Organic Impurities

Solution A: Dissolve 9.1 g of monobasic potassium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0 .

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
10	45	55
10.1	60	40
13	60	40

Diluent: Acetonitrile and water (10:90)

Standard stock solution: 0.03 mg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*. Sonicate for 2–5 min to dissolve before diluting to final volume.

Standard solution: 0.3 µg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-nitrobenzoate RS in *Diluent* from the *Standard stock solution*

Sample solution: Nominally 150 µg/mL of benzocaine in *Diluent* prepared as follows. Transfer 10 Lozenges to an appropriate volumetric flask to obtain a nominal benzocaine concentration of 0.15 mg/mL. Dissolve Lozenges in *Diluent* and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 10 between benzocaine and aminobenzoic acid; NLT 10 between ethyl 4-nitrobenzoate and benzocaine

Relative standard deviation: NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*

r_S = peak response of the corresponding Reference Standard from the *Standard solution*

C_S = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of benzocaine in the *Sample solution* (µg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_{\bar{f}}$ peak response of each unspecified degradation product from the *Sample solution*

$r_{\bar{s}}$ peak response of benzocaine from the *Standard solution*

$C_{\bar{s}}$ concentration of USP Benzocaine RS in the *Standard solution* ($\mu\text{g/mL}$)

$C_{\bar{f}}$ nominal concentration of benzocaine in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.46	0.2
Benzocaine	1.00	—
Ethyl 4-nitrobenzoate	1.86	0.2
Any unspecified degradation product	—	0.1
Total degradation products	—	2.0

▲USP39

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in well-closed containers, ▲and store at controlled room temperature. ▲USP39

Change to read:

- **USP Reference Standards** { 11 }

▲USP Aminobenzoic Acid RS

Benzoic acid, 4-amino.

$\text{C}_7\text{H}_7\text{NO}_2$ 137.14 ▲USP39

USP Benzocaine RS

▲USP Ethyl 4-Nitrobenzoate RS

Benzoic acid, 4-nitro-, ethyl ester.

$\text{C}_9\text{H}_9\text{NO}_4$ 195.17 ▲USP39

BRIEFING

Benzocaine and Menthol Topical Aerosol, USP 38 page 2394. As part of the USP monograph modernization initiative, it is proposed to make the following changes:

1. Replace *Identification test A*, a wet chemistry test, with a test based on the UV spectrum agreement of the major peak in the chromatogram of the sample and standard in the *Assay*.
2. Replace the test procedure for *Benzocaine* in the *Assay* with an HPLC procedure

based on the same HPLC column and mobile phases as the proposed procedure in the test for *Organic Impurities* for consistency and efficiency.

3. Replace the packed GC column test procedure for *Menthol* in the *Assay* with a validated GC procedure which uses a capillary GC column. The proposed GC procedure for menthol is based on analyses performed with the Agilent DB-Wax brand of G16 column. The typical run time is about 13 min.
4. Add the test for *Organic Impurities*. The proposed HPLC procedure in the *Organic Impurities* is based on analyses performed with the Waters Symmetry Shield RP-8 brand of L7 column. The typical retention time for benzocaine is about 15 min.
5. Add acceptance criteria for specified and unspecified degradation products. Aminobenzoic acid and ethyl 4-nitrobenzoate are potential degradation products of benzocaine. Acceptance criteria of NMT 0.2% are proposed for aminobenzoic acid and ethyl 4-nitrobenzoate. For unspecified degradation products, acceptance criteria of NMT 0.1% are proposed. Manufacturers are encouraged to submit their approved specifications to USP if they are different from those proposed in this revision.
6. Replace the *Pressure Test* and *Leakage Test* in *Other Requirements* with the *Pressure Test* and *Leakage Test* in general chapters *Topical Aerosols* 〈 603 〉 and *Leak Rate* 〈 604 〉.
7. Add two USP Reference Standards to the *USP Reference Standards* section to support the proposed revision for the procedure in the test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: G. Hsu, R. Ravichandran.)

Correspondence Number—C136865

Comment deadline: March 31, 2015

Benzocaine and Menthol Topical Aerosol

DEFINITION

Benzocaine and Menthol Topical Aerosol is a solution of Benzocaine and Menthol with suitable propellants in a pressurized container. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of benzocaine ($C_9H_{11}NO_2$) and menthol ($C_{10}H_{20}O$).

IDENTIFICATION

Delete the following:

▲● A:

Sample: An amount of Topical Aerosol equivalent to 5 mg of benzocaine

Analysis: Transfer the *Sample* to a beaker. Add 20 mL of 0.5 N hydrochloric acid, and warm gently to disperse the solution. Cool, and filter if necessary, to obtain a clear solution. To 10 mL of the clear solution add 5 drops of a solution of sodium nitrite (1 in 10) and 2 drops of methyl red TS, and neutralize with 1 N sodium hydroxide. Add 2 mL of a solution of 100 mg of 2-naphthol in 5 mL of 1 N sodium hydroxide.

Acceptance criteria: An orange-red precipitate is formed. ▲USP39

Add the following:

- ▲● **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ▲USP39
- **B.** The retention time of the major peak for benzocaine of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **C.** The retention time of the major peak for menthol of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY**Change to read:**● **Benzocaine**

Mobile phase: ~~Methanol, water, and glacial acetic acid (56:40:4)~~

Diluent: ~~Methanol and water (1:1)~~

Standard solution: ~~0.02 mg/mL of USP Benzocaine RS in Diluent~~

Sample solution: ~~Nominally 0.02 mg/mL of benzocaine in Diluent prepared as follows.~~

~~Spray the contents of the Topical Aerosol into a flask. Transfer an amount of Topical Aerosol containing 200 mg of benzocaine to a suitable volumetric flask, and dilute with Diluent.~~

Chromatographic system

~~(See Chromatography <621>, System Suitability.)~~

Mode: ~~LC~~

Detector: ~~UV 294 nm~~

Column: ~~3.9 mm × 30 cm; packing L1~~

Flow rate: ~~1.5 mL/min~~

Injection volume: ~~50 µL~~

System suitability

Sample: ~~Standard solution~~

Suitability requirements

Relative standard deviation: ~~NMT 2.0%~~

Analysis

Samples: ~~Standard solution and Sample solution~~

~~Calculate the percentage of the labeled amount of benzocaine (C₉H₁₁NO₂) in the portion of Topical Aerosol taken:~~

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

~~r_U peak response from the Sample solution~~

~~r_S peak response from the Standard solution~~

~~C_S concentration of USP Benzocaine RS in the Standard solution (mg/mL)~~

~~C_U nominal concentration in the Sample solution (mg/mL)~~

Acceptance criteria: ~~90.0%–110.0%~~

▲**Solution A:** Dilute 1.0 mL of trifluoroacetic acid with water to 1 L.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
15	72	28
18	50	50
18.1	90	10
20	90	10

Diluent: *Solution A* and *Solution B* (1:1)

Standard solution: 0.1 mg/mL of USP Benzocaine RS in *Diluent*. Sonicate for 2–5 min to dissolve before diluting to final volume.

Sample solution: Nominally 0.1 mg/mL of benzocaine in *Diluent* prepared as follows. Spray the contents of the Topical Aerosol into a flask with stopper. Heat and stir the sprayed Topical Aerosol at 100° for 30 min in an oil bath to obtain a viscous liquid sample. Cool the sample to room temperature. Transfer an amount of Topical Aerosol equivalent to 10 mg of benzocaine to a 100-mL volumetric flask. Dissolve the sample in *Diluent* and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm. For *Identification test A*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 25-cm; 5-μm packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzocaine (C₉H₁₁NO₂) in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%▲*USP39*

Change to read:

● **Menthol**

Internal standard solution: 1 mg/mL of decanol in *n*-hexane

Standard stock solution: 1 mg/mL of USP Menthol RS in *n*-hexane

Standard solution: 0.05 mg/mL each of USP Menthol RS and decanol in ether from *Standard stock solution* and *Internal standard solution*

Sample stock solution: Spray the contents of the Topical Aerosol into a flask. Transfer an amount of Topical Aerosol equivalent to 50 mg of menthol to a separator, and extract with three 15 mL portions of ether, collecting the ether extracts in a 50 mL volumetric flask. Dilute with ether to volume.

Sample solution: Nominally 0.05 mg/mL of menthol prepared as follows. Transfer 5.0 mL of the *Sample stock solution* to a 100 mL volumetric flask, add 5.0 mL of *n*-hexane and 5.0 mL of *Internal standard solution*, and dilute with ether to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2 mm × 1.8 m, packed with 10% phase G16 on support S1AB

Carrier gas: Helium

Temperatures

Column: 170°

Injection port: 260°

Detector: 240°

Flow rate: 50 mL/min

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for menthol and decanol are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between the menthol and decanol peaks

Relative standard deviation: NMT 2% of the ratio of the peak response of menthol to that of decanol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of menthol ($C_{10}H_{20}O$) in the portion of

Topical Aerosol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U peak response ratio of menthol to decanol from the *Sample solution*

R_S peak response ratio of menthol to decanol from the *Standard solution*

C_S concentration of USP Menthol RS in the *Standard solution* (mg/mL)

C_U nominal concentration of menthol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

▲Internal standard solution: 1 mg/mL of decanol in isopropyl alcohol

Standard stock solution: 1 mg/mL of USP Menthol RS in isopropyl alcohol

Standard solution: 0.5 mg/mL each of USP Menthol RS and decanol in isopropyl alcohol from *Internal standard solution* and *Standard stock solution*

Sample solution: Nominally 0.5 mg/mL of menthol prepared as follows. Spray the contents of the Topical Aerosol into a flask. Transfer an amount of Topical Aerosol equivalent to 5 mg of menthol to a 10-mL volumetric flask and add 5.0 mL of *Internal standard solution*. Dilute with isopropyl alcohol to volume.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 30-m; fused-silica capillary column bonded with a 1.0-μm film of phase G16

Carrier gas: Hydrogen

Temperatures

Injection port: 250°

Detector: 250°

Column: See *Table 2*.

Table 2

Initial Temperature (°)	Hold Time at 130° (min)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	7.5	35	240	1.0

Flow rate: 10 mL/min

Injection volume: 1 μL

Injection type: Split, split ratio 10:1

System suitability

Sample: *Standard solution*

[Note—The relative retention times for menthol and decanol are 1.0 and 1.6, respectively.]

Suitability requirements

Resolution: NLT 2.5 between the menthol and decanol peaks

Relative standard deviation: NMT 1.0% of the ratio of the peak response of menthol to that of decanol

Analysis**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of menthol ($C_{10}H_{20}O$) in the portion of Topical Aerosol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

 R_U

= peak response ratio of menthol to decanol from the *Sample solution*

R_S = peak response ratio of menthol to decanol from the *Standard solution*

C_S = concentration of USP Menthol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of menthol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%▲*USP39*

PERFORMANCE TESTS

- **Minimum Fill** (755): Meets the requirements

IMPURITIES

Add the following:

▲• **Organic Impurities**

Solution A: Dilute 1.0 mL of trifluoroacetic acid with water to 1 L.

Solution B: Acetonitrile

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

Diluent: *Solution A* and *Solution B* (1:1)

Standard stock solution: 0.1 mg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*. Sonicate for about 5 min to dissolve before diluting to final volume.

Standard solution: 0.001 mg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent* from the *Standard stock solution*

Sample solution: Nominally 0.5 mg/mL of benzocaine in *Diluent* prepared as follows. Spray the contents of the Topical Aerosol into a flask. Heat and stir the sprayed Topical Aerosol at 100° for 30 min in an oil bath to obtain a viscous liquid sample. Cool the sample to room temperature. Transfer an amount of Topical Aerosol equivalent to 50 mg of benzocaine to

a 100-mL volumetric flask. Dissolve the sample in *Diluent* and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*

r_S peak response of the corresponding Reference Standard from the *Standard solution*

C_S concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (mg/mL)

C_U nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each unspecified degradation product from the *Sample solution*

r_S peak response of benzocaine from the *Standard solution*

C_S concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

C_U nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 4*. Disregard peaks less than 0.05%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.3	0.2
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.2
Any other unspecified degradation product	—	0.1
Total degradation products	—	2.0

SPECIFIC TESTS

- **Microbial Enumeration Tests** 〈 61 〉 and **Tests for Specified Microorganisms** 〈 62 〉: It meets the requirements for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Delete the following:

- ▲● **Other Requirements:** It meets the requirements in *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* 〈 601 〉, *Pressure Test*, and *Leakage Test*. ▲USP39

Add the following:

- ▲● **Pressure Test:** It meets the requirements in *Topical Aerosols* 〈 603 〉. ▲USP39

Add the following:

- ▲● **Leakage Test:** It meets the requirements in *Leak Rate* 〈 604 〉. ▲USP39

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, pressurized containers, and avoid exposure to excessive heat.

Change to read:

- **USP Reference Standards** 〈 11 〉

▲USP Aminobenzoic Acid RS

Benzoic acid, 4-amino.

$C_7H_7NO_2$ 137.14▲USP39

USP Benzocaine RS

▲USP Ethyl 4-Nitrobenzoate RS

Benzoic acid, 4-nitro-, ethyl ester.

$C_9H_9NO_4$ 195.17▲USP39

USP Menthol RS

BRIEFING

Brompheniramine Maleate Oral Solution, USP 38 page 2476. The United States Pharmacopeia (USP) is engaged in the ongoing challenge of obtaining information needed to create and sustain up-to-date drug product quality monographs for drugs marketed under the FDA over-the-counter (OTC) drug monograph system (21 CFR Part 330). The following revisions to the *Assay* and *Identification* tests are being proposed:

1. The *Identification—Organic Nitrogenous Bases* 〈 181 〉 procedure, which uses carbon disulfide and requires extensive sample preparation, has been replaced with retention time agreement using the procedure from the *Assay*.

2. An *Identification* test *B* that uses the photo-diode array detection (190–400 nm) has been added, with the *Assay* procedure as a second orthogonal test.
3. The *Assay*, which uses a titration procedure requiring the use of chloroform, an unsafe solvent, has been replaced with a liquid chromatography procedure from the general chapter *Drug Product Assay Tests—Organic Chemical Medicines* 〈 321 〉, *Procedure 1*. This procedure was validated using the Waters Acquity UPLC HSS T3 brand of L1 column. The retention time of brompheniramine is about 7.0 min.
4. The test for *Alcohol Determination* criteria was modified to reflect “if present”.
5. *Deliverable Volume* 〈 698 〉, *Microbial Enumeration Tests* 〈 61 〉, and *Tests for Specified Microorganisms* 〈 62 〉 have been added to strengthen the monograph.
6. The *Packaging and Storage* section is updated to be consistent with the manufacturers’ package inserts.
7. Additional Reference Standards have been added to the *USP Reference Standards* section to support the revision of the *Assay*.

Chapter 〈 321 〉, also published in this issue of *PF*, is a procedure that was developed and validated for OTC medicines. Further information can be found in the *Stimuli* article [Medicines Marketed under the Food and Drug Administration Over-the-Counter \(FDA OTC\) Drug Monograph System Regulations: Strategy for Developing Compendial Quality Standards](#), also published in this issue of *PF*. This article describes in detail the rationale for the development of general chapters to organize procedures for the assay that can then be referenced in the individual product monographs. USP plans to continue with this initiative to revise or establish standards for OTC drug products.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: A. Potts.)

Correspondence Number—C151302

Comment deadline: March 31, 2015

Brompheniramine Maleate Oral Solution

DEFINITION

Brompheniramine Maleate Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of brompheniramine maleate ($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$).

IDENTIFICATION

Delete the following:

▲• Identification—Organic Nitrogenous Bases ~~〈 181 〉~~

Sample solution: Transfer an equivalent of 50 mg of brompheniramine maleate from a volume of Oral Solution, to a separator. Render distinctly alkaline with 1 N sodium hydroxide, and extract with two 50 mL portions of chloroform, shaking gently to avoid emulsification. Wash the combined chloroform extracts with 10 mL of water, and discard the aqueous phase. Filter the combined chloroform extracts into a conical flask, and evaporate the solvent on a steam bath, with the aid of a current of air. To the residue add 25 mL of dilute hydrochloric acid (1 in 1200), and proceed as directed under

~~Identification—Organic Nitrogenous Bases (181), beginning with "Transfer the liquid to a separator".~~

~~**Acceptance criteria:** The Oral Solution meets the requirements. ▲USP39~~

Add the following:

- ▲● **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP39

Add the following:

- ▲● **B.** The UV-vis spectrum (190-400 nm) of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP39

ASSAY

Change to read:

Procedure

▲Drug Product Assay Tests—Organic Chemical Medicines, Procedure 1 (321):

95.0%–105.0%▲USP39

~~**Sample solution:** Transfer an equivalent of 20 mg of brompheniramine maleate from a volume of Oral Solution, to a separator. Render distinctly alkaline with 1-N sodium hydroxide, and extract with ten 10-mL portions of chloroform, shaking gently to avoid emulsification. Wash the combined chloroform extracts with 10 mL of water, wash the latter with 20 mL of chloroform, and discard the aqueous phase. Quantitatively filter the combined chloroform extracts and washings into a conical flask, and evaporate the solvent on a steam bath, with the aid of a current of air. To the residue add 25 mL of glacial acetic acid and 5 mL of acetic anhydride, agitate, and allow to stand for about 15 min. Add 1 drop of crystal violet TS.~~

~~**Titrimetric system**~~

~~**Mode:** Direct titration~~

~~**Titrant:** 0.01 N perchloric acid VS~~

~~**Endpoint detection:** Visual~~

~~**Analysis:** Titrate the *Sample solution* with *Titrant* to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 2.177 mg of brompheniramine maleate ($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$).~~

▲▲USP39

OTHER COMPONENTS

Change to read:

- **Alcohol Determination, Method I (611):** 2.7%–3.3% of

▲ethanol▲USP39

(C_2H_5OH),

▲if present▲USP39

PERFORMANCE TESTS**Add the following:**

- ▲● **Deliverable Volume** 〈 698 〉: Meets the requirements ▲*USP39*

SPECIFIC TESTS

- **pH** 〈 791 〉: 2.5–3.5

Add the following:

- ▲● **Microbial Enumeration Tests** 〈 61 〉 and **Tests for Specified Microorganisms** 〈 62 〉: It meets the requirements of the test for the absence of *Escherichia coli*. The total aerobic microbial count does not exceed 10² cfu/mL. The total yeasts and molds count does not exceed 10¹ cfu/mL. ▲*USP39*

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in ~~well-closed~~

▲tight, ▲*USP39*

light-resistant containers.

▲Store at controlled room temperature. ▲*USP39*

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Brompheniramine Maleate RS

▲USP Caffeine RS

USP Dextromethorphan Hydrobromide RS

USP Diphenhydramine Hydrochloride RS

USP Doxylamine Succinate RS

USP Pseudoephedrine Hydrochloride RS

▲*USP39*

BRIEFING

Brompheniramine Maleate Tablets, *USP 38* page 2477. The United States Pharmacopeia (USP) is engaged in the ongoing challenge of obtaining information needed to create and sustain up-to-date drug product quality monographs for drugs marketed under the FDA over-the-counter (OTC) drug monograph system (21 CFR Part 330). The following revisions to the *Assay* and *Identification* tests are being proposed:

1. The *Identification* section has been updated to replace the *Organic Nitrogenous Bases* 〈 181 〉 procedure, which uses carbon disulfide and requires extensive sample preparation, with retention time agreement using the procedure from the *Assay*.
2. It is being proposed to add an *Identification* test *B* that uses photo-diode array detection (190–400 nm) with the *Assay* procedure to compare the UV-vis spectra to the USP Reference Standard.

3. The *Assay*, which used an outdated UV procedure, has been replaced with a liquid chromatography procedure in general chapter *Drug Product Assay Tests—Organic Chemical Medicines* $\langle 321 \rangle$, *Procedure 1*. This procedure was validated using the Waters Acquity UPLC HSS T3 brand of L1 column. The retention time of brompheniramine is about 7.0 min.
4. The *Packaging and Storage* section is updated to be consistent with the manufacturers' package inserts.

Chapter $\langle 321 \rangle$, also published in this issue of *PF*, is a procedure that was developed and validated for over-the-counter (OTC) medicines. Further information can be found in the *Stimuli* article [Medicines Marketed under the Food and Drug Administration's Over-the-Counter \(FDA OTC\) Drug Monograph System Regulations: Strategy for Developing Compendial Quality Standards](#), also published in this issue of *PF*. This article describes in detail the rationale for the development of this chapter and other related chapters, as well as the associated impact on drug product monographs. USP plans to continue with this initiative to revise or establish standards for OTC drug products.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: A. Potts.)

Correspondence Number—C151303

Comment deadline: March 31, 2015

Brompheniramine Maleate Tablets

DEFINITION

Change to read:

Brompheniramine Maleate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of \blacktriangle brompheniramine maleate \blacktriangle *USP39*
($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$).

IDENTIFICATION

Delete the following:

\blacktriangle ~~• Identification—Organic Nitrogenous Bases $\langle 181 \rangle$ Meets the requirements.~~ \blacktriangle *USP39*

Add the following:

\blacktriangle • **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. \blacktriangle *USP39*

Add the following:

\blacktriangle • **B.** The UV-vis spectrum (190–400 nm) of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. \blacktriangle *USP39*

ASSAY

Change to read:**Procedure****▲Drug Product Assay Tests—Organic Chemical Medicines, Procedure 1 (321) :**

95.0%–105.0%▲USP39

~~**Standard stock solution:** 160 µg/mL of USP Brompheniramine Maleate RS~~~~**Standard solution:** 20 µg/mL of USP Brompheniramine RS prepared as follows. Transfer 25.0 mL of *Standard stock solution* to a separator containing 25 mL of water, mix, and proceed as directed under *Sample solution*, beginning with “adjust with sodium hydroxide solution (1 in 10) to a pH of 11”.~~~~**Sample solution:** Nominally 20 µg/mL of brompheniramine maleate from Tablets prepared as follows. Weigh and finely powder NLT 20 Tablets. Weigh a portion of Tablets nominally equivalent to 4 mg of brompheniramine maleate, mix with 50 mL of water for 10 min, adjust with sodium hydroxide solution (1 in 10) to a pH of 11, and cool to room temperature. Extract the mixture with two 75 mL portions of solvent hexane, and combine the extracts in a second separator. Extract the solvent hexane solution with three 50 mL portions of dilute hydrochloric acid (1 in 120), combining the acid extracts in a 200 mL volumetric flask, and dilute with hydrochloric acid (1 in 120) to volume.~~~~**Instrumental conditions**~~~~**Mode:** UV~~~~**Analytical wavelength:** 264 nm~~~~**Cell:** 1 cm~~~~**Blank:** Dilute hydrochloric acid (1 in 120)~~~~**Analysis**~~~~**Samples:** *Standard solution*, *Sample solution*, and *Blank*~~~~Calculate the percentage of the labeled amount of brompheniramine maleate ($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$) in the portion of Tablets taken:~~

~~$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$~~

 ~~A_U absorbance from the *Sample solution*~~ ~~A_S absorbance from the *Standard solution*~~ ~~C_S concentration of USP Brompheniramine Maleate RS in the *Standard solution* (µg/mL)~~ ~~C_U nominal concentration of brompheniramine maleate in the *Sample solution* (µg/mL)~~

▲▲USP39

PERFORMANCE TESTS● **Dissolution (711)****Medium:** Water; 500 mL**Apparatus 1:** 100 rpm**Time:** 45 min**Standard solution:** USP Brompheniramine Maleate RS at a known concentration in *Medium***Sample solution:** Sample per *Dissolution (711)*. Filter portions of the solution under test, suitably diluted with 3 N hydrochloric acid.**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* 〈 851 〉.)

Mode: UV

Analytical wavelength: 264 nm

Cell: 5 cm

Analysis

Samples: *Standard solution and Sample solution*

Tolerances: NLT 75% (Q) of the labeled amount of brompheniramine maleate ($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$) is dissolved.

- **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers.

▲Store at controlled room temperature. ▲USP39

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Brompheniramine Maleate RS

▲USP Caffeine RS

USP Dextromethorphan Hydrobromide RS

USP Diphenhydramine Hydrochloride RS

USP Doxylamine Succinate RS

USP Pseudoephedrine Hydrochloride RS

▲USP39

BRIEFING

Candesartan Cilexetil Tablets. Because there is no existing *USP* monograph for this dosage form, a new monograph based on validated methods of analysis is proposed. The liquid chromatographic procedure in both the *Assay* and *Dissolution* tests, is based on analysis performed using the Xterra RP8 brand of L7 column. The typical retention time for candesartan cilexetil is about 5.4 min. The liquid chromatographic procedure in the *Organic Impurities* test is based on analyses performed with the Symmetry C18 brand of L1 column. The typical retention time for candesartan cilexetil is about 16.8 min.

(SM2: S. Ramakrishna.)

Correspondence Number—C127211

Comment deadline: March 31, 2015

Add the following:

▲**Candesartan Cilexetil Tablets**

DEFINITION

Candesartan Cilexetil Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of

candesartan cilexetil (C₃₃H₃₄N₆O₆).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak from the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Mobile phase: Acetonitrile, trifluoroacetic acid, and water (550:1:450)

Diluent: Acetonitrile and water (70:30)

Standard solution: 0.8 mg/mL of USP Candesartan Cilexetil RS in *Diluent*. Sonication may be necessary for complete dissolution. Pass through a suitable filter of 0.45- μ m pore size.

Sample solution: Nominally 0.8 mg/mL of candesartan cilexetil in *Diluent* prepared as follows. Transfer a number of Tablets (see *Table 1*) to a suitable volumetric flask.

Table 1

Tablet Strength (mg)	Number of Tablets (NLT)
4	10
8	10
16	5
32	5

Add *Diluent* to fill about 70% of the total volume, and sonicate for about 25 min with intermittent shaking. Allow to cool and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detectors

Assay: UV 282 nm

Identification B: Diode array

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Column temperature: 30^o

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

Run time: NLT 2.7 times the retention time of candesartan cilexetil

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Candesartan Cilexetil RS in the *Standard solution* (mg/mL)

C_U nominal concentration of candesartan cilexetil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution 〈 711 〉

Medium for tablets labeled to contain 4 mg, 8 mg, and 16 mg: 0.35% polysorbate 20 in 0.05 M phosphate buffer, pH 6.5; 900 mL

Medium for tablets labeled to contain 32 mg: 0.70% polysorbate 20 in 0.05 M phosphate buffer, pH 6.5; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Mobile phase: Acetonitrile, trifluoroacetic acid, and water (550:1:450)

Standard stock solution: 0.45 mg/mL USP Candesartan Cilexetil RS in acetonitrile. Sonication may be necessary for complete dissolution.

Standard solution: Prepare solutions in *Medium* from *Standard stock solution* (see *Table 2* for concentrations).

Table 2

Tablet Strength (mg)	Concentration (mg/mL)
4	0.0045
8	0.009
16	0.018
32	0.036

Sample solution: Pass a portion of solution under test through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Column temperature: 30 $^{\circ}$

Flow rate: 1.5 mL/min

Injection volume: 50 μ L

Run time: NLT 1.8 times the retention time of candesartan cilexetil

System suitability

Sample: *Standard solution*

Suitability requirements**Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution and Sample solution*

Calculate the percentage of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V (1/L)100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = volume of medium, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) is dissolved.

- **Uniformity of Dosage Units** (905): Meet the requirements

IMPURITIES

- **Organic Impurities**

Solution A: Acetonitrile, trifluoroacetic acid, and water (10: 0.1: 90)

Solution B: Acetonitrile, trifluoroacetic acid, and water (90: 0.1: 10)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	65	35
30	5	95
45	5	95
50	65	35
55	65	35

System suitability stock solution A: 0.05 mg/mL each of USP Candesartan Cilexetil Related Compound A RS, USP Candesartan Cilexetil Related Compound B RS, USP Candesartan Cilexetil Related Compound D RS, and USP Candesartan Cilexetil Related Compound F RS in acetonitrile

System suitability stock solution B: 0.1 mg/mL of USP Candesartan Cilexetil RS in acetonitrile

System suitability stock solution C: 0.5 mg/mL of USP Candesartan Cilexetil Related Compound G RS in methanol

System suitability solution: 0.0015 mg/mL each of USP Candesartan Cilexetil Related Compound A RS, USP Candesartan Cilexetil Related Compound B RS, USP Candesartan Cilexetil Related Compound D RS, and USP Candesartan Cilexetil Related Compound F RS, 0.001 mg/mL of USP Candesartan Cilexetil RS, 0.005 mg/mL of USP Candesartan Cilexetil Related Compound G RS from *System suitability stock solution A*, *System suitability stock*

solution B, and *System suitability stock solution C* in acetonitrile

Standard solution: 0.001 mg/mL of USP Candesartan Cilexetil RS in acetonitrile from *System suitability stock solution B*

Sample solution: Nominally 1 mg/mL of candesartan cilexetil in acetonitrile prepared as follows. Transfer a suitable quantity of candesartan cilexetil from NLT 20 powdered Tablets into a suitable volumetric flask. Add acetonitrile to fill 60% of the total volume and sonicate for 15 min with intermittent shaking in cold water. Dilute with acetonitrile to volume and pass through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 10-cm; 3.5- μ m packing L1

Sample cooler temperature: 10^o

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between candesartan cilexetil related compound B and candesartan cilexetil, *System suitability solution*

Tailing factor: NMT 2.0 for candesartan cilexetil peak, *Standard solution*

Relative standard deviation: NMT 10.0% for candesartan cilexetil peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_S peak response of candesartan cilexetil from the *Standard solution*

C_S concentration of USP Candesartan Cilexetil RS in the *Standard solution* (mg/mL)

C_U nominal concentration of candesartan cilexetil in the *Sample solution* (mg/mL)

F = relative response factor of each impurity (see *Table 4*)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Candesartan cilexetil related compound G ^a	0.17	1.30	1.0
Candesartan cilexetil related compound A ^{b,c}	0.46	1.16	—
Candesartan cilexetil related compound B ^d	0.77	1.00	1.5
Candesartan cilexetil	1.0	—	—

Candesartan cilexetil related compound D ^e	1.15	1.00	0.5
Candesartan cilexetil related compound F ^f	1.47	0.88	1.5
Any unspecified impurity	—	1.00	0.2
Total impurities	—	—	4.0
a 1- {[2'-(1 <i>H</i> -Tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylic acid.			

b Ethyl 1- {[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylate.

c Process related impurity not included in *Total impurities*.

d 1-(Cyclohexyloxycarbonyloxy)ethyl 1- {[2'-(1*H*- tetrazol-5- yl)biphenyl-4-yl]methyl}-2-hydroxybenzimidazole-7-carboxylate.

e 1- {[[(Cyclohexyloxy)carbonyl]oxy}ethyl 3- {[2'-(2-ethyl-1*H*-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl]methyl}-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate.

f 1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1- {[2'-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl}benzimidazole-7-carboxylate.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light resistant containers. Store at controlled room temperature.

- **USP Reference Standards** (11)

USP Candesartan Cilexetil RS

USP Candesartan Cilexetil Related Compound A RS

Ethyl 1- {[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylate.

C₂₆H₂₄N₆O₃ 468.51

USP Candesartan Cilexetil Related Compound B RS

1-(Cyclohexyloxycarbonyloxy)ethyl 1- {[2'-(1*H*- tetrazol-5- yl)biphenyl-4-yl]methyl}-2-hydroxybenzimidazole-7-carboxylate.

C₃₁H₃₀N₆O₆ 582.61

USP Candesartan Cilexetil Related Compound D RS

1- {[[(Cyclohexyloxycarbonyloxy)carbonyl]oxy}ethyl 3- {[2'-(2-ethyl-2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate.

C₃₃H₃₄N₆O₆ 610.67

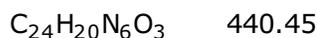
USP Candesartan Cilexetil Related Compound F RS

1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1- {[2'-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl}benzimidazole-7-carboxylate.

C₃₅H₃₈N₆O₆ 638.71

USP Candesartan Cilexetil Related Compound G RS

1- {[2'-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylic acid.



▲USP39

BRIEFING

Candesartan Cilexetil and Hydrochlorothiazide Tablets. Because there is no existing *USP* monograph for this dosage form, a new monograph based on validated methods of analysis is proposed. The liquid chromatographic procedure used in the *Assay* and *Dissolution* test is based on the Xterra RP8 brand of 5- μm L7 column. The typical retention time for hydrochlorothiazide and candesartan cilexetil are about 4.7 and 10.6 min, respectively, based on the *Assay* conditions and about 3.6 and 10 min, respectively, based on the *Dissolution* test conditions. The liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with the Inertsil C8-3 brand of 5- μm L7 column. The typical retention time for hydrochlorothiazide is 7.5 min, and about 36.7 min for candesartan cilexetil.

(SM2: S. Ramakrishna.)

Correspondence Number—C113423

Comment deadline: March 31, 2015**Add the following:****▲Candesartan Cilexetil and Hydrochlorothiazide Tablets****DEFINITION**

Candesartan Cilexetil and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% each of the labeled amount of candesartan cilexetil ($\text{C}_{33}\text{H}_{34}\text{N}_6\text{O}_6$) and hydrochlorothiazide ($\text{C}_7\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the major peaks of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peaks of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **Procedure****Solution A:** Acetonitrile, trifluoroacetic acid, and water (10: 0.1: 90)**Solution B:** Acetonitrile, trifluoroacetic acid, and water (90: 0.1: 10)**Mobile phase:** See *Table 1*.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4.0	90	10
6.0	30	70
15.0	30	70

17.0	90	10
20.0	90	10

Diluent: Acetonitrile and water (70:30)

Standard solution: Prepare solutions of USP Candesartan Cilexetil RS and USP Hydrochlorothiazide RS in *Diluent* at concentrations given in *Table 2* as follows. Transfer suitable amounts of USP Candesartan Cilexetil RS and USP Hydrochlorothiazide RS to a suitable volumetric flask. Add *Diluent*, about 50% of the total volume, and sonicate to dissolve. Dilute with *Diluent* to volume and pass through a suitable filter of 0.45- μ m pore size.

Table 2

Tablet Strength Candesartan Cilexetil/Hydrochlorothiazide (mg/mg)	Concentration of Candesartan Cilexetil (mg/mL)	Concentration of Hydrochlorothiazide (mg/mL)
16/12.5	0.32	0.25
32/12.5	0.64	0.25
32/25	0.32	0.25

Sample solution: Nominally equivalent to the concentration mentioned in *Table 2* prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask. Add about 60% of the total volume of *Diluent*, and sonicate for about 25 min with intermittent shaking. Cool to room temperature and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- μ m pore size.

Chromatographic system:

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector

Assay: UV 282 nm

Identification test B: Diode array, UV 282 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7. [Note—Conditioning of the column with *Solution A* and *Solution B* (80:20) for about 30 min is recommended prior to use.]

Column temperature: 30 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for both candesartan cilexetil and hydrochlorothiazide peaks

Relative standard deviation: NMT 2.0% for both candesartan cilexetil and hydrochlorothiazide peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount each of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_{\overline{f}}$ peak response of candesartan cilexetil or hydrochlorothiazide from the *Sample solution*

$r_{\overline{s}}$ peak response of candesartan cilexetil or hydrochlorothiazide from the *Standard solution*

$C_{\overline{s}}$ concentration of USP Candesartan Cilexetil RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

$C_{\overline{f}}$ nominal concentration of candesartan cilexetil or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of candesartan cilexetil and hydrochlorothiazide

PERFORMANCE TESTS

• Dissolution 〈 711 〉

Medium

For Tablets labeled to contain 16 mg/12.5 mg of candesartan

cilexetil/hydrochlorothiazide: 0.35% Polysorbate 20 in 0.05 M phosphate buffer pH 6.5; 900 mL

For Tablets labeled to contain 32 mg/12.5 mg and 32 mg/25 mg of candesartan

cilexetil/hydrochlorothiazide: 0.70% Polysorbate 20 in 0.05 M phosphate buffer pH 6.5; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Solution A and Solution B: Proceed as directed in the *Assay*.

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	80	20
3.0	80	20
5.0	30	70
10.0	30	70
13.0	80	20
16.0	80	20

Standard stock solution A: 0.72 mg/mL of USP Candesartan Cilexetil RS in acetonitrile prepared as follows. Transfer a quantity of USP Candesartan Cilexetil RS to a suitable volumetric flask. Add acetonitrile, about 50% of volume of the flask, and sonicate. Dilute with acetonitrile to volume.

Standard stock solution B: 0.28 mg/mL of USP Hydrochlorothiazide RS in acetonitrile prepared as follows. Transfer a quantity of USP Hydrochlorothiazide RS to a suitable volumetric flask. Add acetonitrile, about 50% of volume of the flask, and sonicate. Dilute with acetonitrile to volume.

Standard solution: Prepare solutions of concentrations per *Table 4*, from *Standard stock solution A* and *Standard stock solution B* in the appropriate *Medium*. Pass through a suitable filter of 0.45- μ m pore size.

Table 4

Tablet Strength Candesartan Cilexetil/Hydrochlorothiazide (mg/mg)	Concentration of Candesartan Cilexetil (mg/mL)	Concentration of Hydrochlorothiazide (mg/mL)
16/12.5	0.018	0.014
32/12.5 and 32/25	0.036	0.014

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Chromatographic system:

(See *Chromatography* \langle 621 \rangle , *System Suitability*.)

Mode: LC

Detector: UV 264 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7. [Note—Conditioning of the column with *Solution A* and *Solution B* (80:20) for NLT 20 min is recommended prior to use.]

Flow rate: 1 mL/min

Injection volume: 50 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for both candesartan cilexetil and hydrochlorothiazide peaks

Relative standard deviation: NMT 2.0% for both candesartan cilexetil and hydrochlorothiazide peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) or hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of candesartan cilexetil or hydrochlorothiazide from the *Sample solution*

r_S = peak response of candesartan cilexetil or hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Candesartan Cilexetil RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) each of the labeled amounts of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.

- **Uniformity of Dosage Units** \langle 905 \rangle : Meet the requirements

IMPURITIES

- **Organic Impurities**

Solution A and **Solution B:** Proceed as directed in the *Assay*.

Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	95	5
8	95	5
15	60	40
20	60	40
30	40	60
35	30	70
45	20	80
50	0	100
60	0	100
62	95	5
70	95	5

Diluent A: Acetonitrile and water (70:30)

Diluent B: Acetonitrile and water (50:50)

Peak identification stock solution A: 0.05 mg/mL each of USP Candesartan Cilexetil Related Compound A RS, USP Candesartan Cilexetil Related Compound B RS, USP Candesartan Cilexetil Related Compound D RS, and USP Candesartan Cilexetil Related Compound F RS in acetonitrile

Peak identification stock solution B: 0.1 mg/mL of USP Candesartan Cilexetil RS in acetonitrile

Peak identification stock solution C: 0.5 mg/mL of USP Candesartan Cilexetil Related Compound G RS in methanol

Peak identification solution: 0.0015 mg/mL each of USP Candesartan Cilexetil Related Compound A RS, USP Candesartan Cilexetil Related Compound B RS, USP Candesartan Cilexetil Related Compound D RS, and USP Candesartan Cilexetil Related Compound F RS, 0.001 mg/mL of USP Candesartan Cilexetil RS, and 0.005 mg/mL of USP Candesartan Cilexetil Related Compound G RS from *Peak identification stock solution A*, *Peak identification stock solution B*, and *Peak identification stock solution C* in acetonitrile

System suitability stock solution: 0.05 mg/mL each of USP Benzothiadiazine Related Compound A RS and USP Hydrochlorothiazide RS, and 0.1 mg/mL of USP Chlorothiazide RS in *Diluent B*

System suitability solution: 2.5 µg/mL each of USP Benzothiadiazine Related Compound A RS and USP Hydrochlorothiazide RS, and 5 µg/mL of USP Chlorothiazide RS in *Diluent A* from *System suitability stock solution*

Standard stock solution: 1.6 mg/mL of USP Candesartan Cilexetil RS and 0.6 mg/mL of USP Hydrochlorothiazide RS in *Diluent A* prepared as follows. Transfer a quantity of USP Candesartan Cilexetil RS and USP Hydrochlorothiazide RS to a suitable volumetric flask. Add *Diluent A*, about 60% of the total volume, and sonicate to dissolve. Dilute with *Diluent A* to volume.

Standard solution: 0.008 mg/mL of USP Candesartan Cilexetil RS and 0.003 mg/mL of USP Hydrochlorothiazide RS in *Diluent A* from *Standard stock solution*. Pass through a suitable filter of 0.45-µm pore size.

Sample solution: Nominally 1.5 mg/mL of candesartan cilexetil in acetonitrile prepared as follows. Transfer about 75 mg of candesartan cilexetil, from NLT 20 finely powdered Tablets, to a 50-mL volumetric flask. Add 30 mL of *Diluent A* and sonicate for 20 min with

intermittent shaking in cold water. Allow it to reach room temperature, dilute with *Diluent A* to volume, and pass through a suitable filter of 0.45- μm pore size.

Chromatographic system:

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L7

Column temperature: 35 $^{\circ}$

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*. [Note—The relative retention times for benzothiadiazine related compound A and chlorothiazide in *Table 6* are relative to hydrochlorothiazide.]

Suitability requirements

Resolution: NLT 1.5 between benzothiadiazine related compound A and chlorothiazide; NLT 1.5 between chlorothiazide and hydrochlorothiazide, *System suitability solution*

Tailing factor: NMT 2.0 for both candesartan cilexetil and hydrochlorothiazide peaks, *Standard solution*

Relative standard deviation: NMT 10.0% for both candesartan cilexetil and hydrochlorothiazide peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity of candesartan cilexetil and any unspecified impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_S peak response of candesartan cilexetil from the *Standard solution*

C_S concentration of USP Candesartan Cilexetil RS in the *Standard solution* (mg/mL)

C_U nominal concentration of candesartan cilexetil in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 6*)

Calculate the percentage of benzothiadiazine related compound A and chlorothiazide in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of benzothiadiazine related compound A and chlorothiazide from the *Sample solution*

r_S peak response of hydrochlorothiazide from the *Standard solution*

C_S concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

C_U nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 6*)

Acceptance criteria: See *Table 6*.

Table 6

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Candesartan cilexetil related compound G ^a	0.51	1.11	1.0
Candesartan related compound A ^{b,c}	0.73	1.16	—
Benzothiadiazine related compound A ^d	0.75	1.15	1.0
Chlorothiazide ^e	0.85	0.48	0.5
Candesartan cilexetil related compound B ^f	0.89	0.90	1.75
Candesartan cilexetil	1.00	—	—
Candesartan cilexetil related compound D ^g	1.06	0.91	0.5
Candesartan cilexetil related compound F ^h	1.24	0.83	1.5
Any unspecified degradation product	—	1.00	0.2
Total impurities	—	—	4.0

^a 1- {[2'-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylic acid.

^b Ethyl 1- {[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylate.

^c Process related impurity not included in *Total impurities*.

^d 4-Amino-6-chloro-1,3-benzenedisulfonamide.

^e 6-Chloro-2-*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide.

^f 1-(Cyclohexyloxycarbonyloxy)ethyl 1- {[2'-(1*H*- tetrazol-5-yl)biphenyl-4-yl]methyl}-2-hydroxybenzimidazole-7-carboxylate.

^g 1- {[[(Cyclohexyloxycarbonyloxy)carbonyl]oxy}ethyl 3- {[2'-(2-ethyl-2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate.

^h 1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1- {[2'-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl}benzimidazole-7-carboxylate.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

- **USP Reference Standards** { 11 }

USP Benzothiadiazine Related Compound A RS

4-Amino-6-chloro-1,3-benzenedisulfonamide.

C₆ H₈ ClN₃ O₄ S₂ 285.73

USP Candesartan Cilexetil RS

USP Candesartan Cilexetil Related Compound A RS

Ethyl 1-{{[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl}}-2-ethoxybenzimidazole-7-carboxylate.

C₂₆H₂₄N₆O₃ 468.51

USP Candesartan Cilexetil Related Compound B RS

1-(Cyclohexyloxycarbonyloxy)ethyl 1-{{[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl}}-2-hydroxybenzimidazole-7-carboxylate.

C₃₁H₃₀N₆O₆ 582.61

USP Candesartan Cilexetil Related Compound D RS

1-{{[(Cyclohexyloxycarbonyloxy)carbonyl]oxy}}ethyl 3-{{[2'-(2-ethyl-2H-tetrazol-5-yl)biphenyl-4-yl]methyl}}-2-oxo-2,3-dihydro-1H-benzimidazole-4-carboxylate.

USP Candesartan Cilexetil Related Compound F RS

1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-{{[2'-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl}}benzimidazole-7-carboxylate.

C₃₅H₃₈N₆O₆ 638.71

USP Candesartan Cilexetil Related Compound G RS

1-{{[2'-(1H-Tetrazol-5-yl)biphenyl-4-yl]methyl}}-2-ethoxybenzimidazole-7-carboxylic acid.

C₂₄H₂₀N₆O₃ 440.45

USP Chlorothiazide RS

USP Hydrochlorothiazide RS

▲USP39

BRIEFING

Chlorpheniramine Maleate Tablets, *USP 38* page 2779. The United States Pharmacopeia (USP) is engaged in the ongoing challenge of obtaining information needed to create and sustain up-to-date drug product quality monographs for drugs marketed under the FDA over-the-counter (OTC) drug monograph system (21 CFR Part 330). The following revisions to the *Assay* and *Identification* tests are being proposed:

1. Add an *Identification* test *B* procedure based on the retention time comparison of the chlorpheniramine peak from the proposed *Assay* procedure to complement the spectroscopic identification test.
2. Replace the UV procedure in the *Assay* with the liquid chromatography procedure from the general chapter *Drug Product Assay Tests—Organic Chemical Medicines* (321), *Procedure 1*. This procedure was validated using the Waters Acquity UPLC HSS T3 brand of L1 column. The retention time for chlorpheniramine is about 6.1 min.
3. The *Packaging and Storage* section has been updated to be consistent with the manufacturers' package inserts.
4. Additional Reference Standards have been added to the *USP Reference Standards* section to support the revision of the *Assay*.

Chapter (321), also published in this issue of *PF*, is a procedure that was developed and validated for over-the-counter (OTC) medicines. Further information can be found in the *Stimuli* article [Medicines Marketed under the Food and Drug Administration Over-the-Counter \(FDA OTC\) Drug Monograph System Regulations: Strategy for Developing Compendial Quality Standards](#), also published in this issue of *PF*. This article describes in detail the rationale for development of this chapter and other related chapters, as well as the associated impact on drug product monographs. USP plans to continue with this initiative to revise or establish standards for OTC drug products.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: A. Potts.)

Correspondence Number—C151306

Comment deadline: March 31, 2015

Chlorpheniramine Maleate Tablets

DEFINITION

Change to read:

Chlorpheniramine Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ~~▲~~chlorpheniramine maleate ~~▲~~[▲]*USP39* ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$).

IDENTIFICATION

Change to read:

•

~~▲~~[▲]*A. ▲*[▲]*USP39*

Standard solution: 1.25 mg/mL of USP Chlorpheniramine Maleate RS in dilute hydrochloric acid (1 in 100)

Sample solution: 1.25 mg/mL of chlorpheniramine maleate from a portion of powdered Tablets in dilute hydrochloric acid (1 in 100)

Analysis: Treat each solution as follows. Render alkaline, to a pH of about 11, with sodium hydroxide solution (1 in 10). Extract with two 50-mL portions of solvent hexane, collect the extracts in a beaker, and evaporate to dryness. Prepare a mineral oil dispersion of the residue so obtained and determine the infrared absorption spectrum of the preparation in the region between 2 and 12 μm .

Acceptance criteria: The spectrum of the *Sample solution* exhibits maxima only at the same wavelengths as those of the *Standard solution*.

Add the following:

~~▲~~[▲] **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ~~▲~~[▲]*USP39*

ASSAY

Delete the following:

~~▲~~[▲] **Procedure**

~~**Standard solution:** 0.2 mg/mL of USP Chlorpheniramine Maleate RS in dilute hydrochloric acid (1 in 100), and treat 20.0 mL of this solution the same as the *Sample solution* in dilute hydrochloric acid (1 in 100) of the portion of Tablets taken.~~

~~**Sample solution:** Using a portion of powdered Tablets equivalent to 4 mg of chlorpheniramine maleate, proceed as directed under *Salts of Organic Nitrogenous Bases* (501), but using dilute hydrochloric acid (1 in 100) instead of the dilute sulfuric acid (1~~

in 350), and dilute sulfuric acid (1 in 70), and using solvent hexane instead of the ether, and diluting 10 mL of the *Sample solution* with dilute hydrochloric acid (1 in 100) to 25.0 mL.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 264 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of chlorpheniramine maleate in the *Sample solution* (mg/mL)

▲USP39

Add the following:

- ▲● **Drug Product Assay Tests—Organic Chemical Medicines**, *Procedure 1* 〈 321 〉: 90.0%–110.0% ▲USP39

PERFORMANCE TESTS

Change to read:

- **Dissolution** 〈 711 〉

Medium: 0.01 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 30 min

Detector: UV 265 nm

Standard solution: USP Chlorpheniramine Maleate RS of a known concentration in *Medium*

Sample solution: Sample per *Dissolution* 〈 711 〉. Filter a portion of the solution under test, suitably diluted with *Medium*, to a concentration that is similar to the *Standard solution*.

Tolerances: NLT 80% (Q) of the labeled amount of

▲chlorpheniramine maleate▲USP39

($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) is dissolved.

- **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers.

▲Store at room temperature and protect from moisture.▲USP39

Change to read:● **USP Reference Standards** 〈 11 〉

▲USP Caffeine RS ▲USP39

USP Chlorpheniramine Maleate RS

▲USP Dextromethorphan Hydrobromide RS

USP Diphenhydramine Hydrochloride RS

USP Doxylamine Succinate RS

USP Pseudoephedrine Hydrochloride RS ▲USP39

BRIEFING

Cromolyn Sodium Ophthalmic Solution, *USP 38* page 2962. The revisions appearing in *PF 40(1)* have been canceled. It is proposed to modernize the monograph with the following changes.

1. Replace the nonspecific *Assay* by UV with a specific high-performance liquid chromatographic procedure developed and validated for this product. The procedure uses the Zorbax SB C8 brand of L7 column manufactured by Agilent Technologies in which cromolyn elutes at about 8.4 min.
2. Replace the TLC procedure in the test for *Organic Impurities* with a more selective and validated HPLC procedure. The procedure uses the same chromatographic parameters as those proposed in the *Assay*, except the *Autosampler temperature* is set at 4°.
3. Replace *Identification* test *A* based on UV analysis with the retention time agreement based on the *Assay*.
4. Add additional storage requirements based on the drug package insert.
5. Add USP impurity Reference Standards to ensure the system suitability evaluation for the HPLC procedures.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: D. Min.)

Correspondence Number—C145089; C126522

Comment deadline: March 31, 2015

Cromolyn Sodium Ophthalmic Solution**DEFINITION**

Cromolyn Sodium Ophthalmic Solution is a sterile, aqueous solution of Cromolyn Sodium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cromolyn sodium ($C_{23}H_{14}Na_2O_{11}$). It may contain suitable antimicrobial and stabilizing agents.

IDENTIFICATION

Change to read:• **A.**

~~**Sample solution:** A solution (1 in 40,000) in *Buffer* prepared as directed in the *Assay*~~

~~**Acceptance criteria:** The UV absorption spectrum of the *Sample solution* exhibits maxima at the same wavelengths as that of a similar solution of USP Cromolyn Sodium RS, concomitantly measured.~~

▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP39

ASSAY**Change to read:**• **Procedure**

~~**Buffer:** Dissolve 70 g of anhydrous dibasic sodium phosphate in 900 mL of water. Adjust to a pH of 7.4 by the addition of dilute phosphoric acid (1 in 10). Dilute with water to 1000 mL, and mix. Transfer 10 mL of this solution to a 100 mL volumetric flask, and dilute with water to volume.~~

~~**Standard stock solution:** 250 µg/mL of USP Cromolyn Sodium RS in water.~~

~~**Standard solution:** 25 µg/mL of USP Cromolyn Sodium RS prepared by transferring 10 mL of *Standard stock solution* to a 100 mL volumetric flask, add 1 mL of *Buffer*, and dilute with water to volume.~~

~~**Sample stock solution:** Transfer 4 mL of Ophthalmic Solution to a 100 mL volumetric flask and dilute with water.~~

~~**Sample solution:** Nominally equivalent to 32 µg of cromolyn sodium prepared from a portion of *Sample stock solution* to a 250 mL volumetric flask. Add 2.5 mL of *Buffer*, and dilute with water to volume.~~

~~**Spectrometric conditions**~~

~~**Mode:** UV~~

~~**Analytical wavelength:** 326 nm~~

~~**Cell:** 1 cm~~

~~**Blank:** *Buffer* (1 in 100)~~

~~**Analysis**~~

~~**Samples:** *Standard solution* and *Sample solution*~~

Calculate the percentage of cromolyn sodium ($C_{23}H_{14}Na_2O_{11}$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

~~A_S absorbance of the *Sample solution*~~

~~A_U absorbance of the *Standard solution*~~

~~C_S concentration of USP Cromolyn Sodium RS in the *Standard solution* (µg/mL)~~

~~C_U nominal concentration of cromolyn sodium in the *Sample solution* (µg/mL)~~

▲Buffer:

5.6 g/L of monobasic potassium phosphate and 22.2 g/L of myristyltrimethylammonium bromide in water. Adjust with sodium hydroxide solution to a pH of 6.5.

Mobile phase: Methanol and *Buffer* (55:45)

Diluent: Acetonitrile and water (30:70)

System suitability solution: 0.5 mg/mL of USP Cromolyn Sodium RS and 0.02 mg/mL each of USP Cromolyn Related Compound A RS and USP Cromolyn Related Compound B RS in *Diluent*

Standard solution: 0.5 mg/mL of USP Cromolyn Sodium RS in *Diluent*. Sonication may be needed to aid dissolution.

Sample solution: Nominally equivalent to 0.5 mg/mL of cromolyn sodium from a volume of Ophthalmic Solution in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 326 nm

Column: 4.6-mm × 10-cm; 3.5-μm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between cromolyn related compound B and cromolyn related compound A; NLT 2.0 between cromolyn related compound A and cromolyn sodium, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cromolyn sodium (C₂₃H₁₄Na₂O₁₁) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of cromolyn from the *Sample solution*

r_S

= peak response of cromolyn from the *Standard solution*

C_S

= concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

▲USP39

Acceptance criteria: 90.0%–110.0%

IMPURITIES**Change to read:**● **Organic Impurities**

Diluent: Stabilizer-free tetrahydrofuran, acetone, and water (4:1:6)

Standard solution A: 10 mg/mL of USP Cromolyn Sodium RS in *Diluent*

Standard solution B: 0.1 mg/mL of USP Cromolyn Sodium RS in *Diluent*

Sample solution: A portion of Ophthalmic Solution

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Developing solvent system: Chloroform, methanol, and glacial acetic acid (9:9:2)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* in a TLC plate and allow the spots to dry. Position the plate in a developing chamber, and develop the chromatograms in *Developing solvent system* until the solvent front have moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*. Any spot of the *Sample solution* moving ahead of the principal spot is not more intense than the spot of *Standard solution B* (1.0%).

▲**Mobile phase, Diluent, and System suitability solution:** Proceed as directed in the *Assay*.

Standard solution: 0.002 mg/mL each of USP Cromolyn Related Compound B RS, USP Cromolyn Related Compound A RS, and USP Cromolyn Sodium RS in *Diluent*

Sample solution: Nominally equivalent to 2 mg/mL of cromolyn sodium from a volume of Ophthalmic Solution in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 326 nm

Column: 4.6-mm \times 10-cm; 3.5- μ m packing L7

Temperatures

Autosampler: 4 $^{\circ}$

Column: 40 $^{\circ}$

Flow rate: 1.5 mL/min

Injection volume: 20 μL

Run time: 2 times the retention time of cromolyn

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between cromolyn related compound B and cromolyn related compound A; NLT 2.0 between cromolyn related compound A and cromolyn sodium, *System suitability solution*

Relative standard deviation: NMT 3% for six replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each cromolyn related compound in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of cromolyn related compound A or cromolyn related compound B from the *Sample solution*

r_S

= peak response of cromolyn related compound A or cromolyn related compound B from the *Standard solution*

C_S

= concentration of the corresponding cromolyn related compound in the *Standard solution* (mg/mL)

C_U

= nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of each unspecified degradation product from the *Sample solution*

r_S

= peak response of cromolyn from the *Standard solution*

C_S

= concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)**Acceptance criteria:** See *Table 1*.**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cromolyn related compound B	0.4	0.75
Cromolyn related compound A	0.5	0.75
Cromolyn sodium	1.0	—
Any unspecified individual degradation product	—	0.75
Total impurities	—	2.0

▲*USP39***SPECIFIC TESTS**

- **pH** 〈 791 〉: 4.0–7.0
- **Sterility Tests** 〈 71 〉: Meets the requirements

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in tight, light-resistant, single-dose or multiple-dose containers. Ophthalmic Solution that is packaged in multiple-dose containers contains a suitable antimicrobial agent.

▲Store between 15°–30°. ▲*USP39***Change to read:**

- **USP Reference Standards** 〈 11 〉

▲USP Cromolyn Related Compound A RS

1,3-Bis(2-acetyl-3-hydroxyphenoxy) propan-2-ol.

C₁₉H₂₀O₇ 360.36

USP Cromolyn Related Compound B RS

Diethyl 5,5'-[(2-hydroxypropane-1,3-diyl)bis(oxy)]bis(4-oxo-4H-chromene-2-carboxylate).

C₂₇H₂₄O₁₁ 524.48 ▲*USP39*USP Cromolyn Sodium RS **BRIEFING**

Cyanocobalamin Tablets, *USP 38* page 2968. It is proposed to make the following changes in the monograph:

1. Add a new HPLC procedure in the *Assay*. The liquid chromatographic *Procedure 2* is based on analyses performed with the Waters Acquity UPLC BEH C18 brand of L1 column.
2. Add performance specifications for Tablets recommended to be disintegrated in the mouth before swallowing.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(DS: N. Davydova.)

Correspondence Number—C153943

Comment deadline: March 31, 2015

Cyanocobalamin Tablets

DEFINITION

Cyanocobalamin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$).

IDENTIFICATION

Change to read:

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*,

▲*Procedure 1* or *Procedure 2*. ▲*USP39*

ASSAY

Change to read:

▲[

Note—Where more than one assay procedure is given in the monograph, the requirements may be met by following any one of the specified procedures. The procedure used is stated in the labeling only if *Procedure 1* is not used.] ▲*USP39*

Change to read:

- **Procedure**

▲**Procedure 1** ▲*USP39*

[Note—Use low-actinic glassware throughout this procedure.]

Mobile phase: Methanol and water (7:13)

Standard solution: 5 µg/mL of cyanocobalamin from USP Cyanocobalamin RS in water

Sample solution: Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 500 µg of cyanocobalamin, to a 100-mL volumetric flask, add 60 mL of water, and sonicate for 5 min. Dilute with water to volume, and filter.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC**Detector:** 361 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L1**Flow rate:** 0.5 mL/min**Injection volume:** 100 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution and Sample solution*

Calculate the percentage of the labeled amount of cyanocobalamin (C₆₃H₈₈CoN₁₄O₁₄P) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of cyanocobalamin from USP Cyanocobalamin RS in the *Standard solution* (μg/mL) C_U = nominal concentration of cyanocobalamin in the *Sample solution* (μg/mL)**Acceptance criteria:** 90.0%–110.0%**Add the following:****▲● Procedure 2**

[Note—Use low-actinic glassware throughout this procedure. Inject samples within 30 min.]

Buffer: Dissolve 470.5 mg of low UV hexanesulfonic acid sodium salt in water, add 1 mL of phosphoric acid, dilute with water to 1000 mL, and mix. Adjust with 50% potassium hydroxide to a pH of 3.5.

Mobile phase: Acetonitrile and *Buffer*. See *Table 1* for gradient.

Table 1

Time (min)	Acetonitrile (%)	Buffer (%)
0	1.0	99.0
0.5	1.0	99.0
1.2	2.3	97.7
1.4	5.0	95.0
2.5	7.0	93.0
5.0	18.0	82.0
5.5	25.0	75.0
6.5	25.0	75.0
7.0	1.0	99.0
8.0	1.0	99.0

Standard solution: 1 μg/mL of cyanocobalamin from USP Cyanocobalamin RS in water

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder,

equivalent to 25 µg of cyanocobalamin, to a suitable Erlenmeyer flask with a stopper, pipet 25 mL of water, sonicate for 5 min, and shake vigorously for 2 min. Pass through a membrane filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: UPLC

Detector: UV 361 nm

Column: 2.1-mm × 10-cm; 1.7-µm packing L1

Column temperature: 35°

Flow rate: 0.5 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cyanocobalamin (C₆₃H₈₈CoN₁₄O₁₄P) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of cyanocobalamin from USP Cyanocobalamin RS in the *Standard solution* (µg/mL)

C_U nominal concentration of cyanocobalamin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%▲*USP39*

PERFORMANCE TESTS

Change to read:

- **Disintegration** 〈 701 〉

Medium: Water

Time: 30 min

▲If the label recommends to disintegrate the Tablets in the mouth before swallowing: NMT 3 min

▲*USP39*

Acceptance criteria: Meet the requirements

- **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Add the following:

- ▲● **Labeling:** The label states whether the Tablet is to be disintegrated in the mouth. The labeling states with which assay procedure the product complies only if *Procedure 1* is not used. ▲USP39

- **USP Reference Standards** { 11 }

USP Cyanocobalamin RS

BRIEFING

Daunorubicin Hydrochloride for Injection, USP 38 page 3004. As part of the USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. The current HPLC procedure in the *Assay* is replaced with a stability-indicating UHPLC procedure that employs a mass spectroscopy-compatible *Mobile phase* and a modern analytical column. The procedure was validated using the Acquity UPLC BEH C18 brand of L1 column manufactured by Waters. Daunorubicin elutes at about 7 min.
2. Add a validated *Organic Impurities* method that is used to monitor the impurities/degradants. The procedure uses the same chromatographic parameters as those proposed in the *Assay*.
3. Add the required A designation to the existing *Identification* test. Add *Identification* test B based on the UV spectrum as proposed in the *Assay*.
4. Delete the test for *Water Determination, Method I* because water content is formulation dependent.
5. Add the *Sterility Test* to be consistent with the drug product sterility requirement.
6. Add an additional requirement in the *Packaging and Storage* section based on the drug package insert.
7. Add two USP Reference Standards to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM1: D. Min.)

Correspondence Number—C135803

Comment deadline: March 31, 2015

Daunorubicin Hydrochloride for Injection**DEFINITION**

Daunorubicin Hydrochloride for Injection is a sterile mixture of Daunorubicin Hydrochloride and Mannitol. It contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of daunorubicin ($C_{27}H_{29}NO_{10}$).

IDENTIFICATION**Change to read:**

-

▲A.▲USP39

The retention time of the main peak of the *Sample solution* corresponds to that of the

Standard solution, as directed in the Assay.

Add the following:

- ▲● **B.** The UV spectrum of the main peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the proposed Assay. ▲*USP39*

ASSAY

Change to read:

● **Procedure**

Mobile phase: Acetonitrile and water (19:31). Adjust with phosphoric acid to a pH of 2.2 ± 0.2.

Standard solution: 250 µg/mL of daunorubicin from USP Daunorubicin Hydrochloride RS in *Mobile phase*

System suitability solution: 250 µg/mL of doxorubicin hydrochloride in the *Standard solution*

Sample solution: Nominally 250 µg/mL of daunorubicin from Daunorubicin Hydrochloride for Injection prepared as follows. Transfer the contents of 1 vial of Daunorubicin Hydrochloride for Injection with the aid of *Mobile phase* to a suitable volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6 mm × 30 cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 5 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for doxorubicin and daunorubicin are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3 between the doxorubicin and the daunorubicin peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of daunorubicin ($C_{27}H_{29}NO_{10}$) in the portion of Daunorubicin Hydrochloride for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Daunorubicin Hydrochloride RS in the *Standard solution* (µg/mL)

C_U nominal concentration of daunorubicin in the *Sample solution* (µg/mL)

~~P = potency of daunorubicin in USP Daunorubicin Hydrochloride RS ($\mu\text{g}/\text{mg}$)~~

~~F = conversion factor, 0.001 $\text{mg}/\mu\text{g}$~~

▲Solution A:

0.1% Trifluoroacetic acid

Solution B: Acetonitrile, methanol, and trifluoroacetic acid (800:200:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
3	70	30
13	40	60
13.1	70	30
15	70	30

Diluent: *Solution A* and *Solution B* (50:50)

System suitability solution: 0.1 mg/mL each of USP Daunorubicin Hydrochloride RS and USP Daunorubicinone RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Daunorubicin Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.1 mg/mL of daunorubicin from Daunorubicin Hydrochloride for Injection prepared as follows. Reconstitute NLT 5 vials of Daunorubicin Hydrochloride for Injection using a suitable volume of *Diluent*. Transfer the contents of the reconstituted vials with the aid of *Diluent* to a suitable volumetric flask. Rinse the vials with *Diluent* NLT 2 times. Dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm. For *Identification* test B, use a diode array detector in the range of 200–300 nm.

Column: 2.1-mm × 10-cm; 1.7- μm packing L1

Autosampler temperature: 4°

Flow rate: 0.4 mL/min

Injection volume: 1.7 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.5 between daunorubicin and daunorubicinone, *System suitability solution*

Tailing factor: NMT 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of daunorubicin ($\text{C}_{27}\text{H}_{29}\text{NO}_{10}$) in the

portion of Daunorubicin Hydrochloride for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Daunorubicin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of daunorubicin in the *Sample solution* (mg/mL)

P

= potency of daunorubicin in USP Daunorubicin Hydrochloride RS ($\mu\text{g}/\text{mg}$)

F

= conversion factor, 0.001 mg/ μg

▲USP39

Acceptance criteria: 90.0%–115.0%

IMPURITIES

Add the following:

▲● Organic Impurities

Mobile phase, Diluent, System suitability solution, and Chromatographic system:

Proceed as directed in the *Assay*.

Standard solution: 2 $\mu\text{g}/\text{mL}$ each of USP Dihydrodaunorubicin Hydrochloride RS, USP Daunorubicin Hydrochloride RS, and USP Daunorubicinone RS in *Diluent*

Sample solution: Nominally 0.4 mg/mL of daunorubicin from Daunorubicin Hydrochloride for Injection prepared as follows. Reconstitute NLT 5 vials using a suitable volume of *Diluent*. Transfer the contents of the reconstituted vials with the aid of *Diluent* to a suitable volumetric flask. Rinse the vials with *Diluent* NLT 2 times. Dilute with *Diluent* to volume.

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.5 between daunorubicin and daunorubicinone, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dihydrodaunorubicin hydrochloride in the portion of Daunorubicin Hydrochloride for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of dihydrodaunorubicin from the *Sample solution*

r_S = peak response of dihydrodaunorubicin from the *Standard solution*

C_S = concentration of USP Dihydrodaunorubicin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of daunorubicin in the *Sample solution* (mg/mL)

P = potency of dihydrodaunorubicin in USP Dihydrodaunorubicin Hydrochloride RS (mg/mg)

Calculate the percentage of daunorubicinone in the portion of Daunorubicin Hydrochloride for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of daunorubicinone from the *Sample solution*

r_S = peak response of daunorubicinone from the *Standard solution*

C_S = concentration of USP Daunorubicinone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of daunorubicin in the *Sample solution* (mg/mL)

P = potency of daunorubicinone in USP Daunorubicinone RS (mg/mg)

Calculate the percentage of any individual unspecified impurity in the portion of Daunorubicin Hydrochloride for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*

r_S = peak response of daunorubicin from the *Standard solution*

C_S = concentration of USP Daunorubicin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of daunorubicin in the *Sample solution* (mg/mL)

P = potency of daunorubicin in USP Daunorubicin Hydrochloride RS ($\mu\text{g}/\text{mg}$)

F = conversion factor, 0.001 mg/ μg

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Doxorubicin ^a	0.50	—
Dihydrodaunorubicin ^{b,c}	0.72	0.5
	0.75	
Daunorubicin	1.0	—
Daunorubicinone ^d	1.2	0.5
Any individual unspecified impurity	—	0.5
Total impurities	—	2.5

a Process impurities are controlled in the drug substance and are not to be reported here. They are not included in total impurities.

b The system resolves two diastereoisomers of dihydrodaunorubicin. The limit is for the sum of the two diastereoisomers.

c (8S,10S)-10-[(3-Amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-(1-hydroxyethyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione hydrochloride.

d Daunorubicinol; (8S,10S)-8-Acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.

▲USP39

SPECIFIC TESTS

- pH 〈 791 〉

Sample solution: Constitute as directed in the labeling

Acceptance criteria: 4.5–6.5

Delete the following:

- ▲• ~~Water Determination, Method I 〈 921 〉~~

~~**Analysis:** Proceed as directed for a hygroscopic specimen~~

~~**Acceptance criteria:** NMT 3.0%▲USP39~~

- **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products 〈 1 〉*, *Constituted Solutions*.

- **Bacterial Endotoxins Test 〈 85 〉:** It contains NMT 4.3 USP Endotoxin Units/mg of daunorubicin.

Add the following:

- ▲• **Sterility Tests 〈 71 〉:** Meets the requirements ▲USP39

- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products 〈 1 〉*.

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in light-resistant *Containers for Sterile Solids* as described in *Injections and Implanted Drug Products 〈 1 〉*.

▲Store in a refrigerator.▲USP39

Change to read:

- **USP Reference Standards** (11)

USP Daunorubicin Hydrochloride RS

▲USP Daunorubicinone RS

(8S,10S)-8-Acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.

C₂₁H₁₈O₈ 398.36

USP Dihydrodaunorubicin Hydrochloride RS

(8S,10S)-10-[(3-Amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-(1-hydroxyethyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione hydrochloride.

C₂₇H₃₁NO₁₀HCl 566.00

▲USP39

USP Endotoxin RS

BRIEFING

Diltiazem Hydrochloride Extended-Release Capsules, *USP 38* page 3128. As part of the monograph modernization effort, it is proposed to make the following changes:

1. Add a new stability indicating UHPLC procedure for the test of *Organic Impurities*. The UHPLC procedure is based on analyses performed with the Waters Acquity UPLC BEH C18 brand of L1 column. The typical retention time for the diltiazem peak in the *Organic Impurities* test is 16 min.
2. Replace the current HPLC Assay with a procedure using the same UHPLC parameters as proposed in the test for *Organic Impurities*.
3. Replace the *Identification* test A by visual detection with UV spectrum as proposed in the Assay.
4. Add an additional storage condition in *Packaging and Storage* based on information from the approved drug product.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: D. Min.)

Correspondence Number—C120149

Comment deadline: March 31, 2015

Diltiazem Hydrochloride Extended-Release Capsules

DEFINITION

Diltiazem Hydrochloride Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of diltiazem hydrochloride (C₂₂H₂₆N₂O₄S·HCl).

IDENTIFICATION

Change to read:

- **A.**

~~**Indicator solution:** Transfer 17.4 g of ammonium thiocyanate and 2.8 g of cobalt chloride~~

to a 100-mL volumetric flask, add 50 mL of water, and sonicate for 10 min. Dilute with water to volume.

Analysis: Grind the content of 1 Capsule, and transfer to a 15-mL screw-capped test tube. Add 10 mL of 0.1 N hydrochloric acid, shake, and filter. Add 2 mL of *Indicator solution* to 2 mL of the filtrate, and shake. Add 5 mL of chloroform, and shake.

Acceptance criteria: A blue color develops in the chloroform layer.

▲The UV-Vis spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP39

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

Buffer: 6.9 g of monobasic potassium phosphate in 1000 mL of water. Adjust with 0.1 N hydrochloric acid to a pH of 3.0, and add 0.50 mL of triethylamine.

Mobile phase: Acetonitrile and *Buffer* (50:50)

Standard stock solution: 1.2 mg/mL of USP Diltiazem Hydrochloride RS and 0.02 mg/mL of USP Desacetyl Diltiazem Hydrochloride RS in methanol

Standard solution: 24 µg/mL of USP Diltiazem Hydrochloride RS from *Standard stock solution*, in *Mobile phase*

Sample stock solution: Nominally 1.2 mg/mL of diltiazem hydrochloride in methanol prepared as follows. Transfer the equivalent to 120 mg of diltiazem hydrochloride from NLT 20 Capsules (mix and grind the contents thoroughly) to a 100-mL volumetric flask. Add approximately 60 mL of methanol, and shake by mechanical means for 30 min. Sonicate the resulting solution for 10 min to complete the extraction. Dilute with methanol to volume.

Sample solution: Nominally 24 µg/mL of diltiazem hydrochloride in *Mobile phase* prepared from *Sample stock solution*

Chromatographic system

(See *Chromatography* ~~621~~, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6 mm × 15 cm; 5 µm packing L7

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for desacetyl diltiazem and diltiazem are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between desacetyl diltiazem and diltiazem

Relative standard deviation: NMT 2.0% for diltiazem

Analysis

Samples: ~~Standard solution and Sample solution~~

Calculate the percentage of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the ~~Sample solution~~

r_S peak response from the ~~Standard solution~~

C_S concentration of USP Diltiazem Hydrochloride RS in the ~~Standard solution~~ ($\mu\text{g/mL}$)

C_U nominal concentration of diltiazem hydrochloride in the ~~Sample solution~~ ($\mu\text{g/mL}$)

▲Solution A:

0.79 g/L of ammonium bicarbonate in water. Adjust with diluted ammonium solution to a pH of 8.0.

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2.0	95	5
5.0	60	40
13.0	60	40
16.0	30	70
20.0	30	70
20.1	95	5
25.0	95	5

Diluent: Acetonitrile and water (40:60)

Standard solution: 0.05 mg/mL of USP Diltiazem Hydrochloride RS in *Diluent*

Sample stock solution: Nominally 0.5 mg/mL of diltiazem hydrochloride from the Capsules in *Diluent* prepared as follows. Transfer a portion of finely powdered contents of NLT 20 Capsules to a suitable volumetric flask. Add *Diluent* equivalent to 80% of the flask volume, mechanically shake for 30 min, and sonicate for 60 min. Dilute with the *Diluent* to volume. Centrifuge and use the supernatant.

Sample solution: Nominally 0.05 mg/mL of diltiazem hydrochloride prepared in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 240 nm. For *Identification* test A, use a diode-array detector in the range of 190–400 nm.

Column: 2.1-mm × 15-cm; 1.7- μm packing L1

Flow rate: 0.3 mL/min

Injection volume: 2.0 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of diltiazem from the *Sample solution*

r_S

= peak response of diltiazem from the *Standard solution*

C_S

= concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of diltiazem hydrochloride in the *Sample solution* (mg/mL)

▲USP39

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution 〈 711 〉

For products labeled for dosing every 12 h

Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

Medium: Water; 900 mL

Apparatus 2: 100 rpm

Times: 3, 9, and 12 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
3	10–25
9	45–85
12	NLT 70

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈 711 〉.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: Water; 900 mL

Apparatus 2: 100 rpm

Times: 4, 8, 12, and 24 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Dissolved (%)
4	10–25
8	35–60
12	55–80
24	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈 711 〉.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium: 0.05 M phosphate buffer, pH 7.2; 900 mL

Apparatus 2: 50 rpm

Times: 1, 3, and 8 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 4*.

Table 4

Time (h)	Amount Dissolved (%)
1	NMT 15
3	45–70
8	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈 711 〉.

Test 10: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 10*.

Buffer: Dissolve 7.1 g of anhydrous dibasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 6.5.

Medium: *Buffer*; 900 mL

Apparatus 1: 100 rpm

Times: 1, 6, 9, and 24 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved (%)
1	NMT 10
6	10–30
9	34–60
24	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈 711 〉.

For products labeled for dosing every 24 h

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Water; 900 mL

Apparatus 2: 100 rpm

Times: 1, 4, 10, and 15 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 6*.

Table 6

Time (h)	Amount Dissolved (%)
1	5–20
4	30–50
10	70–90
15	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈 711 〉.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 100 rpm

Times: 6, 12, 18, 24, and 30 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 7*.

Table 7

Time (h)	Amount Dissolved (%)
6	20–45
12	25–50
18	35–70
24	NLT 70
30	NLT 85

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈 711 〉.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Times: 2, 4, 8, 12, and 16 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 8*.

Table 8

Time (h)	Amount Dissolved (%)
2	NMT 25
4	25–50
8	60–85
12	NLT 70
16	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈 711 〉.

Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

Buffer: Transfer 115 mL of acetic acid to a 10-L volumetric flask, dilute with water to volume, and mix (*Solution A*). Transfer 165.4 g of anhydrous sodium acetate to a 10-L volumetric flask, dilute with water to volume, and mix (*Solution B*). Mix 4410 mL of

Solution A with 1590 mL of *Solution B*. Adjust, if necessary, with the addition of *Solution A* or *Solution B* to a pH of 4.2 ± 0.05 .

Medium: *Buffer*; 900 mL

Apparatus 2: 100 rpm

Times: 1, 4, 10, and 15 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈711〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 9*.

Table 9

Time (h)	Amount Dissolved (%)
1	NMT 10
4	15–35
10	65–85
15	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈711〉.

Test 8: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

Medium: Water; 900 mL

Apparatus 2: 100 rpm

Times: 1, 4, 10, and 15 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈711〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 10*.

Table 10

Time (h)	Amount Dissolved (%)
1	5–20
4	30–50
10	60–90
15	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈711〉.

Test 9: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.

[Note—Perform the test separately in each of the two media.]

Medium 1: 0.1 N hydrochloric acid; 900 mL

Medium 2: Simulated intestinal fluid TS, prepared without enzyme and adjusted to a pH of 7.5 ± 0.1 ; 900 mL

Apparatus 2: 75 rpm

Time for Medium 1: 2 h

Times for Medium 2: 2, 12, 18, and 24 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈711〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 11*.

Table 11

Time (h)	Amount Dissolved, <i>Medium 1</i> (%)	Amount Dissolved, <i>Medium 2</i> (%)
2	0–5	20–45
12	—	35–55
18	—	NLT 60
24	—	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$)

dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈711〉.

Test 11: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 11*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 100 rpm

Times: 1, 6, 12, and 18 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈711〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 12*.

Table 12

Time (h)	Amount Dissolved (%)
1	NMT 10
6	30–40
12	36–58
18	NLT 85

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$)

dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈711〉.

Test 12: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 12*. Proceed as directed for *Extended-Release Dosage Forms*.

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Times: 2, 8, 14, and 24 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 13*.

Table 13

Time (h)	Amount Dissolved (%)
2	NMT 20
8	30–55
14	NLT 65
24	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈 711 〉.

Test 13: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 13*. Proceed as directed for *Extended-Release Dosage Forms*.

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Times: 2, 8, 14, and 24 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 14*.

Table 14

Time (h)	Amount Dissolved (%)
2	NMT 20
8	30–55
14	60–80
24	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈 711 〉.

Test 14: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 14*. Proceed as directed for *Extended-Release Dosage Forms*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 100 rpm

Times: 6, 12, 18, 24, and 30 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium* to a

concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 15*.

Table 15

Time (h)	Amount Dissolved (%)
6	20–45
12	25–50
18	35–70
24	NLT 70
30	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈711〉.

Test 15: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 15*. Proceed as directed for *Extended-Release Dosage Forms*.

Medium: 0.05 M phosphate buffer, pH 7.5; 900 mL

Apparatus 2: 75 rpm

Times: 2, 4, 8, 12, and 16 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈711〉. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: See *Table 16*.

Table 16

Time (h)	Amount Dissolved (%)
2	NMT 25
4	20–40
8	60–85
12	NLT 70
16	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈711〉.

Test 16: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 16*.

Medium, Apparatus, Times, Standard solution, and Sample solution: Proceed as directed for *Test 3*.

Detector: UV 238 nm

Tolerances: See *Table 17*.

Table 17

Time (h)	Amount Dissolved (%)
6	20–45

12	30–55
18	40–75
24	NLT 70
30	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈711〉.

- **Uniformity of Dosage Units** 〈905〉: Meet the requirements

IMPURITIES

Add the following:

▲• Organic Impurities

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 2.5 µg/mL each of USP Desacetyl Diltiazem Hydrochloride RS and USP Diltiazem Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.5 mg/mL of diltiazem hydrochloride from the Capsules in *Diluent* prepared as follows. Transfer a portion of the finely powdered contents of NLT 20 Capsules to a suitable volumetric flask. Add *Diluent* equivalent to 80% of the flask volume, mechanically shake for 30 min, and sonicate for 60 min. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.

System suitability

Sample: *Standard solution*

[Note—For relative retention times see *Table 18*.]

Suitability requirements

Resolution: NLT 2.0 between desacetyl diltiazem and diltiazem

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of desacetyl diltiazem in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of desacetyl diltiazem from the *Sample solution*

r_S peak response of desacetyl diltiazem from the *Standard solution*

C_S concentration of USP Desacetyl Diltiazem Hydrochloride RS in the *Standard solution* (µg/mL)

C_U nominal concentration of diltiazem hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each unspecified impurity from the *Sample solution*

r_S peak response of diltiazem from the *Standard solution*

C_s concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* ($\mu\text{g/mL}$)

C_T nominal concentration of diltiazem hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 18*. Disregard limit: 0.05%.

Table 18

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Diltiazem related compound H ^{a,b}	0.44	—
Diltiazem related compound G ^{c,b}	0.52	—
Diltiazem related compound C ^{d,b}	0.58	—
Diltiazem related compound D ^{e,b}	0.61	—
Diltiazem related compound E ^{f,b}	0.66	—
Desacetyl diltiazem	0.75	0.5
Diltiazem related compound A ^{g,b}	0.83	—
Diltiazem related compound B ^{h,b}	0.89	—
Diltiazem	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities	—	1.0

^a (2*S*,3*S*)-5-(2-Aminoethyl)-3-hydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine-4(5*H*)-one.

^b These are impurities related to the drug substance. They are not to be reported for the drug product and should not be included in the total impurities.

^c (2*S*,3*S*)-3-Hydroxy-2-(3-methoxyphenyl)-5-(2-(methylamino)ethyl)-2,3-dihydrobenzo[*b*][1,4]thiazepin-4(5*H*)-one.

^d (2*S*,3*S*)-5-[2-(Dimethylamino)ethyl]-2-(4-hydroxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate.

^e (2*S*,3*S*)-2-(4-Methoxyphenyl)-5-[2-(methylamino)ethyl]-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl acetate.

^f (2*S*,3*S*)-3-Hydroxy-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepine-4(5*H*)-one.

^g (2*R*,3*S*)-5-[2-(Dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl acetate.

^h (2*S*,3*S*)-2-(4-Methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl acetate.

▲USP39

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers.

▲Store at controlled room temperature. ▲USP39

- **Labeling:** The labeling indicates the *Dissolution Test* with which the product complies.

- **USP Reference Standards** { 11 }

USP Desacetyl Diltiazem Hydrochloride RS

$C_{20}H_{24}N_2O_3S \cdot HCl$ 408.95

USP Diltiazem Hydrochloride RS

BRIEFING

Diltiazem Hydrochloride Tablets, USP 38 page 3133. As part of the USP monograph modernization effort, it is proposed to make the following changes:

1. Add a new stability-indicating UHPLC procedure in the test for *Organic Impurities*. The UHPLC procedure is based on analyses performed with the Waters Acquity UPLC BEH C18 brand of L1 column. The typical retention time for the diltiazem peak in the test for *Organic Impurities* is 16 min.
2. Replace the current HPLC Assay with a procedure using the same UHPLC parameters as proposed in the test for *Organic Impurities*.
3. Replace the current *Identification test A* using wet chemistry and visual detection with UV spectrum as proposed in the *Assay*.
4. Add an additional storage requirement in the *Packaging and Storage* section.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM2: D. Min.)

Correspondence Number—C141048

Comment deadline: March 31, 2015

Diltiazem Hydrochloride Tablets**DEFINITION**

Diltiazem Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$).

IDENTIFICATION**Delete the following:**

▲● **A. Procedure**

Indicator solution: Transfer 17.4 g of ammonium thiocyanate and 2.8 g of cobalt chloride to a 100 mL volumetric flask, add 50 mL of water, and sonicate for 10 min. Dilute with water to volume.

Analysis: Finely powder 1 Tablet, and transfer to a 15 mL screw capped test tube. Add 10 mL of 0.1 N hydrochloric acid, shake, and filter. Add 2 mL of *Indicator solution* to 2 mL of the filtrate, and shake. Add 5 mL of chloroform, and shake.

Acceptance criteria: A blue color develops in the chloroform layer. ▲*USP39*

Add the following:

- ▲● **A.** The UV-Vis spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP39*
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

● **Procedure**

Solution A: Dissolve 1.16 mg/mL of *d*-10-camphorsulfonic acid in 0.1 M sodium acetate, and adjust with 0.1 N sodium hydroxide to a pH of 6.2.

Mobile phase: Acetonitrile, methanol, and *Solution A* (1:1:2)

System suitability solution: 12 µg/mL of USP Diltiazem Hydrochloride RS and 12 µg/mL of USP Desacetyl Diltiazem Hydrochloride RS, in methanol

Standard solution: 1.2 mg/mL of USP Diltiazem Hydrochloride RS in methanol

Sample solution: Nominally 1.2 mg/mL of diltiazem hydrochloride from Tablets in methanol prepared as follows. Transfer an equivalent to 600 mg of diltiazem hydrochloride from finely powdered Tablets (NLT 20) to a 500 mL volumetric flask. Add 200 mL of methanol, and sonicate for 1 h. Cool, and dilute with methanol to volume. Centrifuge a 25 mL aliquot at 3500 rpm for 15 min, and use the clear supernatant.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 3.9 mm × 30 cm; packing L1

Flow rate: 1.6 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for desacetyl diltiazem and diltiazem are about 0.65 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3 between desacetyl diltiazem and diltiazem, *System suitability solution*

Column efficiency: NLT 1200 theoretical plates for the diltiazem peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

▲**Solution A:** 0.79 g/L of ammonium bicarbonate in water. Adjust with diluted ammonium solution to a pH of 8.0.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2.0	95	5
5.0	60	40
13.0	60	40
16.0	30	70
20.0	30	70
20.1	95	5
25.0	95	5

Diluent: Acetonitrile and water (40:60)

Standard solution: 0.05 mg/mL of USP Diltiazem Hydrochloride RS in *Diluent*

Sample stock solution: Nominally 0.5 mg/mL of diltiazem hydrochloride from Tablets in *Diluent* prepared as follows. Transfer an appropriate portion of finely powdered Tablets (NLT 20) to a suitable volumetric flask. Add *Diluent* equivalent to 80% of the flask volume, and sonicate for 60 min. Dilute with *Diluent* to volume. Centrifuge the solution for 20 min. Use the supernatant.

Sample solution: Nominally 0.05 mg/mL of diltiazem hydrochloride in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm. For *Identification test A*, use a diode-array detector in the range of 190–400 nm.

Column: 2.1-mm × 15-cm; 1.7-μm packing L1

Flow rate: 0.3 mL/min

Injection volume: 2.0 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

▲USP39

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of diltiazem from the *Sample solution*

r_S peak response of diltiazem from the *Standard solution*

C_S concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of diltiazem hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Dissolution** 〈 711 〉

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 30 min and 3 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

Tolerances: See *Table 2* for the 30-min time point. Use the criteria in *Dissolution* 〈 711 〉, *Acceptance Table 1* for the 3-h time point. NMT 60% (*Q*) of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) is dissolved in 30 min, and NLT 75% (*Q*) is dissolved in 3 h.

Table 2

Stage	Acceptance Criteria
S_1	No unit is more than <i>Q</i> .
S_2	Average value is equal to or less than <i>Q</i> , and no unit is greater than <i>Q</i> + 10%.
S_3	Average value is equal to or less than <i>Q</i> , and NMT 2 units are more than <i>Q</i> + 10%, and no unit is more than <i>Q</i> + 25%.

- **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

IMPURITIES

Add the following:

- ▲• **Organic Impurities**

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 2.5 µg/mL each of USP Desacetyl Diltiazem Hydrochloride RS and USP Diltiazem Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.5 mg/mL of diltiazem hydrochloride from Tablets in *Diluent* prepared as follows. Transfer an appropriate portion of the powdered Tablets (NLT 20) to a suitable volumetric flask. Add *Diluent* equivalent to 80% of the flask volume, and sonicate for 60 min. Dilute with *Diluent* to volume. Centrifuge the solution for 20 min. Use the supernatant.

System suitability

Sample: *Standard solution*

[Note—See *Table 3* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between desacetyl diltiazem and diltiazem

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of desacetyl diltiazem in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of desacetyl diltiazem from the *Sample solution*

r_S peak response of desacetyl diltiazem from the *Standard solution*

C_S concentration of USP Desacetyl Diltiazem Hydrochloride RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U nominal concentration of diltiazem hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

Calculate the percentage of any individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each unspecified impurity from the *Sample solution*

r_S peak response of diltiazem from the *Standard solution*

C_S concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U nominal concentration of diltiazem hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See Table 3. The disregard limit is 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Diltiazem related compound H ^{a,h}	0.44	—
Diltiazem related compound G ^{b,h}	0.52	—
Diltiazem related compound C ^{c,h}	0.58	—
Diltiazem related compound D ^{d,h}	0.61	—
Diltiazem related compound E ^{e,h}	0.66	—
Desacetyl diltiazem	0.75	0.5
Diltiazem related compound A ^{f,h}	0.83	—
Diltiazem related compound B ^{g,h}	0.89	—
Diltiazem	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities	—	1.0

^a (2S,3S)-5-(2-Aminoethyl)-3-hydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine-4(5H)-one.

^b (2S,3S)-3-Hydroxy-2-(3-methoxyphenyl)-5-[2-(methylamino)ethyl]-2,3-dihydrobenzo[*b*][1,4]thiazepin-4(5H)-one.

^c (2S,3S)-5-[2-(Dimethylamino)ethyl]-2-(4-hydroxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate.

d (2*S*,3*S*)-2-(4-Methoxyphenyl)-5-[2-(methylamino)ethyl]-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl acetate.

e (2*S*,3*S*)-3-Hydroxy-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepine-4(5*H*)-one.

f (2*R*,3*S*)-5-[2-(Dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl acetate.

g (2*S*,3*S*)-2-(4-Methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl acetate.

h These are impurities related to the drug substance. They are not to be reported for the drug product and should not be included in the total impurities.

▲*USP39*

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

▲Store at controlled room temperature. ▲*USP39*

- **USP Reference Standards** { 11 }

USP Desacetyl Diltiazem Hydrochloride RS

$C_{20}H_{24}N_2O_3S \cdot HCl$ 408.95

USP Diltiazem Hydrochloride RS

BRIEFING

Diphenhydramine Hydrochloride Capsules, *USP 38* page 3150 and *PF 40(2)* [Mar.–Apr. 2014]. The revision proposal for this monograph, which appeared in *PF 40(2)*, is being canceled and replaced with a new proposal based on the comments received. The proposal changes are the following:

1. The current *Assay* procedure is replaced with a validated new HPLC procedure. The liquid chromatographic procedure in the *Assay* is based on analyses performed with the YMC-Pack Pro C8 brand of L7 column. The typical retention time for diphenhydramine is 5 min.
2. The test for *Organic Impurities*, based on the validated HPLC procedure, is added. The liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with the YMC-Pack Pro C8 brand of L7 column. The typical retention time for diphenhydramine is 5 min.
3. USP Diphenhydramine Related Compound A RS is added to the *USP Reference Standards* section to support the proposed revision in the test for *Organic Impurities*.
4. Update the *Packaging and Storage* section to include storage conditions.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R.S. Prasad.)

Correspondence Number—C149941; C148242; C149100; C123170

Comment deadline: March 31, 2015**Diphenhydramine Hydrochloride Capsules****DEFINITION**

Diphenhydramine Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$).

IDENTIFICATION

- **A. Identification—Organic Nitrogenous Bases** (181): The contents of the Capsules meet the requirements.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**Change to read:**• **Procedure**

Mobile phase: ~~Acetonitrile, triethylamine, and water (100:1:100). Adjust with glacial acetic acid to a pH of 6.5.~~

System suitability solution: ~~5 mg of benzophenone in 5 mL of acetonitrile. Dilute with water to 100 mL. Transfer 1.0 mL of this solution and 5 mg of diphenhydramine hydrochloride to a 10 mL volumetric flask and dilute with water to volume.~~

Standard solution: ~~0.5 mg/mL of USP Diphenhydramine Hydrochloride RS in water~~

Sample solution: ~~Nominally equivalent to 0.5 mg/mL of diphenhydramine hydrochloride from Capsule contents in water is prepared as follows. Transfer an accurately weighed portion of the combined Capsule contents (NLT 20 capsules), equivalent to 50 mg of diphenhydramine hydrochloride, to a 100 mL volumetric flask. Dissolve in and dilute with water to volume, mix, and filter.~~

Chromatographic system

~~(See Chromatography (621), System Suitability.)~~

Mode: ~~LC~~

Detector: ~~UV 254 nm~~

Column: ~~4.6 mm x 25 cm, packing L10~~

Flow rate: ~~1 mL/min~~

Injection volume: ~~10 µL~~

System suitability

Samples: ~~System suitability solution and Standard solution~~

Suitability requirements

Resolution: ~~NLT 2.0 between benzophenone and diphenhydramine, System suitability solution~~

Tailing factor: ~~NMT 2.0, Standard solution~~

Relative standard deviation: ~~NMT 2.0%, Standard solution~~

Analysis

Samples: ~~Standard solution and Sample solution~~

Calculate the percentage of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) in the portion of Capsule contents taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

~~r_U~~ peak response from the ~~Sample solution~~

~~r_S~~ peak response from the ~~Standard solution~~

~~C_S~~ concentration of USP Diphenhydramine Hydrochloride RS in the ~~Standard solution~~ (mg/mL)

~~C_U~~ nominal concentration of diphenhydramine hydrochloride in the ~~Sample solution~~ (mg/mL)

▲Buffer:

5.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Solution A: Buffer

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	65	35
4	65	35
7	20	80
9	65	35
13	65	35

Diluent: Acetonitrile and Buffer (35:65)

System suitability solution: 0.1 mg/mL each of USP Diphenhydramine Related Compound A RS and USP Diphenhydramine Hydrochloride RS in Diluent

Standard solution: 0.07 mg/mL of USP Diphenhydramine Hydrochloride RS in Diluent

Sample stock solution: Weigh and combine the contents of NLT 20 Capsules. Transfer an accurately weighed portion of the combined Capsule contents, equivalent to about 50 mg of diphenhydramine hydrochloride, to a 100-mL volumetric flask. Dissolve in and dilute with water to volume, and filter. Alternatively, dissolve NLT 20 Capsules in water at 50° and pipet the solution equivalent to about 50 mg of diphenhydramine hydrochloride to a 100-mL volumetric flask. Dissolve in and dilute with water to volume, and filter.

Sample solution: 0.07 mg/mL of diphenhydramine hydrochloride in Diluent from the Sample stock solution

Chromatographic system

(See Chromatography { 621 }, System Suitability.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm column; 5-μm packing L7

Flow rate: 1.2 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for diphenhydramine related compound A and diphenhydramine are about 0.9 and 1.0, respectively.]

System suitability

Resolution: NLT 2.0 between diphenhydramine and diphenhydramine related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diphenhydramine hydrochloride ($\text{C}_{17}\text{H}_{21}\text{NO}\cdot\text{HCl}$) in the portion of Capsule contents taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of diphenhydramine from the *Sample solution*

r_S

= peak response of diphenhydramine from the *Standard solution*

C_S

= concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

▲USP39

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Dissolution**, *Procedure for a Pooled Sample* { 711 }

Medium: Water; 500 mL

Apparatus 1: 100 rpm

Time: 30 min

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

Injection volume: 50 μL

Standard solution: USP Diphenhydramine Hydrochloride RS in *Medium*, at a known concentration similar to that of the *Sample solution*

Sample solution: Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diphenhydramine hydrochloride

(C₁₇H₂₁NO·HCl) dissolved.

Tolerances: NLT 80% (Q) of the labeled amount of diphenhydramine hydrochloride

(C₁₇H₂₁NO·HCl) is dissolved.

- **Uniformity of Dosage Units** (905): Meet the requirements

IMPURITIES

Add the following:

▲• Organic Impurities

Buffer, Diluent, System suitability solution, and Chromatographic system: Proceed as directed in the Assay with a run time that is 10 times the retention time of diphenhydramine.

Mobile phase: Acetonitrile and Buffer (35:65)

Standard solution: 0.02 mg/mL of USP Diphenhydramine Hydrochloride RS and 0.01 mg/mL of USP Diphenhydramine Related Compound A RS in Diluent

Sample solution: Nominally equivalent to 2 mg/mL of diphenhydramine hydrochloride in Diluent prepared as follows. Remove the contents of NLT 20 Capsules as completely as possible, and weigh. Transfer a portion of the powder, nominally equivalent to 100 mg of diphenhydramine hydrochloride, to a 50-mL volumetric flask. Dilute with Diluent to volume. Sonicate the solution for 5 min.

System suitability

Sample: System suitability solution

[Note—See Table 2 for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between diphenhydramine and diphenhydramine related compound A

Relative standard deviation: NMT 5.0% for diphenhydramine and diphenhydramine related compound A

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of diphenhydramine related compound A in the portion of Capsule contents taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of diphenhydramine related compound A from the Sample solution

r_S peak response of diphenhydramine related compound A from the Standard solution

C_S concentration of USP Diphenhydramine Related Compound A RS in the Standard solution (mg/mL)

C_U nominal concentration of diphenhydramine hydrochloride in the Sample solution (mg/mL)

Calculate the percentage of each degradation product in the portion of Capsule contents taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of each degradation product from the Sample solution

r_S peak response of diphenhydramine from the Standard solution

C_S concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)

C_T nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Diphenhydramine related compound A	0.9	1.0	0.5
Diphenhydramine	1.0	—	—
Diphenhydramine <i>N</i> -oxide ^a	1.2	1.0	3.0
Benzhydrol ^b	4.7	1.5	2.0
Benzophenone ^c	9.3	0.8	0.4
Individual unspecified impurity	—	—	1.0

^a 2-(Benzhydryloxy)-*N,N*-dimethylethan-1-amine oxide.

^b Diphenylmethanol.

^c Diphenylmethanone.

▲USP39

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers.

▲Store at controlled room temperature. ▲USP39

Change to read:

- **USP Reference Standards** { 11 }

USP Diphenhydramine Hydrochloride RS

▲USP Diphenhydramine Related Compound A RS

2-(Diphenylmethoxy)-*N*-methylethanamine hydrochloride.

$C_{16}H_{19}NO \cdot HCl$ 277.79 ▲USP39

BRIEFING

Diphenhydramine Hydrochloride Injection, *USP 38* page 3150 and *PF 39(6)* [Nov.–Dec. 2013]. The revision proposal for this monograph, which appeared in *PF 39(6)*, is being canceled and replaced with a new proposal based on the comments received. The proposal changes are the following:

1. The current *Assay* procedure is replaced with a validated new HPLC procedure. The

liquid chromatographic procedure in the *Assay* is based on analyses performed with the YMC-Pack Pro C8 brand of L7 column. The typical retention time for diphenhydramine is 5 min.

2. The test for *Organic Impurities*, based on the validated HPLC procedure, is added. The liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with the YMC-Pack Pro C8 brand of L7 column. The typical retention time for diphenhydramine is 5 min.
3. USP Diphenhydramine Related Compound A RS is added to the *USP Reference Standards* section to support the proposed revision in the test for *Organic Impurities*.
4. Update the *Packaging and Storage* section to include storage conditions.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R.S. Prasad.)

Correspondence Number—C123268; C144119; C142767; C149942

Comment deadline: March 31, 2015

Diphenhydramine Hydrochloride Injection

DEFINITION

Diphenhydramine Hydrochloride Injection is a sterile solution of Diphenhydramine Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$).

IDENTIFICATION

• **A.**

Sample solution: Dilute a volume of Injection equivalent to 50 mg of diphenhydramine hydrochloride with 0.03 N sulfuric acid to 25 mL.

Analysis: Proceed as directed in *Identification—Organic Nitrogenous Bases* (181), beginning with "Transfer the liquid to a separator".

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **Procedure**

~~**Mobile phase:** Acetonitrile, triethylamine, and water (100:1:100). Adjust with glacial acetic acid to a pH of 6.5.~~

~~**System suitability solution:** 5 mg of benzophenone in 5 mL of acetonitrile. Dilute with water to 100 mL. Transfer 1.0 mL of this solution and 5 mg of diphenhydramine hydrochloride to a 10 mL volumetric flask and dilute with water to volume.~~

~~**Standard solution:** 0.5 mg/mL of USP Diphenhydramine Hydrochloride RS in water~~

~~**Sample solution:** Transfer a volume of Injection equivalent to 50 mg of diphenhydramine hydrochloride to a 100 mL volumetric flask and dilute with water to volume.~~

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6 mm × 25 cm; packing L10

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between benzophenone and diphenhydramine, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) in each mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

▲Buffer:

5.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Solution A: *Buffer*

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	65	35
4	65	35
7	20	80
9	65	35
13	65	35

Diluent: Acetonitrile and *Buffer* (35:65)

System suitability solution: 0.1 mg/mL each of USP Diphenhydramine Hydrochloride Related Compound A RS and USP Diphenhydramine Hydrochloride RS in *Diluent*

Standard solution: 0.07 mg/mL of USP Diphenhydramine Hydrochloride RS in *Diluent*

Sample solution: Nominally equivalent to 0.07 mg/mL of diphenhydramine hydrochloride in *Diluent* prepared as follows. Transfer 5.0 mL of Injection, equivalent to 250 mg of diphenhydramine hydrochloride, to a 500-mL volumetric flask and dilute with water to volume. Transfer 7.0 mL of this solution to a 50-mL volumetric flask and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Flow rate: 1.2 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for diphenhydramine hydrochloride related compound A and diphenhydramine hydrochloride are about 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between diphenhydramine and diphenhydramine related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

▲USP39

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

▲● Organic Impurities

Buffer, System suitability solution, and Chromatographic system: Proceed as directed in the Assay with a run time that is 10 times the retention time of diphenhydramine.

Mobile phase: Acetonitrile and Buffer (35:65)

Standard solution: 0.002 mg/mL of USP Diphenhydramine Hydrochloride RS in *Mobile phase*

Sample solution: Nominally equivalent to 2 mg/mL of diphenhydramine hydrochloride in water prepared as follows. Transfer a volume of Injection, equivalent to 500 mg of diphenhydramine hydrochloride, to a 250-mL volumetric flask and dilute with water to volume.

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See Table 2 for the relative retention times. Inject a blank injection between the *System suitability solution* and *Standard solution*.]

Suitability requirements

Resolution: NLT 2.0 between diphenhydramine and diphenhydramine related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of each degradation product from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see Table 2)

Acceptance criteria: See Table 2. Disregard any peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Diphenhydramine related compound A	0.9	1.1	0.4
Diphenhydramine	1.0	—	—
Benzhydrol ^a	4.3	1.5	0.4
Benzophenone ^b	8.2	0.8	0.2
Individual unspecified impurity	—	—	0.2
Total impurities	—	—	0.8
^a Diphenylmethanol.			

^b Diphenylmethanone.

SPECIFIC TESTS

- **pH** 〈 791 〉: 4.0–6.5
- **Bacterial Endotoxins Test** 〈 85 〉: NMT 3.4 USP Endotoxin Units/mg of diphenhydramine hydrochloride
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* 〈 1 〉.

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

▲Store at controlled room temperature. ▲*USP39*

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Diphenhydramine Hydrochloride RS

▲USP Diphenhydramine Related Compound A RS

2-(Diphenylmethoxy)-*N*-methylethanamine hydrochloride.

C₁₆H₁₉NO·HCl 277.79 ▲*USP39*

USP Endotoxin RS

BRIEFING

Dopamine Hydrochloride, *USP 38* page 3200. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Replace the titration procedure in the *Assay* with a stability-indicating UHPLC method using the Thermo Hypersil Gold aQ C18 brand of L1 column. The retention time of dopamine in the analysis is 1.3 min.
2. Replace the *Ultraviolet Absorption* test in *Identification test B* using a retention time agreement based upon on the proposed method for the *Assay*.
3. Replace the current TLC procedure for the determination in the test for *Organic Impurities* with the chromatographic method proposed in the *Assay*. The current TLC method in the test for *Organic Impurities* uses an undesirable solvent, chloroform, in the procedure. This proposed chromatographic test monitors specified impurities, namely, dopamine related compound A, dopamine related compound B, and dopamine related compound C (as per the equivalent monograph in the *European Pharmacopoeia*), as well as any other unspecified impurities.
4. Delete the test for *Readily Carbonizable Substances* given the impurities detection capabilities of the proposed test for *Organic Impurities*.
5. Add the following three new Reference Standards, introduced by the proposed procedure in the *Assay* and test for *Organic Impurities*, to the *USP Reference Standards* section to support the proposed changes for the *Assay* and *Impurities* sections: USP Dopamine Related Compound A RS, USP Dopamine Related Compound B RS, and USP Dopamine Related Compound C RS.

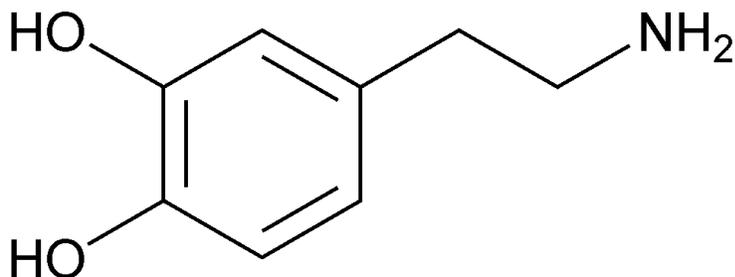
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: D.A. Porter.)

Correspondence Number—C115767

Comment deadline: March 31, 2015

Dopamine Hydrochloride



• HCl

$C_8H_{11}NO_2 \cdot HCl$ 189.64

1,2-Benzenediol, 4-(2-aminoethyl)-, hydrochloride;
4-(2-Aminoethyl)pyrocatechol hydrochloride [62-31-7].

DEFINITION

Dopamine Hydrochloride contains NLT 98.0% and NMT 102.0% of dopamine hydrochloride ($C_8H_{11}NO_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** $\langle 197K \rangle$

Delete the following:

- ~~▲• **B. Ultraviolet Absorption** $\langle 197U \rangle$~~

~~**Medium:** Sodium bisulfite in water (1 in 1000)~~

~~**Sample solution:** 40 μ g/mL in *Medium* ▲*USP39*~~

Add the following:

- ▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP39*

- **C. Identification Tests—General, Chloride** $\langle 191 \rangle$: Meets the requirements

ASSAY

Change to read:

- **Procedure**

~~**Sample:** 75 mg of Dopamine Hydrochloride~~

~~**Titrimetric system:** See *Titrimetry* $\langle 541 \rangle$~~

~~**Mode:** Direct titration~~

~~**Titrant:** 0.1 N perchloric acid VS~~

~~**Endpoint detection:** Potentiometric~~

~~**Analysis:** Dissolve the *Sample* in 5 mL of formic acid, add 25 mL of acetic anhydride, and titrate with *titrant*, determining the endpoint potentiometrically. Perform a blank determination and make any necessary corrections. Each mL of 0.1 N perchloric acid is equivalent to 18.96 mg of dopamine hydrochloride ($C_8H_{11}NO_2 \cdot HCl$.)~~

~~**Acceptance criteria:** 98.0%–102.0% on the dried basis~~

▲Solution A: Dilute 1.0 mL of heptafluorobutyric acid with water to 1000 mL.

Solution B: Dilute 1.0 mL of heptafluorobutyric acid with methanol to 1000 mL.

Mobile phase: *Solution A* and *Solution B* (85:15)

System suitability solution: 0.2 mg/mL of USP Dopamine Hydrochloride RS and 0.02 mg/mL of USP Dopamine Related Compound B RS in *Solution A*

Standard solution: 0.2 mg/mL of USP Dopamine Hydrochloride RS in *Solution A*, sonicated to fully dissolve solids

Sample solution: 0.2 mg/mL of Dopamine Hydrochloride in *Solution A*. Sonication may be necessary for complete dissolution.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 2.1-mm × 10-cm; 1.9- μ m packing L1

Column temperature: 35°

Flow rate: 0.5 mL/min

Injection volume: 2 μ L

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between dopamine and dopamine related compound B

Tailing factor: NMT 2.0 for the dopamine peak

Relative standard deviation: NMT 0.73% for dopamine hydrochloride

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dopamine hydrochloride ($C_8H_{11}NO_2 \cdot HCl$) in the portion of Dopamine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of dopamine from the *Sample solution*

r_S

= peak response of dopamine from the *Standard solution*

C_S

= concentration of USP Dopamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= concentration of Dopamine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ▲*USP39*

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

Delete the following:

-

- **Heavy Metals, Method I** (231)

Sample solution: 40 mg/mL of Dopamine Hydrochloride in water

Acceptance criteria: NMT 20 ppm ● (Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

Standard stock solution: 30 mg/mL of USP Dopamine Hydrochloride RS in methanol

Standard solutions: Prepare a series of dilutions of the *Standard stock solution* in methanol to contain 0.6, 0.3 and 0.15 mg/mL of USP Dopamine Hydrochloride RS, corresponding to 2.0%, 1.0%, and 0.5% of impurities, respectively.

Sample solution: 30 mg/mL of Dopamine Hydrochloride in methanol

Chromatographic system

(See *Chromatography* (621); *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 20 cm × 20 cm chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: 13 volumes of chloroform, 9 volumes of methanol, and 4 volumes of dilute glacial acetic acid (3 in 10)

Spray reagent: Freshly prepared mixture containing equal volumes of ferric chloride solution (1 in 10) and potassium ferricyanide solution (1 in 20)

Analysis

Samples: *Standard stock solution*, *Standard solutions*, and *Sample solution*

Place the *Developing solvent system* in a chromatographic chamber arranged for thin-layer chromatography. Line the chamber with filter paper, and allow to equilibrate. Separately apply portions of the *Standard stock solution*, the *Standard solutions*, and the *Sample solution* to the chromatographic plate. Develop the chromatogram until the solvent front has moved about 15 cm. Remove the plate from the developing chamber, allow to dry at room temperature for several min, and spray evenly with *Spray reagent*. [Note—Dopamine and its related impurities appear as blue spots under visible light.]

Estimate the concentration of any secondary spots exhibited by the *Sample solution* in comparison to the *Standard solutions*.

Acceptance criteria: ~~The *Sample solution* exhibits its principal spot at an R_f value corresponding to that of the *Standard solution* and NMT three secondary spots. The sum of the impurities is NMT 1.0%.~~

▲Solution A, Solution B, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

Standard stock solution: 50 µg/mL each of USP Dopamine Hydrochloride RS, USP Dopamine Related Compound A RS, USP Dopamine Related Compound B RS, and USP Dopamine Related Compound C RS in *Solution A*

Standard solution: 2 µg/mL each of USP Dopamine Hydrochloride RS, USP Dopamine Related Compound A RS, USP Dopamine Related Compound B RS, and USP Dopamine Related Compound C RS from the *Standard stock solution* in *Solution A*

Sample solution: 2.0 mg/mL of Dopamine Hydrochloride in *Solution A*. Sonication may be necessary for complete dissolution.

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.5 between dopamine and dopamine related compound B peaks

Relative standard deviation: NMT 5.0% for all peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Dopamine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of each specified impurity from the *Sample solution*

r_S

= peak response of the corresponding specified impurity from the *Standard solution*

C_S

= concentration of the corresponding specified impurity in the *Standard solution* (mg/mL)

C_U

= concentration of Dopamine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Dopamine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*

r_S = peak response of dopamine from the *Standard solution*

C_S = concentration of USP Dopamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Dopamine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dopamine hydrochloride	1.0	—
Dopamine related compound B ^a	1.8	0.1
Dopamine related compound A ^b	2.3	0.1
Dopamine related compound C ^c	4.6	0.1
Any individual unspecified impurity	—	0.1
Total impurities	—	1.0
a 4-(2-Aminoethyl)-2-methoxyphenol.		

b 5-(2-Aminoethyl)-2-methoxyphenol.

c 2-(3,4-Dimethoxyphenyl)ethan-1-amine.

▲USP39

SPECIFIC TESTS

- pH 〈 791 〉

Sample solution: 1 in 25 solution

Acceptance criteria: 3.0–5.5

- Loss on Drying 〈 731 〉

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.5%

- Chloride and Sulfate, Sulfate 〈 221 〉

Sample solution: 500 mg in 40 mL of water

Acceptance criteria: Any turbidity produced is NMT that produced in a solution containing 0.10 mL of 0.020 N sulfuric acid.

- Clarity and Color of Solution

Sample solution: 400 mg of Dopamine Hydrochloride in 10 mL of sodium bisulfite solution (1 in 1000)

Acceptance criteria: The solution is clear and colorless, or practically colorless.

Delete the following:

- ▲ ~~Readily Carbonizable Substances 〈 271 〉~~

~~**Sample solution:** 100 mg of Dopamine Hydrochloride in 5 mL of sulfuric acid~~

~~**Acceptance criteria:** The solution has no more color than *Matching Fluid A* (see *Color and Achromicity* 〈 631 〉, *Color Determination and Standards*). ▲USP39~~

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at room temperature.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Dopamine Hydrochloride RS

▲USP Dopamine Related Compound A RS

5-(2-Aminoethyl)-2-methoxyphenol.

C₉H₁₃NO₂ 167.21

USP Dopamine Related Compound B RS

3-*O*-Methyldopamine;

4-(2-Aminoethyl)-2-methoxyphenol.

C₉H₁₃NO₂ 167.21

USP Dopamine Related Compound C RS

2-(3,4-Dimethoxyphenyl)ethan-1-amine.

C₁₀H₁₅NO₂ 181.23▲USP39

BRIEFING

Dopamine Hydrochloride Injection, *USP 38* page 3200. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Replace the current HPLC procedure in the *Assay* with the proposed stability-indicating UHPLC method used in *Dopamine Hydrochloride*. The proposed liquid chromatographic procedure is based on analyses using the Thermo Hypersil Gold aQ C18 brand of L1 column.
2. Replace the current TLC method in *Identification* test *A* with the retention time agreement from the proposed *Assay* procedure.
3. Add *Identification* test *B* in which the UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the proposed *Assay*.
4. Add a UHPLC procedure in the test for *Organic Impurities* to be consistent with the proposed *Assay* method.
5. Add a storage requirement in the *Packaging and Storage* section that is consistent with FDA-approved drug products.
6. Add USP Dopamine Related Compound B RS to the *USP Reference Standards* section in support of the proposed changes to the *Assay* and *Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: D.A. Porter.)

Correspondence Number—C115768

Comment deadline: March 31, 2015

Dopamine Hydrochloride Injection**DEFINITION**

Dopamine Hydrochloride Injection is a sterile solution of Dopamine Hydrochloride in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of dopamine hydrochloride ($C_8H_{11}NO_2 \cdot HCl$). It may contain a suitable antioxidant.

[Note—Do not use the Injection if it is darker than slightly yellow or discolored in any other way.]

IDENTIFICATION

Delete the following:

▲• A. Thin-Layer Chromatographic Identification Test ~~(201)~~

Standard solution: 1.6 mg/mL of USP Dopamine Hydrochloride RS in dilute methanol (1 in 5)

Sample solution: 1.6 mg/mL from volume of Injection in dilute methanol (1 in 5)

Developing solvent system: *n*-butyl alcohol, glacial acetic acid, and water (4:1:1)

Analysis

Samples: ~~Standard solution and Sample solution~~

~~Proceed as directed in the chapter.~~

Acceptance criteria: Meets the requirements ▲*USP39*

Add the following:

▲• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP39*

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP39*

ASSAY

Change to read:

● **Procedure**

Solution A: 20 mg/mL of benzoic acid in methanol

Mobile phase: 0.005 M sodium 1-octanesulfonate in 1% glacial acetic acid and acetonitrile (87:13)

System suitability stock solution: 5 mg/mL of benzoic acid prepared by diluting 1 volume of *Solution A* with 3 volumes of the *Mobile phase*

Standard stock solution: 1.6 mg/mL of USP Dopamine Hydrochloride RS in *Mobile phase*

Standard solution: 0.16 mg/mL of dopamine hydrochloride, from *Standard stock solution* in *Mobile phase*

System suitability solution: 0.5 mg/mL of benzoic acid and 0.16 mg/mL of dopamine hydrochloride prepared by transferring 10.0 mL of *System suitability stock solution* and 10.0 mL of *Standard stock solution* to a 100 mL volumetric flask, and diluting with *Mobile phase* to volume

Sample solution: 0.16 mg/mL of dopamine hydrochloride from a volume of Injection, diluted with *Mobile phase*

Chromatographic system

(See *Chromatography* ~~(621)~~, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4 mm × 30 cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 40 µL

System suitability

Samples: *Standard solution and System suitability solution*

Suitability requirements

Resolution: NLT 4.0 between benzoic acid and dopamine hydrochloride, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of dopamine hydrochloride ($C_8H_{11}NO_2 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of dopamine from the *Sample solution*

r_S peak response of dopamine from the *Standard solution*

C_S concentration of USP Dopamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of dopamine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

▲Solution A: Dilute 1.0 mL of heptafluorobutyric acid with water to 1 L.

Solution B: Dilute 1.0 mL of heptafluorobutyric acid with methanol to 1 L.

Mobile phase: *Solution A* and *Solution B* (85:15)

System suitability solution: 0.2 mg/mL of USP Dopamine Hydrochloride RS and 0.02 mg/mL of USP Dopamine Related Compound B RS in *Solution A*, sonicated to fully dissolve solids

Standard solution: 0.2 mg/mL of USP Dopamine Hydrochloride RS in *Solution A*, sonicated to fully dissolve solids

Sample solution: Nominally 0.2 mg/mL of dopamine hydrochloride from Injection in *Solution A*

Chromatographic system

(See *Chromatography* ~~(621)~~, *System Suitability*.)

Mode: LC

Detector: UV 280 nm. For *Identification test B*, use a diode array detector in the range of 200–400 nm.

Column: 2.1-mm × 10-cm; 1.9-µm packing L1

Column temperature: 35°

Flow rate: 0.5 mL/min

Injection volume: 2 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 5.0 between dopamine hydrochloride and dopamine related compound B

Tailing factor: NMT 2.0 for the dopamine hydrochloride peak

Relative standard deviation: NMT 0.73% for dopamine hydrochloride

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dopamine hydrochloride ($C_8H_{11}NO_2 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of dopamine from the *Sample solution*

r_S

= peak response of dopamine from the *Standard solution*

C_S

= concentration of USP Dopamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of dopamine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% ▲*USP39*

IMPURITIES

Add the following:

▲● **Organic Impurities**

Solution A, Solution B, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 0.2 mg/mL of USP Dopamine Hydrochloride RS and 0.02 mg/mL of USP Dopamine Related Compound B RS in *Solution A*

Standard stock solution: 50 µg/mL of USP Dopamine Hydrochloride RS in *Solution A*. Sonication may be necessary for complete dissolution.

Standard solution: 2 µg/mL of USP Dopamine Hydrochloride RS from the *Standard stock solution* in *Diluent*

Sample solution: Nominally 2 mg/mL of dopamine hydrochloride in *Diluent*

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 5.0 between dopamine hydrochloride and dopamine related compound B peaks

Relative standard deviation: NMT 5.0% for dopamine hydrochloride

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any unspecified impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any unspecified impurity from the *Sample solution*

r_S peak response of dopamine from the *Standard solution*

C_S concentration of USP Dopamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of dopamine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: Disregard any impurity peak less than 0.05% (see *Table 1*).

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dopamine	1.0	—
Dopamine related compound B ^{a,b}	1.8	—
Any individual unspecified impurity	—	0.1
Total impurities	—	1.0
^a 4-(2-Aminoethyl)-2-methoxyphenol.		

^b This is a process impurity which is included for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.

▲USP39

SPECIFIC TESTS

- **pH** 〈 791 〉: 2.5–5.0
- **Injections and Implanted Drug Products** 〈 1 〉: Meets the requirements
- **Particulate Matter in Injections** 〈 788 〉: Meets the requirements for small-volume injections
- **Bacterial Endotoxins Test** 〈 85 〉: It contains NMT 16.67 USP Endotoxin Units/mg of dopamine hydrochloride.

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in single-dose containers of Type I glass.

▲Store at controlled room temperature. ▲USP39

- **Labeling:** Label it to indicate that the Injection is to be diluted with a suitable parenteral vehicle prior to intravenous infusion.

Change to read:

- **USP Reference Standards** { 11 }

USP Dopamine Hydrochloride RS

▲USP Dopamine Related Compound B RS

3-*O*-Methyldopamine;

4-(2-Aminoethyl)-2-methoxyphenol.

C₉H₁₃NO₂ 167.21▲USP39

USP Endotoxin RS

BRIEFING

Enalapril Maleate Tablets, *USP 38* page 3292. On the basis of comments received, it is proposed to include the *Standard solution* concentrations in the *Dissolution* test to be consistent with the validation data.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: S. Ramakrishna.)

Correspondence Number—C152191

Comment deadline: March 31, 2015

Enalapril Maleate Tablets**DEFINITION**

Enalapril Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of enalapril maleate (C₂₀H₂₈N₂O₅·C₄H₄O₄).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: Dissolve 1.38 g of monobasic sodium phosphate in 800 mL of water, adjust with phosphoric acid to a pH of 2.2, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (250:750)

Enalapril diketopiperazine solution: Place 20 mg of USP Enalapril Maleate RS in a 100-mL beaker to form a mound on the bottom of the beaker. Place the beaker on a hot plate at one-half the maximum hot plate temperature setting to melt the solid. When melting is observed (after 5–10 min of heating), immediately remove the beaker from the hot plate, and allow it to cool. Avoid overheating beyond the melting initially observed to prevent heat-induced degradation, which would give rise to a brown color.

To the cooled residue in the beaker add 50 mL of acetonitrile, and sonicate for a few min to dissolve the residue. The solution typically contains between 0.2 and 0.4 mg/mL of enalapril diketopiperazine.

Enalaprilat stock solution: 0.4 mg/mL of USP Enalaprilat RS in water

Standard solution: 0.2 mg/mL of USP Enalapril Maleate RS and 0.002 mg/mL of USP

Enalaprilat RS in *Buffer* prepared as follows. To a suitable amount of USP Enalapril Maleate RS in a suitable volumetric flask add an appropriate amount of *Enalaprilat stock solution* to the flask, and add 50% of the total volume of *Buffer* to dissolve. Sonicate if necessary, then dilute with *Buffer* to volume.

System suitability solution: Dilute 0.5 mL of *Enalapril diketopiperazine solution* with *Standard solution* to a final volume of 25 mL.

Sample solution: Nominally 0.2 mg/mL of enalapril maleate in *Buffer* prepared as follows.

Transfer NLT 10 Tablets to a volumetric flask of capacity such that when filled to volume it will produce a 0.2-mg/mL solution. Add a volume of *Buffer* that is about one-half the nominal volume of the flask, sonicate for 15 min, and shake by mechanical means for 30 min. Dilute with *Buffer* to volume, shake well, and sonicate for another 15 min. Pass the solution through a suitable filter of 0.45- μ m pore size, and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Column temperature: 50 $^{\circ}$

Flow rate: 2 mL/min

Injection volume: 50 μ L

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for maleic acid, enalaprilat, enalapril, and enalapril diketopiperazine are about 0.3, 0.5, 1.0, and 1.5, respectively, *System suitability solution*. A peak response for a heat-induced degradation product of enalapril diketopiperazine (if present with a relative retention time of about 1.2) is NMT 15% of the response for enalapril diketopiperazine.]

Suitability requirements

Resolution: NLT 2.0 between maleic acid and enalaprilat; NLT 2.0 between enalaprilat and enalapril; NLT 2.0 between enalapril and enalapril diketopiperazine, *System suitability solution*

Column efficiency: NLT 1000 theoretical plates for enalaprilat; NLT 300 theoretical plates for enalapril; NLT 2500 theoretical plates for enalapril diketopiperazine, *System suitability solution*

Tailing factor: NMT 2.0 for enalapril, *System suitability solution*

Relative standard deviation

Enalapril peak: NMT 2.0%, *Standard solution*

Enalaprilat peak: Responses agree within 5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_{\bar{F}}$ peak response from the *Sample solution*

$r_{\bar{S}}$ peak response from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Enalapril Maleate RS in the *Standard solution* (mg/mL)

$C_{\bar{F}}$ nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **Dissolution** 〈 711 〉

Medium: pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: 0.1 mg/mL of USP Enalapril Maleate RS in *Medium*. Sonicate if necessary.

▲ Dilute in *Medium* per *Table 1*.

Tablet Strength (mg)	Volume of Standard solution (mL)	Volumetric Flask Size (mL)
2.5	5	200
5	10	100
10	10	100
20	10	50
40	10	25

▲ USP39

Sample solution: Pass a portion of solution under test through a suitable filter. Dilute as needed with *Medium* to a concentration that is similar to that of the *Standard solution*.

Analysis: Determine the amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) dissolved as directed in *Uniformity of Dosage Units* 〈 905 〉.

Tolerances: NLT 80% (*Q*) of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) is dissolved.

- **Uniformity of Dosage Units** 〈 905 〉

Procedure for content uniformity

Buffer and Mobile phase: Prepare as directed in the *Assay*.

Standard solution: 0.1 mg/mL of USP Enalapril Maleate RS in *Buffer*

Sample solution: 0.1 mg/mL of enalapril maleate from 1 Tablet in *Buffer*. Add a volume of *Buffer* that is one-half the nominal volume of the flask, sonicate for 15 min, and shake by mechanical means for 30 min. Dilute with *Buffer* to volume, shake well, and sonicate for an additional 15 min. Pass through a suitable filter of 0.45- μ m pore size, and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Column temperature: 50°

Flow rate: 2 mL/min

Injection volume: 50 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 300 theoretical plates

Tailing factor: NMT 2.0

Capacity factor, *k'*: NLT 1.5

Relative standard deviation: NMT 2.0%

[Note—The enalapril peak tailing factor may be minimized by controlling the column temperature between 45° and 50° and by raising the pH of the aqueous component of the *Mobile phase* from 2.2 to 2.6; the capacity factor may be increased by decreasing the amount of acetonitrile in the *Mobile phase*.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of enalapril maleate (C₂₀H₂₈N₂O₅·C₄H₄O₄) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Enalapril Maleate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **Organic Impurities**

Buffer, Mobile phase, Enalapril diketopiperazine solution, Standard solution, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Diluted standard solution: Dilute 1.0 mL of *Standard solution* with *Buffer* to 100 mL.

Analysis

Samples: *Buffer, Standard solution, Sample solution, and Diluted standard solution*

Measure the responses for all of the peaks in the *Sample solution* greater than 0.1% of the response of the enalapril peak that are not observed in the *Buffer*.

Calculate the percentage of anhydrous enalaprilat (as enalapril maleate) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Enalaprilat RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of enalapril maleate, 492.52

M_{r2} = molecular weight of anhydrous enalaprilat, 348.39

Calculate the percentage of enalapril diketopiperazine (as enalapril maleate) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of enalapril diketopiperazine from the *Sample solution*

r_S = peak response of enalapril from the *Diluted standard solution*

C_S = concentration of USP Enalapril Maleate RS in the *Diluted standard solution* (mg/mL)

C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

F = relative response factor of enalapril diketopiperazine, 1.25

M_{r1} = molecular weight of enalapril maleate, 492.52

M_{r2} = molecular weight of enalapril diketopiperazine, 358.44

Calculate the percentage of the sum of all other individual impurities in the portion of Tablets taken:

$$\text{Result} = (r_T/r_S) \times (C_S/C_U) \times 100$$

r_T = sum of the responses of all other individual impurities other than maleic acid, enalapril, enalaprilat, and enalapril diketopiperazine from the *Sample solution*

r_S = peak response of enalapril maleate from the *Diluted standard solution*

C_S = concentration of USP Enalapril Maleate RS in the *Diluted standard solution* (mg/mL)

C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 5.0% for the sum of all impurities including those from enalaprilat and enalapril diketopiperazine

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** { 11 }

USP Enalaprilat RS

USP Enalapril Maleate RS

BRIEFING

Ephedrine Hydrochloride, *USP 38* page 3312. As part of the USP monograph modernization initiative, the following changes are proposed:

1. Add the chemical structure of ephedrine hydrochloride to the chemical information section.
2. Delete the extraction procedure in *Identification* test A because it is no longer

necessary and add a reference to *Infrared Absorption* 〈197K〉 to strengthen the monograph.

3. Replace the titration method in the *Assay* with the titration procedure from the *European Pharmacopoeia 8.4* that eliminates the use of mercuric acetate, which is a safety hazard.
4. Add a reference to *Chloride and Sulfate* 〈221〉 in the test for *Sulfate*.
5. Replace the *Ordinary Impurities* test with an HPLC method in the test for *Organic Impurities* based on the *Related substances* procedure in the *Ephedrine Hydrochloride* monograph in the *European Pharmacopoeia 8.4*. The procedure uses the Hypersil phenyl brand of L11 column. The typical retention time for ephedrine is about 10 min.
6. Delete the nonspecific *Melting Range or Temperature* test. This test is no longer necessary because a specific HPLC-based test for *Organic Impurities* is added.
7. Delete USP Ephedrine Sulfate RS from the *USP Reference Standards* section because it is no longer needed for *Identification* test A.
8. Add USP Ephedrine Hydrochloride RS and USP Pseudoephedrine Hydrochloride RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities* and the change in the USP Reference Standard for *Identification* test A.

Additionally, minor editorial changes have been made to update the monograph to the current *USP* style.

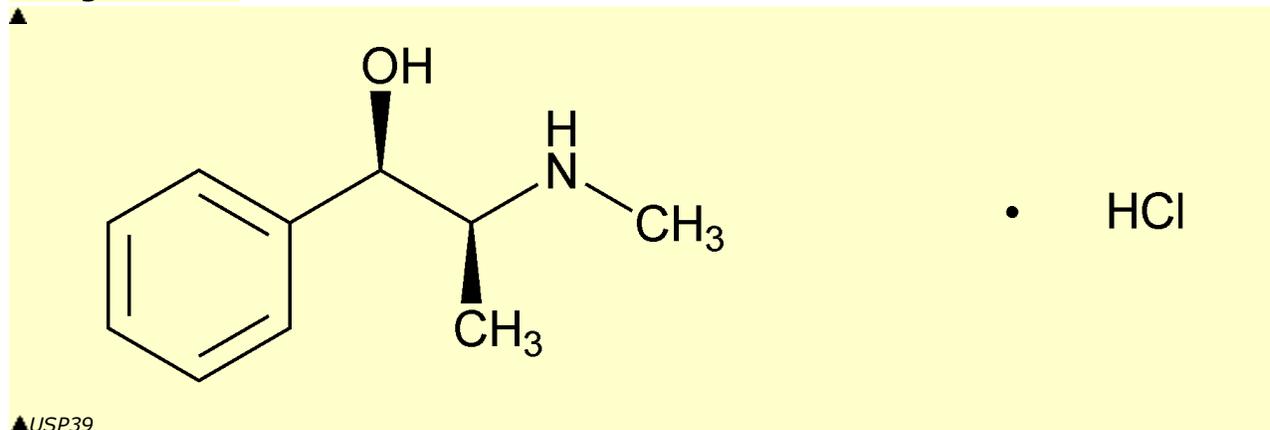
(SM4: M. Koleck, D. Vicchio.)

Correspondence Number—C107330

Comment deadline: March 31, 2015

Ephedrine Hydrochloride

Change to read:



▲USP39

$C_{10}H_{15}NO \cdot HCl$ 201.69

Benzenemethanol, α -[1-(methylamino)ethyl]-, hydrochloride, [*R*-(*R*^{*},*S*^{*})]-;
 (-)-Ephedrine hydrochloride [50-98-6].

DEFINITION

Ephedrine Hydrochloride contains NLT 98.0% and NMT 100.5% of ephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.**

▲**Infrared Absorption** 〈 197K〉 ▲*USP39*

~~**Sample solution:** Dissolve 100 mg of Ephedrine Hydrochloride in 5 mL of water, add 1 mL of potassium carbonate solution (1 in 5), and extract with 2 mL of chloroform.~~

~~**Acceptance criteria:** The *Sample solution* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Ephedrine Sulfate RS.~~

▲▲*USP39*

- **B. Identification Tests—General, Chloride** 〈 191〉: Meets the requirements

ASSAY

Change to read:

- **Procedure**

~~**Sample solution:** 500 mg of Ephedrine Hydrochloride in 25 mL of glacial acetic acid~~

~~**Analysis:** To the *Sample solution*, add 10 mL of mercuric acetate TS and 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to an emerald-green endpoint. Perform a blank determination and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 20.17 mg of ephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).~~

~~**Acceptance criteria:** 98.0%–100.5% on the dried basis~~

▲**Sample solution:** Dissolve 150 mg of Ephedrine Hydrochloride in 50 mL of alcohol. Add 5.0 mL of 0.01 N hydrochloric acid.

Titrimetric system

(See *Titrimetry* 〈 541〉.)

Titrant: 0.1 N sodium hydroxide VS

Endpoint detection: Potentiometric

Analysis

Sample: *Sample solution*

Titrate the *Sample solution* with *Titrant*, determining the endpoint potentiometrically.

Read the volume added between the two points of inflection. Each mL of 0.1 N sodium hydroxide is equivalent to 20.17 mg of ephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

Acceptance criteria: 98.0%–100.5% on the dried basis ▲*USP39*

IMPURITIES

- **Residue on Ignition** 〈 281〉: NMT 0.1%

Change to read:

-

▲Chloride and Sulfate, Sulfate 〈 221 〉 ▲*USP39*

Sample solution: 1.25 mg/mL of Ephedrine Hydrochloride in water

Analysis: Add 1 mL of 3 N hydrochloric acid and 1 mL of barium chloride TS to 40 mL of *Sample solution*.

Acceptance criteria: No turbidity develops within 10 min.

Change to read:

• **Organic Impurities**

• ~~Ordinary Impurities~~ 〈 466 〉

~~Test solution:~~ Alcohol

~~Standard solution:~~ Alcohol

~~Eluant:~~ Isopropyl alcohol, chloroform, and ammonium hydroxide (80:5:15)

~~Visualization:~~ 1, followed by 4

~~Acceptance criteria:~~ Meets the requirements

▲Buffer: 11.6 g/L of ammonium acetate adjusted with glacial acetic acid to a pH of 4.0

Mobile phase: Methanol and *Buffer* (6:94)

System suitability solution: 0.1 mg/mL each of USP Ephedrine Hydrochloride RS and USP Pseudoephedrine Hydrochloride RS in *Mobile phase*

Sensitivity solution: 3.8 µg/mL of USP Ephedrine Hydrochloride RS in *Mobile phase*

Standard solution: 30 µg/mL of USP Ephedrine Hydrochloride RS in *Mobile phase*

Sample solution: 7.5 mg/mL of Ephedrine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 257 nm

Column: 4.6-mm × 15-cm; 3-µm packing L11

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 2.5 times the retention time of ephedrine

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between ephedrine and pseudoephedrine, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Ephedrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U

= peak response of each impurity from the *Sample solution*

r_S = peak response of ephedrine from the *Standard solution* C_S = concentration of USP Ephedrine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = concentration of Ephedrine Hydrochloride in the *Sample solution* (mg/mL) F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard peaks that are less than 0.05% of the ephedrine peak.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ephedrine	1.0	—	—
Pseudoephedrine ^a	1.1	—	—
α -Acetylbenzyl alcohol ^b	1.4	2.5	0.2
Any individual unspecified impurity	—	1.0	0.1
Total impurities ^c	—	—	0.5

^a Included for identification only. It is not to be reported and not to be included in the total impurities.

^b (-)-(1R)-1-Hydroxy-1-phenylpropan-2-one.

^c Excludes α -acetylbenzyl alcohol.

▲USP39

SPECIFIC TESTS**Delete the following:**

▲● ~~Melting Range or Temperature, Class 1 (741): 217°–220°~~ ▲USP39

● **Optical Rotation, Specific Rotation** (781S)

Sample solution: 50 mg/mL of Ephedrine Hydrochloride in water

Acceptance criteria: -33.0° to -35.5°

● **Loss on Drying** (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.5%

- **Acidity or Alkalinity**

Sample solution: 50 mg/mL of Ephedrine Hydrochloride in water

Analysis: To 20 mL of *Sample solution*, add 1 drop of methyl red TS.

Acceptance criteria: If the solution is yellow, it is changed to red by NMT 0.10 mL of 0.020 N sulfuric acid. If the solution is pink, it is changed to yellow by NMT 0.20 mL of 0.020 N sodium hydroxide.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers.

Change to read:

- **USP Reference Standards** { 11 }

~~USP Ephedrine Sulfate RS~~

▲USP Ephedrine Hydrochloride RS

USP Pseudoephedrine Hydrochloride RS▲*USP39*

BRIEFING

Fluticasone Propionate and Salmeterol Inhalation Aerosol. Because there is no existing *USP* monograph for this dosage form, the following monograph is proposed.

1. The isocratic liquid chromatographic procedure used in the *Assay* and the tests for *Aerodynamic Size Distribution* and *Delivered-Dose Uniformity* is based on validations performed with the Zorbax StableBondSB-C18 column using dual detectors. UV absorbance is used for fluticasone propionate, and fluorescence emission is used for salmeterol. The typical retention times for fluticasone propionate and salmeterol are about 1.4 and 2.2 min, respectively.
2. The equipment used for *Aerodynamic Size Distribution* and the dose collection in *Delivered-Dose Uniformity* are modified from what was provided in general test chapter *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* { 601 }, but consistent with other new monographs such as *Fluticasone Propionate Inhalation Aerosol*. The details of the modification and the engineering diagrams have been included in the proposal to facilitate evaluation. Interested parties are invited to submit comments.
3. The gradient elution liquid chromatographic procedure used in the test for *Organic Impurities* is based on validations performed with the Inertsil ODS 2 brand of L1 column. The typical retention times for salmeterol and fluticasone propionate are about 15 and 37 min, respectively.

(SM4: R. Ravichandran.)

Correspondence Number—C64167

Comment deadline: March 31, 2015

Add the following:

▲**Fluticasone Propionate and Salmeterol Inhalation Aerosol**

DEFINITION

Fluticasone Propionate and Salmeterol Inhalation Aerosol is a suspension of Fluticasone Propionate and Salmeterol with suitable propellants in a pressurized container. The mean content per actuation contains NLT 88% and NMT 112% of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$). The mean content per actuation contains NLT 88% and NMT 112% of the labeled amount of salmeterol ($C_{25}H_{37}NO_4$) as salmeterol xinafoate.

IDENTIFICATION

- **A. Infrared Absorption** (197A)

Wavenumber range: 4000 cm^{-1} – 600 cm^{-1}

Sample: Discharge an appropriate number of actuations from two containers into an agate mortar. Allow the propellant to evaporate and dry if necessary. Transfer the residue to the sample window.

Acceptance criteria: Meets the requirements. In addition, the ratio of the fluticasone propionate band at 833 cm^{-1} to that of the salmeterol band at 744 cm^{-1} meets the requirements in *Table 1*.

Table 1

Fluticasone Propionate/Salmeterol ($\mu\text{g}/\mu\text{g}$ for each dose)	Ratio (band at 833 cm^{-1} /band at 744 cm^{-1})
45/21	NMT 2.5
115/21	2.5–4.0
230/21	NLT 4.0

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Delivered-Dose Uniformity*.

ASSAY

- **Procedure**

Buffer: 0.01 M sodium dodecyl sulfate containing 0.1% glacial acetic acid

Solution A: Methanol and *Buffer* (20:80)

Mobile phase: Acetonitrile and *Solution A* (50:50)

Diluent: Methanol and water (70:30)

Standard solution: 50 $\mu\text{g}/\text{mL}$ of USP Fluticasone Propionate RS and 15 $\mu\text{g}/\text{mL}$ of USP Salmeterol Xinafoate RS in *Diluent*

Sample solution: Nominally 20–110 $\mu\text{g}/\text{mL}$ of fluticasone propionate and 10 $\mu\text{g}/\text{mL}$ of salmeterol prepared as follows. Shake the canister vigorously, and cool for 10 min in a dry ice–methanol bath. Remove the canister from the bath, and shake vigorously. Using a suitable device, carefully remove and keep the valve, and pour the contents into a suitable container. Allow the propellant to evaporate. Dissolve the canister contents in a minimum amount of methanol and quantitatively transfer to a suitable volumetric flask containing 30% of the flask volume of water. Rinse the canister and valve with methanol into the same volumetric flask. Allow the flask to come to room temperature and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detectors

Fluticasone propionate: UV 239 nm

Salmeterol: Fluorescence with excitation at 225 nm and emission at 305 nm. Use emission response for quantification.

Column: 4.6-mm × 5-cm; 3.5-μm packing L1

Column temperature: 40°

Flow rate: 2 mL/min

Injection volume: 5 μL

Run time: NLT 2 times the retention time of salmeterol

System suitability

Sample: *Standard solution*

[Note—The relative retention times for fluticasone propionate and salmeterol are 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.5 between fluticasone propionate and salmeterol

Tailing factor: NMT 1.5 for fluticasone propionate and salmeterol

Relative standard deviation: NMT 2.0% for fluticasone propionate and salmeterol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluticasone propionate (C₂₅H₃₁F₃O₅S) in the portion of Inhalation Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluticasone propionate from the *Sample solution*

r_S = peak response of fluticasone propionate from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (μg/mL)

C_U = nominal concentration of fluticasone propionate in the *Sample solution* (μg/mL)

Calculate the percentage of the labeled amount of salmeterol (C₂₅H₃₇NO₄) in the portion of Inhalation Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of salmeterol from the *Sample solution*

r_S = peak response of salmeterol from the *Standard solution*

C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (μg/mL)

C_U = nominal concentration of salmeterol free base in the *Sample solution* (μg/mL)

M_{r1} = molecular weight of salmeterol free base, 415.57

M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria: 88%–112% each for fluticasone propionate and salmeterol

PERFORMANCE TESTS

• **Aerodynamic Size Distribution**

(See *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* { 601 }, *Aerodynamic Size Distribution—Inhalation Aerosols, Sprays, and Powders.*)

Sampling apparatus: Use *Apparatus 1* in { 601 } with a modified *Induction port* (Figure 1) and *Entrance cone* (Figure 2).

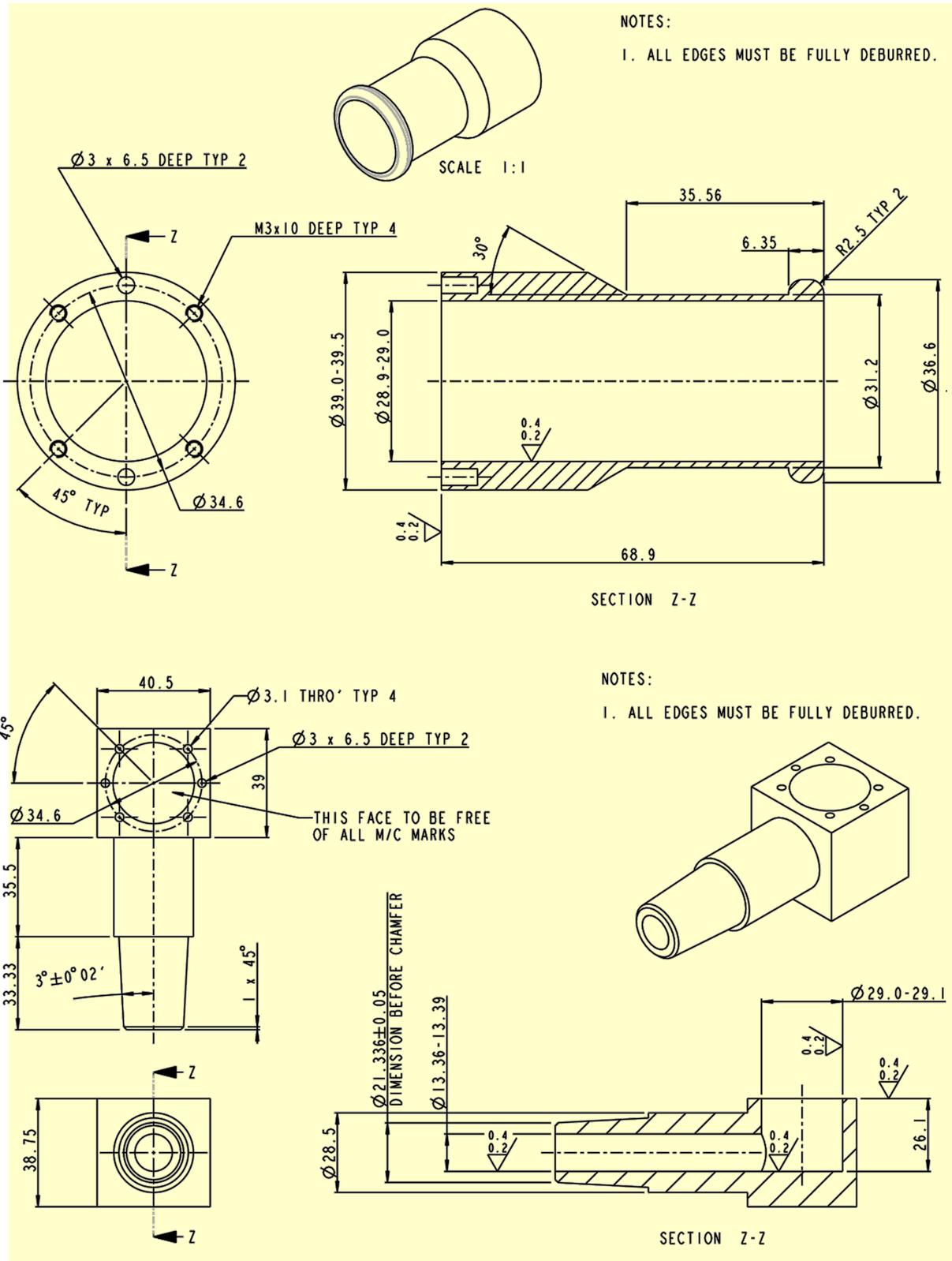


Figure 1. Expanded view of the modified *Induction port*.

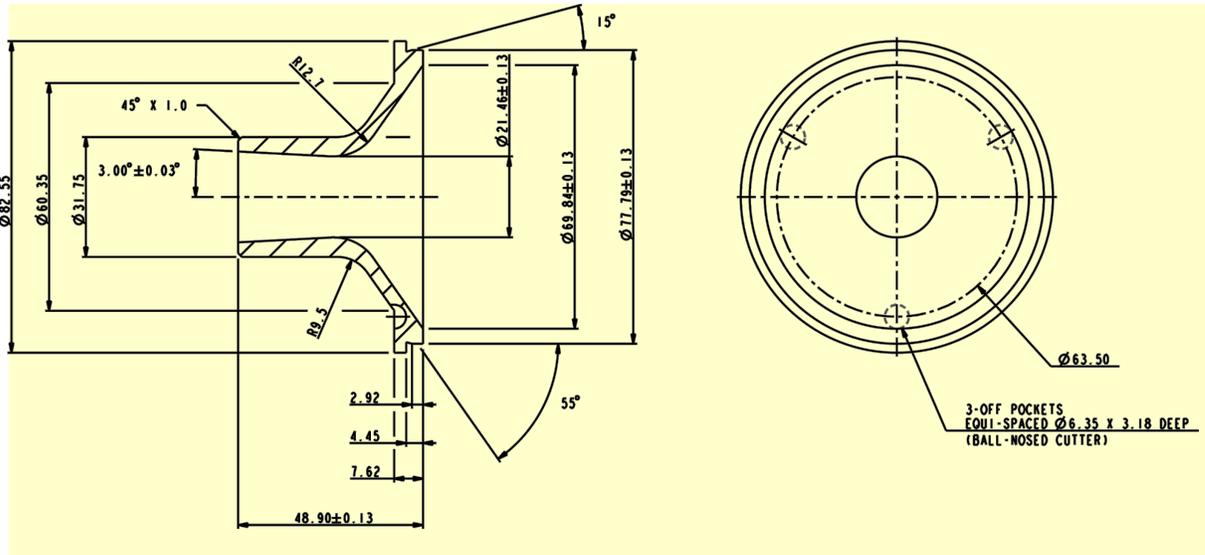


Figure 2. Expanded view of the modified *Entrance cone*.

Buffer, Solution A, and Mobile phase: Proceed as directed in the Assay.

Diluent: Methanol and water (70:30)

Standard solution: 1.25 µg/mL of USP Fluticasone Propionate RS and 0.38 µg/mL of USP Salmeterol Xinafoate RS in *Diluent*

Sample solutions: Prime the valve by discharging a predetermined number of actuations to waste. Discharge the 10 actuations into the cascade impaction *Sampling apparatus* described in *Figure 1* and *Figure 2*. Detach the inhaler, and rinse each piece of the apparatus with methanol into a suitable volumetric flask containing 30% of the flask volume of water. The final concentration of the expected amount of fluticasone propionate should be in the range of 0.05–3.5 µg/mL. Allow the solutions to equilibrate, and dilute with methanol to volume. Repeat these steps on an additional four Inhalation Aerosols for a total of five *Sample solutions*.

Chromatographic system and System suitability: Proceed as directed in the Assay, except for the *Injection volume*.

Injection volume: 50 µL

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the quantity, in µg/actuation, of fluticasone propionate (C₂₅H₃₁F₃O₅S) in the *Sample solutions*:

$$\text{Result} = [(r_U/r_S) \times C_S] \times (V/N)$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

V = total volume of the *Sample solution* (mL)

N = number of actuations discharged into the apparatus

Calculate the quantity, in µg/actuation, of salmeterol (C₂₅H₃₇NO₄) in the *Sample solutions*:

$$\text{Result} = [(r_U/r_S) \times C_S] \times (V/N) \times (M_{r1}/M_{r2})$$

r_U = peak response of salmeterol from the *Sample solution*

r_S = peak response of salmeterol from the *Standard solution*

C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (µg/mL)

V = total volume of the *Sample solution* (mL)

N = number of actuations discharged into the apparatus

M_{r1} = molecular weight of salmeterol free base, 415.57

M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria: The mass of fluticasone propionate and salmeterol deposited in each grouping of the *Sampling apparatus* for each individual inhaler is given in *Table 2*.

Table 2

Parameter	Amount of Fluticasone Propionate Deposited (µg/actuation)			Amount of Salmeterol Deposited (µg/actuation)		
Label claim of fluticasone propionate/salmeterol (µg/actuation)	45/21	115/21	230/21	45/21	115/21	230/21
Mass of mouthpiece adapter, and induction port	10–21	24–54	59–109	2–10	3–11	4–12
Sum of Stages 0–2	NMT 8	NMT 25	NMT 59	NMT 5	NMT 6	NMT 7
Sum of Stages 3, 4, and 5	15–27	39–67	77–124	7–13	7–13	7–13
Sum of Stages 6, 7, and filter	NMT 2	NMT 3	NMT 4	NMT 1	NMT 1	NMT 1

The mass of fluticasone propionate and salmeterol deposited in each grouping of the *Sampling apparatus* for the mean of the five inhalers is given in *Table 3*.

Table 3

Parameter	Amount of Fluticasone Propionate Deposited (µg/actuation)			Amount of Salmeterol Deposited (µg/actuation)		
Label claim of fluticasone propionate/salmeterol (µg/actuation)	45/21	115/21	230/21	45/21	115/21	230/21
Mass of mouthpiece adapter, and induction port	11–20	26–52	64–104	3–9	4–10	5–11
Sum of Stages 0, 1, and 2	NMT 7	NMT 22	NMT 52	NMT 4	NMT 5	NMT 6
Sum of Stages 3, 4, and 5	16–26	41–65	81–120	7–12	7–12	7–12
Sum of Stages 6, 7, and filter	NMT 2	NMT 3	NMT 4	NMT 1	NMT 1	NMT 1

● **Delivered-Dose Uniformity**

(See *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* 〈601〉, *Delivered-Dose Uniformity—Inhalation Aerosols and Inhalation Sprays*.)

Sampling apparatus: Use the apparatus (*Figure 3A*) with modified glass sampling device

(Figure 3B).

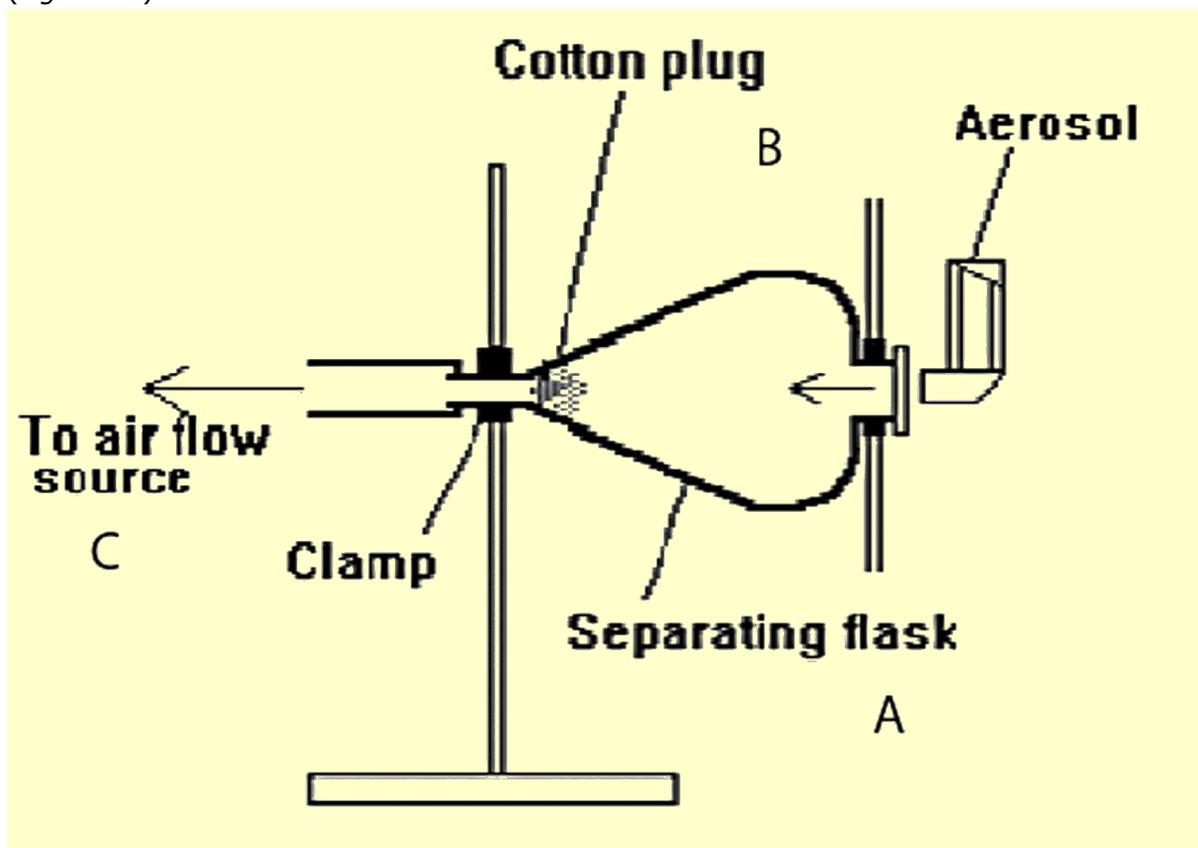


Figure 3A. Sampling apparatus.

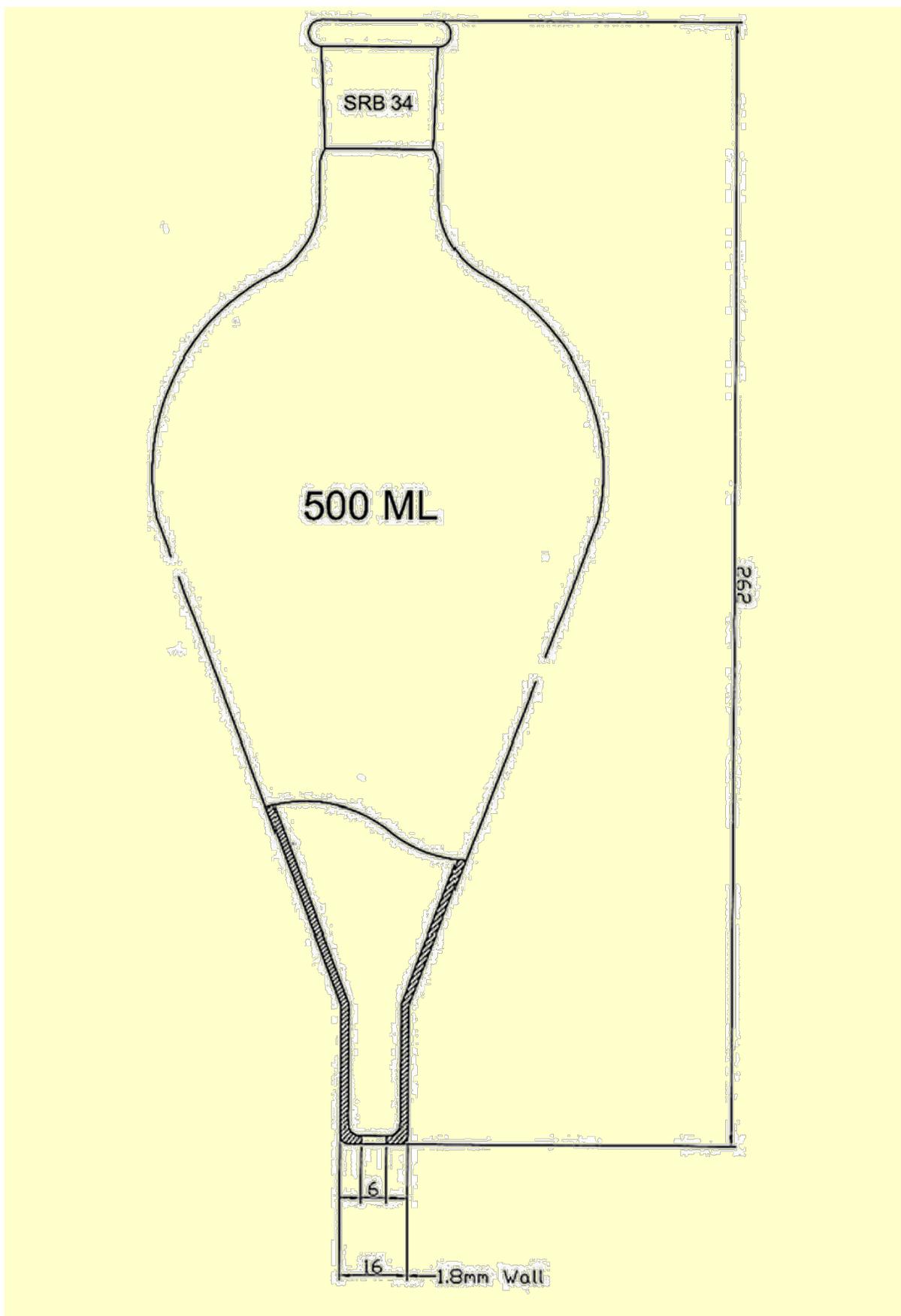


Figure 3B. Expanded view of the modified glass sample collection apparatus.

Buffer, Solution A, Mobile phase, and Diluent: Prepare as directed in the Assay.

Standard solution: 1.25 µg/mL of USP Fluticasone Propionate RS and 0.38 µg/mL of USP Salmeterol Xinafoate RS in *Diluent*

Sample solutions: Prime the valve by discharging a predetermined number of actuations to waste. Discharge the recommended dose at the beginning of use (BOU) into the *Sampling apparatus*, and detach the inhaler. Rinse the apparatus with methanol, and quantitatively transfer the solutions to a 100-mL volumetric flask containing 30 mL of water. Allow the solution to equilibrate, and dilute with methanol to volume. Discharge the requisite number of actuations to waste, shaking the inhaler before each discharge, until the last recommended dose remains. Discharge the remaining recommended dose at end of use (EOU) corresponding to the labeled number of actuations into the *Sampling apparatus*, and detach the inhaler. Rinse the apparatus with methanol, and quantitatively transfer the solution to a 100-mL volumetric flask containing 30 mL of water. Allow the solution to equilibrate, and dilute with methanol to volume. Repeat these steps on an additional nine inhalers for a total of 20 *Sample solutions*.

Chromatographic system and System suitability: Proceed as directed in the Assay, except for the *Injection volume*.

Injection volume: 50 µL

Analysis

Samples: *Standard solution and Sample solutions*

Calculate the percentage of the labeled amount of fluticasone propionate (C₂₅H₃₁F₃O₅S) delivered by the inhaler in each *Sample solution*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

C_U nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL)

Calculate the percentage of the labeled amount of salmeterol (C₂₅H₃₇NO₄) delivered by the inhaler in each *Sample solution*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U peak response of salmeterol from the *Sample solution*

r_S peak response of salmeterol from the *Standard solution*

C_S concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (µg/mL)

C_U nominal concentration of salmeterol free base in the *Sample solution* (µg/mL)

M_{r1} molecular weight of salmeterol free base, 415.57

M_{r2} molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria: See *Table 4* and *Table 5*.

Table 4

Parameter	Acceptance Criteria
-----------	---------------------

Label claim of fluticasone propionate/salmeterol ($\mu\text{g}/\text{actuation}$)	45 /21	115/21	230/21
Mean of 10 BOU contents	80%–120% of label claim	80%–120% of label claim	80%–120% of label claim
Mean of 10 EOU contents	80%–120% of label claim	80%–120% of label claim	80%–120% of label claim
Number of individual contents outside 80%–120% of label claim	NMT 2 of 20	NMT 2 of 20	NMT 2 of 20
Mean of 20 contents/actuations	88%–112% of label claim	88%–112% of label claim	88%–112% of label claim
Each individual content	70%–130% of label claim	75%–125% of label claim	75%–125% of label claim

Test an additional 20 inhalers (BOU and EOU) if up to six of the 20 individual results are outside of 80%–120% of label claim, but none are outside 70%–130% of the label claim for the 45/21 $\mu\text{g}/\text{actuation}$ product strength, or none are outside 75%–125% of the label claim for the 115/21 $\mu\text{g}/\text{actuation}$ and 230/21 $\mu\text{g}/\text{actuation}$.

Table 5

Parameter	Acceptance Criteria		
	45 /21	115/21	230/21
Label claim of fluticasone propionate/salmeterol ($\mu\text{g}/\text{actuation}$)			
Mean of 30 BOU contents	80%–120% of label claim	80%–120% of label claim	80%–120% of label claim
Mean of 30 EOU contents	80%–120% of label claim	80%–120% of label claim	80%–120% of label claim
Number of individual contents outside 80%–120% of label claim	NMT 6 of 60	NMT 6 of 60	NMT 6 of 60
Mean of 60 contents/actuations	88%–112% of label claim	88%–112% of label claim	88%–112% of label claim
Each individual content	70%–130% of label claim	75%–125% of label claim	75%–125% of label claim

IMPURITIES

- **Organic Impurities**

[Note—Protect all solutions containing fluticasone propionate or salmeterol from light.]

Buffer: 0.05 M monobasic ammonium phosphate adjusted with 10% (v/v) of phosphoric acid to a pH of 2.9

Solution A: Acetonitrile and *Buffer* (30:70)

Solution B: Acetonitrile and *Buffer* (78:22)

Mobile phase: See *Table 6*.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	100	0

60	0	100
61	100	0
70	100	0

Diluent 1: Methanol, water, and phosphoric acid (70: 30: 0.05)

Diluent 2: Methanol and water (70:30)

Diluent 3: 0.05% Phosphoric acid in methanol

System suitability solution: 0.075 mg/mL of USP Salmeterol Xinafoate RS, 0.025 mg/mL of USP Fluticasone Propionate RS, and 0.2 µg/mL each of USP Fluticasone Propionate Related Compound D RS and USP Fluticasone Propionate Related Compound J RS in *Diluent 1*

Sensitivity solution: 0.05 µg/mL of USP Salmeterol Xinafoate RS and 0.1 µg/mL of USP Fluticasone Propionate RS in *Diluent 2*

Sample solution: Nominally 50–100 µg/mL of salmeterol prepared as follows. Place a canister into a freezing mixture of dry ice and methanol, and cool for approximately 5 min. Carefully remove the valve from the canister. Pour the contents into a suitable container and allow the propellant to evaporate. Dissolve the residue in *Diluent 3* and dilute with water to the required volume to obtain the nominal concentration.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 228 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[Note—See *Table 7* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between salmeterol and fluticasone propionate related compound J; NLT 1.5 between fluticasone propionate related compound D and fluticasone propionate, *System suitability solution*

Tailing factor: NMT 1.5 for fluticasone propionate and salmeterol, *System suitability solution*

Signal-to-noise ratio: NLT 10 for both fluticasone propionate and salmeterol, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each degradation product in the portion of Inhalation Aerosol taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_S peak response of either salmeterol or fluticasone propionate from the *Sample solution*

Acceptance criteria: See *Table 7*. Disregard any peak less than 0.05%. [Note—Any

unspecified degradation product eluting before salmeterol is related to salmeterol. Any unspecified degradation product eluting after salmeterol is related to fluticasone propionate.]

Table 7

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salmeterol- <i>N</i> -phenylbutyl aminoalcohol ^{a,b}	0.14	—
Salmeterol-phenylethoxy ^{a,c}	0.25	—
Salmeterol-phenylpropoxy ^{a,d}	0.32	—
Salmeterol-phenyl-2-butoxy ^{a,e}	0.37	—
Fluticasone propionate related compound J ^a	0.38	—
Salmeterol	0.41	—
Hydroxynaphthoic acid ^f	0.50	—
Salmeterol-deoxy ^{a,g}	0.55	—
Fluticasone propionate dithioacid ^{a,h}	0.67	—
Salmeterol- <i>N</i> -alkyl ⁱ	0.71	0.2
Fluticasone propionate related compound D	0.97	—
Fluticasone propionate	1.0	—
Fluticasone dimer ^{a,j}	1.09	—
Any unspecified degradation product	—	0.10
Total degradation products	—	0.2
<p>^a This is a process impurity that is included in this table for identification purposes only. This impurity is controlled in drug substance. This impurity is not to be reported for the drug product or to be included in total degradation products.</p>		

^b 4-[1-Hydroxy-2-(4-phenylbutylamino)ethyl]-2-(hydroxymethyl)phenol.

^c 4-[1-Hydroxy-2-(6-phenethoxyhexylamino)ethyl]-2-(hydroxymethyl)phenol.

^d 4-{1-Hydroxy-2-[6-(3-phenylpropoxy)hexylamino]ethyl}-2-(hydroxymethyl)phenol.

^e 4-{1-Hydroxy-2-[6-(4-phenylbutan-2-yloxy)hexylamino]ethyl}-2-(hydroxymethyl)phenol.

^f This is a counter ion of salmeterol that is included in this table for identification purposes only. It is not to be reported for the drug product or to be included in the total degradation products.

^g 4-{1-Hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}-2-methylphenol.

^h 6 α ,9 α -Difluoro-11 β -hydroxy-16 α -methyl-3-oxo-17 α -propionyloxyandrosta-1,4-diene-17 β -carbodithioic acid.

i 4-{1-Hydroxy-2-[(2-hydroxy-5-{1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}benzyl)[6-(4-phenylbutoxy)hexyl]amino]ethyl}-2-(hydroxymethyl)phenol.

j $6\alpha, 9\alpha$ -Difluoro- $11\beta, 17\alpha$ -dihydroxy- 16α -methyl-3-oxoandrosta-1,4-diene- 17β -carboxylic acid $6\alpha, 9\alpha$ -difluoro- 17β -(fluoromethylthio)carbonyl- 11β -hydroxy- 16α -methyl-3-oxoandrosta-1,4-diene- 17β -yl ester.

SPECIFIC TESTS

- **Microbial Enumeration Tests** $\langle 61 \rangle$ and **Tests For Specified Microorganisms** $\langle 62 \rangle$: The total aerobic microbial count does not exceed 10^1 cfu/g of formulation. The total aerobic yeasts and molds count does not exceed 10^1 cfu/g of formulation. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.

- **Foreign Particulate Matter**

The test described below and the specification is only applicable to a microscopic particle count test methodology. General test chapter *Particulate Matter in Injections* $\langle 788 \rangle$ describes details of the test apparatus to be used for the determination of particulate matter using a microscopic particle count test methodology. Samples should be carefully prepared to avoid environmental contamination, and testing should be performed with suitable controls, including the appropriate use of blank determinations.

Filter: Mixed cellulose and ester; 25-mm diameter and 0.45- μm pore size

Sample solution: Perform testing on two previously unused inhalers. Prime each inhaler by discharging a predetermined number of actuations to waste. Discharge, and dissolve 16 actuations, eight from each of two canisters in 50 mL of methanol.

Analysis

Sample: *Sample solution*

Pass the *Sample solution* through the *Filter*, and allow the *Filter* to dry under conditions that will limit particulate contamination. Using a microscopic particle count test method, enumerate the number of particles present in the *Sample solution*. Calculate the number of particles per actuation:

$$\text{Result} = (N_{<10} + N_{10-100} + N_{>100})/16$$

$N_{<10}$ = total number of particles $<10 \mu\text{m}$ present in the *Sample solution*

N_{10-100} = total number of particles between 10 and 100 μm present in the *Sample solution*

$N_{>100}$ = total number of particles $>100 \mu\text{m}$ present in the *Sample solution*

Acceptance criteria: See *Table 8*.

Table 8

Particle Size Range (μm)	Number of Particles per Actuation
<10	140
10–100	50
>100	5

Total	185
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ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in nonreactive, light-resistant aerosol containers with metered valves fitted with a dose counter and provided with oral inhalation actuators. Avoid exposure to heat. Store at controlled room temperature.
- **USP Reference Standards** 〈 11 〉
 - USP Fluticasone Propionate RS
 - USP Fluticasone Propionate Related Compound D RS
 - S-Methyl 6 α , 9 α -difluoro-11 β -hydroxy-16 α -methyl-3-oxo-17 α -propionyloxyandrost-1,4-diene-17 β -carbothioate.
 - C₂₅H₃₂F₂O₅S 482.58
 - USP Fluticasone Propionate Related Compound J RS
 - 6 α , 9 α -Difluoro-11 β ,17 α -dihydroxy-16 α -methyl-3-oxoandrost-1,4-diene-17 β -carboxylic acid.
 - C₂₁H₂₆F₂O₅ 396.42
 - USP Salmeterol Xinafoate A RS

▲USP39

BRIEFING

Fluticasone Propionate and Salmeterol Inhalation Powder. Because there is no existing *USP* monograph for this dosage form, the following monograph is proposed.

1. The isocratic liquid chromatographic procedure used in the *Assay* and the tests for *Aerodynamic Size Distribution* and *Delivered-Dose Uniformity* is based on validations performed with the Zorbax StableBond SB-C18 column using dual detectors. UV absorbance is used for fluticasone propionate, and fluorescence emission is used for salmeterol. The typical retention times for salmeterol and fluticasone propionate are about 1.4 and 2.2 min, respectively.
2. The equipment used for *Aerodynamic Size Distribution* and dose collection in *Delivered-Dose Uniformity* are modified from what is provided in general chapter *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* 〈 601 〉, but consistent with other official monographs such as *Salmeterol Inhalation Powder* and *Fluticasone Propionate Inhalation Powder*. The details of modification and the engineering diagrams have been included in the proposal to facilitate evaluation. Interested parties are invited to submit comments.
3. The gradient elution liquid chromatographic procedure used in the test for *Organic Impurities* is based on validations performed with the Inertsil ODS 2 brand of L1 column. Typical retention times for salmeterol and fluticasone propionate elute at about 15 and 37 min, respectively.

(SM4: R. Ravichandran.)

Correspondence Number—C64175

Comment deadline: March 31, 2015**Add the following:**▲**Fluticasone Propionate and Salmeterol Inhalation Powder**

DEFINITION

Fluticasone Propionate and Salmeterol Inhalation Powder is a mixture of fluticasone propionate and salmeterol xinafoate for use in dry powder inhalers. The Inhalation Powder contains NLT 90% and NMT 110% of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) and NLT 90% and NMT 110% of the labeled amount of salmeterol ($C_{25}H_{37}NO_4$) as salmeterol xinafoate.

IDENTIFICATION**• A. Ultraviolet Absorption** 〈 197U〉

Diluent: Methanol and water (70:30)

Standard solution: A mixture of USP Fluticasone Propionate RS and USP Salmeterol Xinafoate RS according to the individual product strengths in the Inhalation Powder under test in *Diluent*

Sample solution: Dissolve a suitable number of unit doses of the Inhalation Powder under test in a suitable volume of *Diluent*.

Acceptance criteria: Meets the requirements

• B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Delivered-Dose Uniformity*.**ASSAY****• Procedure**

Buffer: 0.01 M sodium dodecyl sulfate containing 0.1% glacial acetic acid

Solution A: Methanol and *Buffer* (20:80)

Mobile phase: Acetonitrile and *Solution A* (50:50)

Diluent: Methanol and water (70:30)

Standard solution: 10 µg/mL of USP Fluticasone Propionate RS and 3 µg/mL of USP Salmeterol Xinafoate RS in *Diluent*

Sample solution: Nominally 5–25 µg/mL of fluticasone propionate and 2.4 µg/mL of salmeterol from NLT 12 unit doses in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621〉, *System Suitability*.)

Mode: LC

Detectors

Fluticasone propionate: UV 239 nm

Salmeterol: Fluorescence with excitation at 225 nm and emission at 305 nm. Use emission response for quantification.

Column: 4.6-mm × 5-cm; 3.5-µm packing L1

Flow rate: 2 mL/min

Column temperature: 40°

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[Note—The retention times for salmeterol and fluticasone propionate are 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.5 between salmeterol and fluticasone propionate

Tailing factor: NMT 1.5 for salmeterol and fluticasone propionate

Relative standard deviation: NMT 2.0% for salmeterol and fluticasone propionate

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the portion of Inhalation Powder taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluticasone propionate from the *Sample solution*

r_S = peak response of fluticasone propionate from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of fluticasone propionate in the *Sample solution* ($\mu\text{g/mL}$)

Calculate the percentage of the labeled amount of salmeterol ($C_{25}H_{37}NO_4$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of salmeterol from the *Sample solution*

r_S = peak response of salmeterol from the *Standard solution*

C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of salmeterol free base in the *Sample solution* ($\mu\text{g/mL}$)

M_{r1} = molecular weight of salmeterol free base, 415.57

M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria: 90%–110% each for fluticasone propionate and salmeterol

PERFORMANCE TESTS

• Aerodynamic Size Distribution

(See *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* { 601 }, *Aerodynamic Size Distribution—Inhalation Aerosols, Sprays, and Powders*.)

Sampling apparatus: Modified *Apparatus 3* (Figure 1) in { 601 } with a modified induction port (Figure 2), and preseparator lid (Figure 3) are to be used.

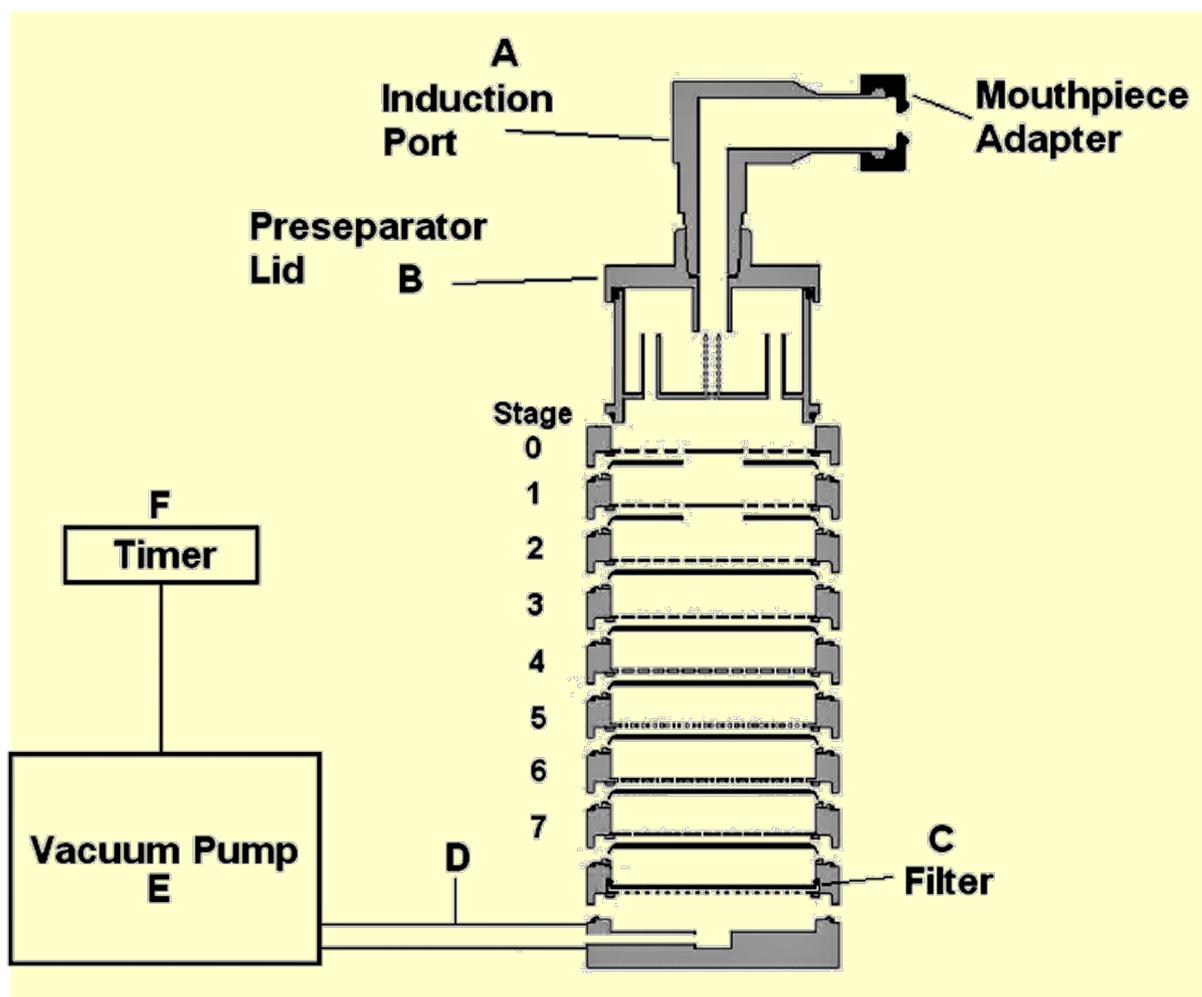


Figure 1. Cascade impaction sampling apparatus (modified *Apparatus 3* in $\langle 601 \rangle$) including *Induction Port* and *Preseparator Lid*.

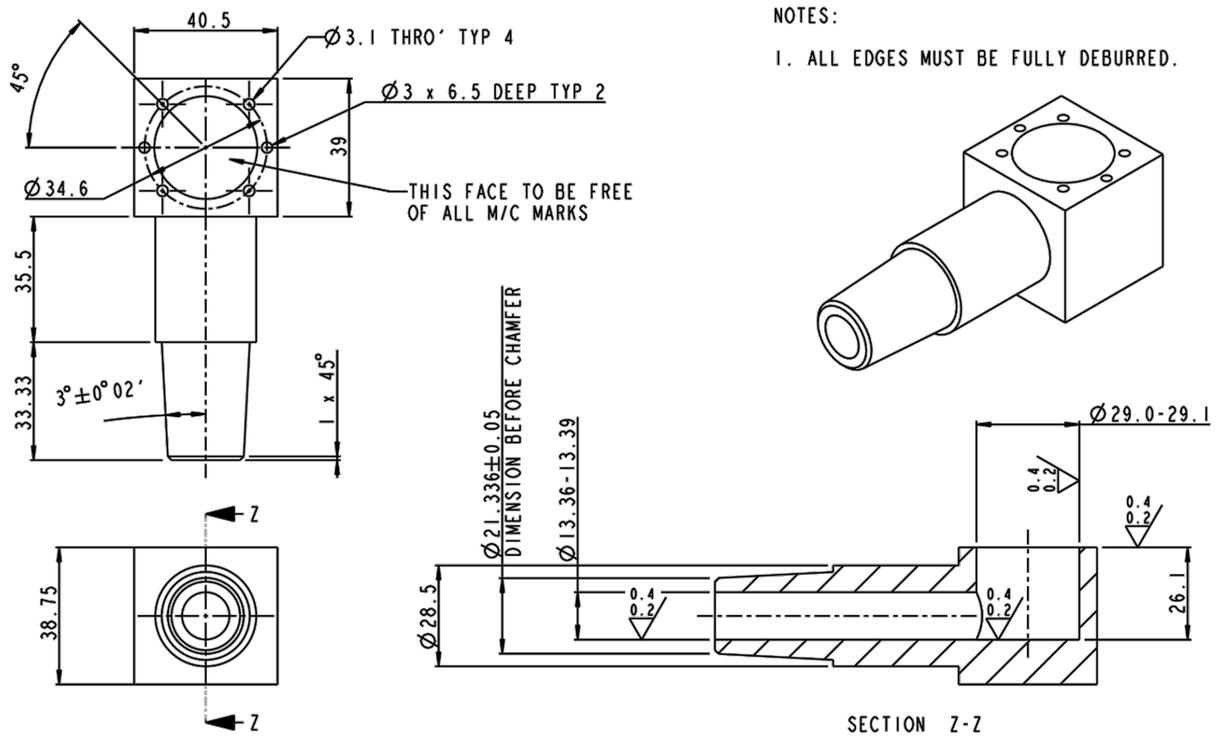
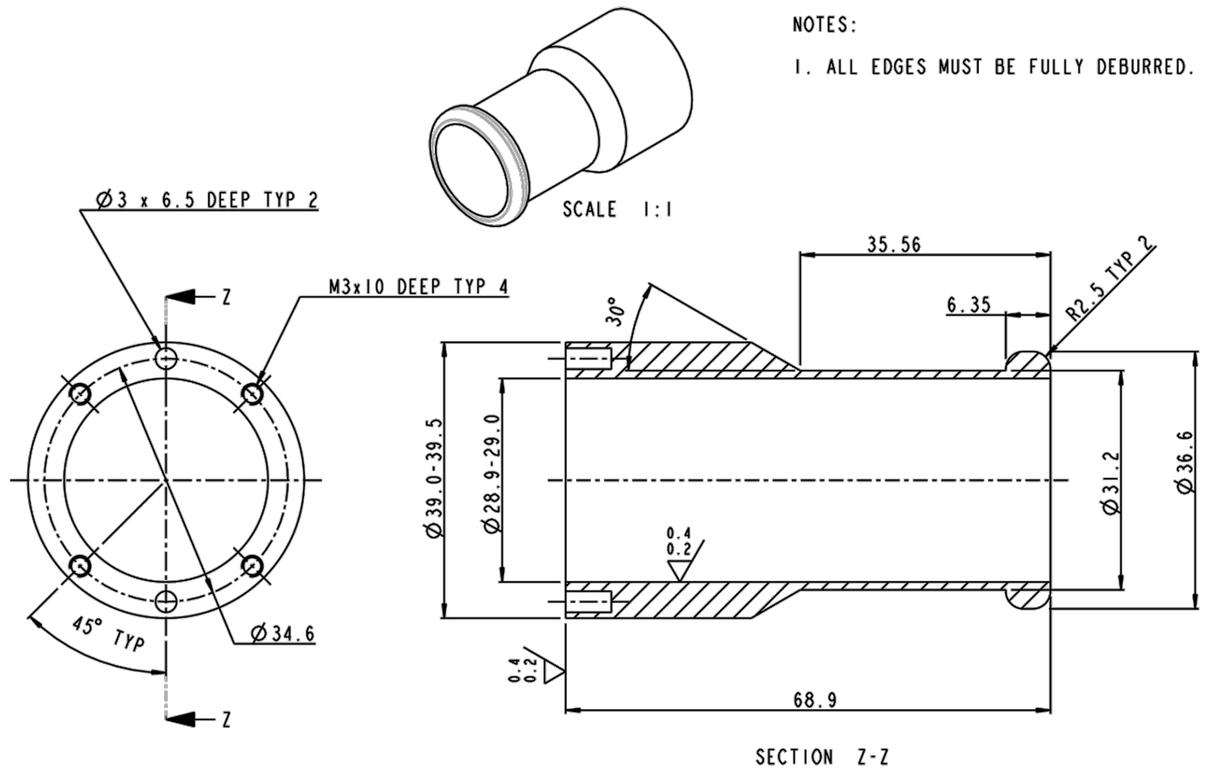


Figure 2. Expanded view of the modified induction port.

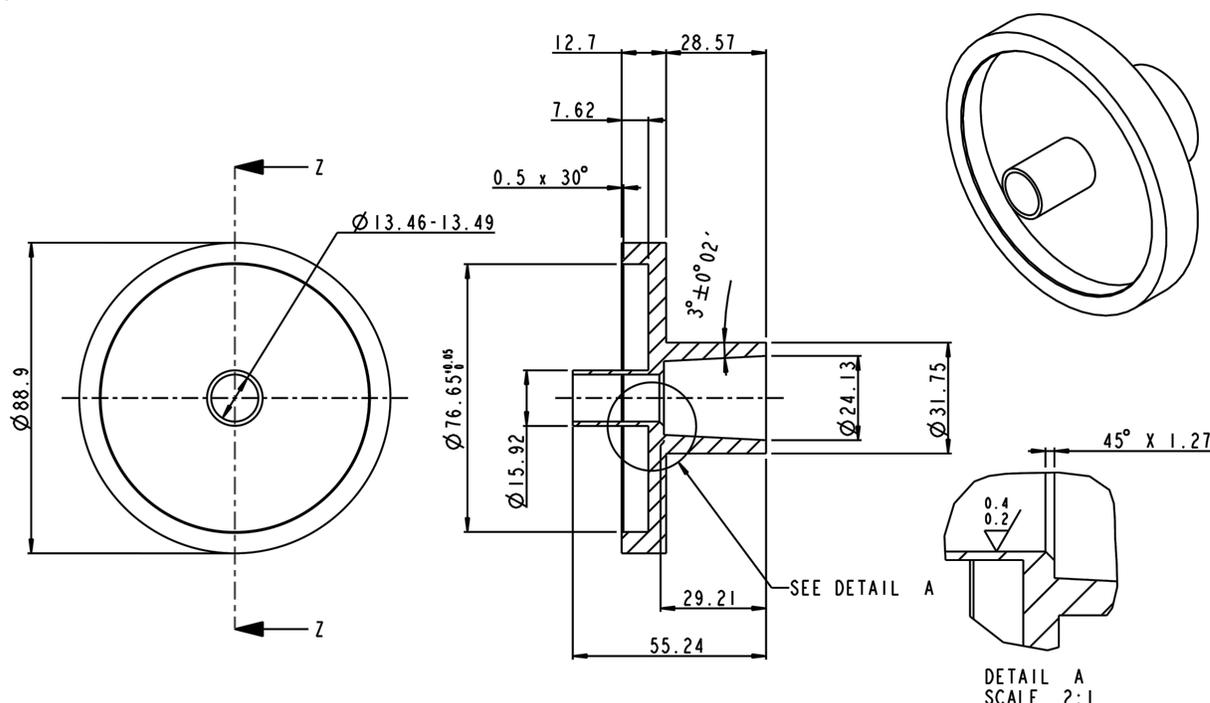


Figure 3. Expanded view of the preseparator lid.

Buffer, Solution A, and Mobile phase: Proceed as directed in the Assay.

Diluent: Methanol and water (70:30)

Standard solution: 2.5 µg/mL of USP Fluticasone Propionate RS and 0.75 µg/mL of USP Salmeterol Xinafoate RS in *Diluent*

Sample solutions: Discharge 10 unit doses given into the cascade impaction sampling apparatus described in *Figure 1*.

Operate the pump for 3 s at an airflow rate of 60 L/min for each dose discharged. Detach the inhaler, and rinse each piece of the apparatus with methanol into a separate suitable volumetric flask containing 30% of the flask volume of water. The final expected amount of fluticasone propionate should be in the concentration range of 0.1–5 µg/mL. Allow the solutions to equilibrate, and dilute with methanol to volume. Repeat these steps for three additional sample preparations, for a total of four *Sample solutions*.

Chromatographic system and System suitability: Proceed as directed in the Assay, except for *Injection volume*.

Injection volume: 50 µL

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the quantity, in µg/actuation, of fluticasone propionate (C₂₅H₃₁F₃O₅S) in the *Sample solutions*:

$$\text{Result} = [(r_U/r_S) \times C_S] \times V/N$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

V = total volume of the *Sample solution* (mL)

N = number of unit doses discharged into the apparatus

Calculate the quantity, in µg/actuation, of salmeterol (C₂₅H₃₇NO₄) in the *Sample solutions*:

$$\text{Result} = [(r_U/r_S) \times C_S] \times V/N \times (M_{r1}/M_{r2})$$

r_U = peak response of salmeterol from the *Sample solution*

r_S = peak response of salmeterol from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

V = total volume of the *Sample solution* (mL)

N = number of unit doses discharged into the apparatus

M_{r1} = molecular weight of salmeterol free base, 415.57

M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria

The mass of fluticasone propionate and salmeterol deposited in each grouping of the *Sampling apparatus* for each inhaler is given in *Table 1*.

Table 1

Parameter	Amount of Fluticasone Propionate Deposited (µg)			Amount of Salmeterol Deposited (µg)		
	100/50 µg/actuation	250/50 µg/actuation	500/50 µg/actuation	100/50 µg/actuation	250/50 µg/actuation	500/50 µg/actuation
Label Claim of fluticasone propionate/salmeterol (µg/actuation)	100/50 µg/actuation	250/50 µg/actuation	500/50 µg/actuation	100/50 µg/actuation	250/50 µg/actuation	500/50 µg/actuation
Mass of mouthpiece adapter, induction port, preseparator, and Stage 0	55-80	140-200	290-400	28-42	28-42	28-42
Sum of Stages 1-5	15-30	42-73	96-150	7-13	7-13	7-13
Sum of Stages 3 and 4	6-18	19-45	43-92	3-8	3-8	4-8
Sum of Stages 6, 7, and filter	NMT 1	NMT 2	NMT 2	NMT 0.5	NMT 0.5	NMT 0.5

All the groupings for each sample preparation must meet the criteria in *Table 1*.

If NMT one of the four sample preparations fails to meet the requirements in *Table 1*, but is within 25% of either the lower or upper specification limit being tested, analyze two additional samples. The batch meets the requirements if five of the six sample preparations meet the limits in *Table 1* for the individual sample preparations.

● **Delivered-Dose Uniformity**

(See *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* (601), *Delivered-Dose Uniformity, Inhalation Powders*.)

Sampling apparatus: Use the apparatus in *Figure 4A* with modified glass sampling device (*Figure 4B*).

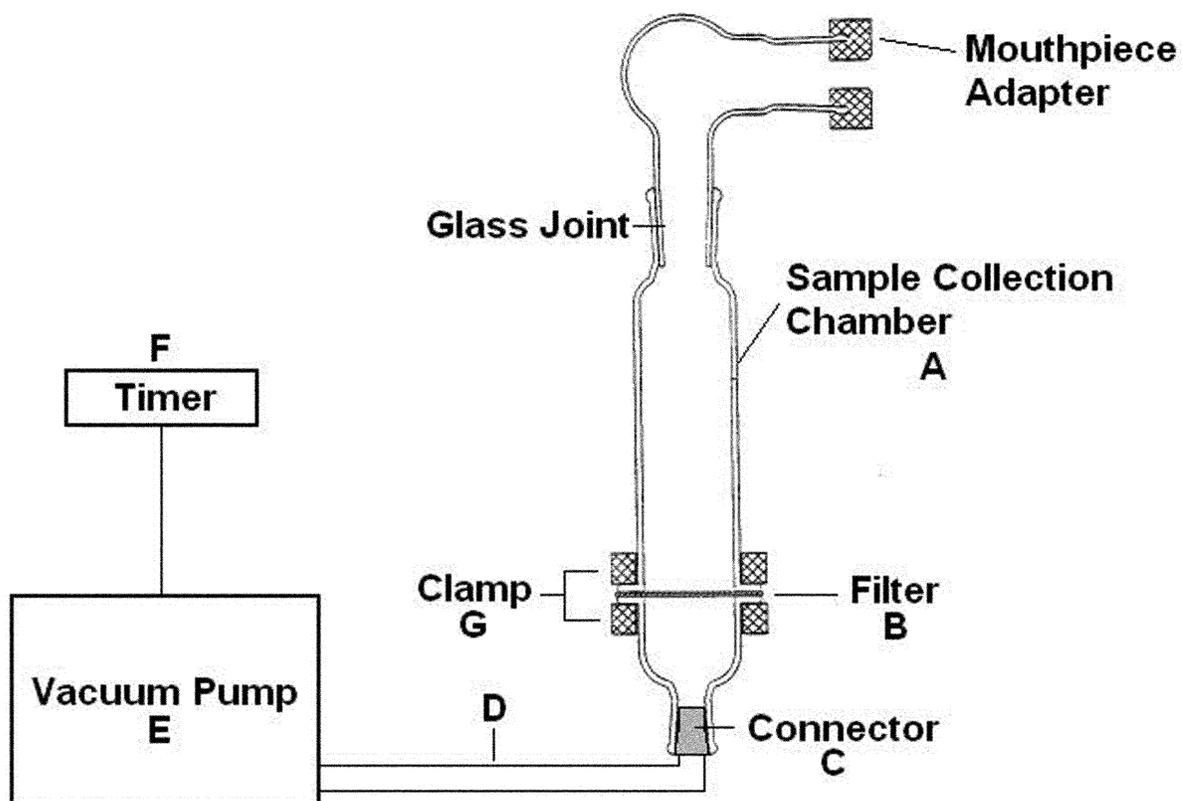


Figure 4A. Sampling apparatus.

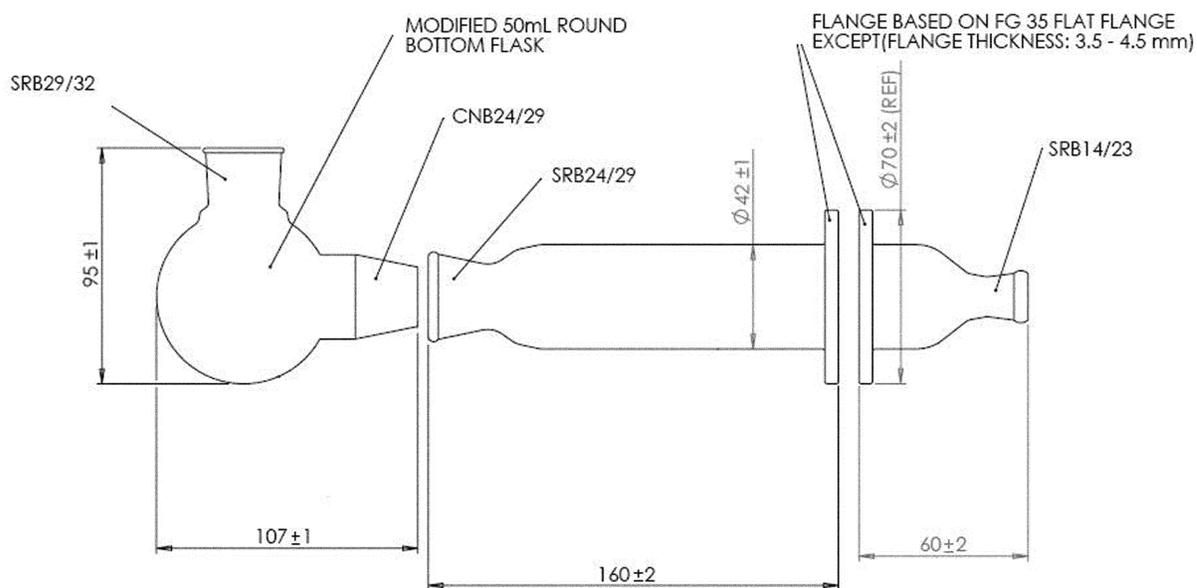


Figure 4B. Expanded view of the modified glass sample collection apparatus.

Buffer, Solution A, Mobile phase, and Diluent: Proceed as directed in the Assay.

Standard solution: 2.5 µg/mL of USP Fluticasone Propionate RS and 0.75 µg/mL of USP Salmeterol Xinafoate RS in *Diluent*

Sample solutions: Discharge a single unit dose into the apparatus shown in *Figure 4A*.

Operate the pump for 2 s at an airflow of 60 L/min to collect the dose. Detach the inhaler. Rinse the mouthpiece adapter and each piece of the sample collection chamber with methanol. Place the filter and washings into a container. Sonicate for 5 min. Quantitatively transfer the contents to a 200-mL volumetric flask containing 60 mL of

water. Allow the solution to equilibrate, and dilute with methanol to volume. Prepare nine additional *Sample solutions* from nine additional unit doses. For multi-dose inhalers, collect one dose from each of 10 inhalers with the 10 doses collected across the minimum number of recommended doses on the label of the inhaler.

Chromatographic system and System suitability: Proceed as directed in the *Assay*, except for the *Injection volume*.

Injection volume: 50 µL

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) delivered by the inhaler in each *Sample solution*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL), based on target emitted dose from *Table 2*

Calculate the percentage of the labeled amount of salmeterol ($C_{25}H_{37}NO_4$) delivered by the inhaler in each *Sample solution*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of salmeterol from the *Sample solution*

r_S = peak response of salmeterol from the *Standard solution*

C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of salmeterol free base in the *Sample solution* (µg/mL), based on target emitted dose from *Table 2*

M_{r1} = molecular weight of salmeterol free base, 415.57

M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Table 2. Target Emitted Dose

Label Claim of Fluticasone Propionate/Salmeterol (µg/unit dose)	Fluticasone Propionate Target Emitted Dose (µg/unit dose)	Salmeterol Target Emitted Dose (µg/unit dose)
100/50	93	45
250/50	233	45
500/50	465	45

Acceptance criteria

- 1.The mean content of fluticasone propionate and salmeterol from 10 doses is NLT 85% and NMT 115% of the target emitted dose.
- 2.NMT 1 emitted dose is outside 80%–120% of the target emitted dose.
- 3.No dose is outside 75%–125% of the target emitted dose.

If requirements 1 and 2 described above are not met, test an additional 20 unit doses.

The mean dose of fluticasone propionate from 30 doses is:

• NLT 85% and NMT 115% of the target emitted dose.

• NMT 3 doses are outside 80%–120% of the target emitted dose.

• No dose is outside 75%–125% of the target emitted dose.

IMPURITIES

• Organic Impurities

[Note—Protect all solutions containing fluticasone propionate or salmeterol from light.]

Solution A: 0.05 M monobasic ammonium phosphate adjusted with 10% (v/v) of phosphoric acid to a pH of 2.9

Solution B: Acetonitrile

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	70	30
60	22	78
61	70	30
70	70	30

Diluent: Methanol, water, and phosphoric acid (70: 30: 0.05)

Acidified methanol: 0.05% v/v of phosphoric acid in methanol

System suitability solution: 0.15 mg/mL of USP Salmeterol Xinafoate RS, 0.05 mg/mL of USP Fluticasone Propionate RS, and 0.4 µg/mL each of USP Fluticasone Propionate Related Compound D RS and USP Fluticasone Propionate Related Compound J RS in *Diluent*

Standard solution: 2 µg/mL of USP Salmeterol Related Compound H RS and 4 µg/mL of USP Fluticasone Propionate RS in *Diluent*

Sensitivity solution: 0.05 µg/mL of USP Salmeterol Related Compound H RS and 0.1 µg/mL of USP Fluticasone Propionate RS from *Standard solution* in *Diluent*

Sample solution: Nominally 200–500 µg/mL of fluticasone propionate prepared as follows.

Transfer the contents of NLT 10 unit doses to a 10-mL volumetric flask. Add 6 mL of acidified methanol and sonicate for 10 min. Add 3 mL of water, mix, and allow the solution to equilibrate. Dilute with acidified methanol to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 228 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Column temperature: 35°

Injection volume: 50 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[Note—See *Table 4* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between fluticasone propionate related compound J and

salmeterol; NLT 1.5 between fluticasone propionate related compound D and fluticasone propionate, *System suitability solution*

Tailing factor: NMT 2.0 for salmeterol related compound H and fluticasone propionate, *Standard solution*

Relative standard deviation: NMT 5.0% for salmeterol related compound H and fluticasone propionate, *Standard solution*

Signal-to-noise ratio: NLT 10 for both fluticasone propionate and salmeterol related compound H, *Sensitivity solution*

Analysis

Samples: *Standard solution, Sensitivity solution, and Sample solution*

Calculate the percentage of each fluticasone propionate related degradation product in the portion of Inhalation Powder taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (W_N/W_U) \times (1/L) \times 100$$

$r_{\bar{r}}$ peak response of each fluticasone propionate related degradation product from the *Sample solution*

$r_{\bar{s}}$ peak response of fluticasone propionate from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Fluticasone Propionate RS in the *Standard solution* ($\mu\text{g/mL}$)

V = volume of the *Sample solution* (mL)

$W_{\bar{N}}$ nominal weight of each unit dose (mg)

$W_{\bar{U}}$ weight of the unit doses in the *Sample solution* (mg)

L = label claim of fluticasone propionate ($\mu\text{g/unit dose}$)

Disregard any fluticasone propionate related degradation product peak less than the area of fluticasone propionate in the *Sensitivity solution*.

Calculate the percentage of each salmeterol related degradation product in the portion of Inhalation Powder taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (W_N/W_U) \times (1/L) \times 100$$

$r_{\bar{r}}$ response of each salmeterol related degradation product from the *Sample solution*

$r_{\bar{s}}$ response of salmeterol related compound H from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Salmeterol Related compound H RS in the *Standard solution* ($\mu\text{g/mL}$)

V = volume of the *Sample solution* (mL)

$W_{\bar{N}}$ nominal weight of each unit dose (mg)

$W_{\bar{U}}$ weight of the unit doses in in the *Sample solution* (mg)

L = label claim of salmeterol free base ($\mu\text{g/unit dose}$)

Acceptance criteria: See *Table 4*. Disregard any salmeterol related degradation product peak less than the area of salmeterol related compound H in the *Sensitivity solution*. [Note —Any unspecified degradation product eluting before salmeterol is related to salmeterol. Any unspecified degradation product eluting after salmeterol is related to fluticasone propionate.]

Table 4

Name	Relative Retention Time	Acceptance Criteria (NMT %)
Salmeterol- <i>N</i> -phenylbutyl aminoalcohol ^{a,b}	0.14	—
Salmeterol-phenylethoxy ^{a,c}	0.25	—
Salmeterol-phenylpropoxy ^{a,d}	0.32	—
Salmeterol-phenyl-2-butoxy ^{a,e}	0.37	—
Fluticasone propionate related compound J ^a	0.38	—
Salmeterol ^a	0.41	N/A
Hydroxynapthoic acid ^f	0.5	—
Salmeterol-deoxy ^{a,g}	0.55	—
Fluticasone propionate dithioacid ^{a,h}	0.67	—
Salmeterol- <i>N</i> -alkyl ⁱ	0.71	0.2
Salmeterol related compound H	0.74	0.9
Fluticasone propionate related compound D ^a	0.97	—
Fluticasone propionate	1.0	N/A
Fluticasone dimer ^{a,j}	1.09	—
Any fluticasone propionate related unspecified degradation product	—	0.1
Any salmeterol related unspecified degradation product	—	0.1
Total degradation products	—	1.3

^a This is a process impurity that is included in this table for identification only. This impurity is controlled in the drug substance. This impurity is not to be reported for the drug product or to be included in the total degradation products.

^b 4-[1-Hydroxy-2-(4-phenylbutylamino)ethyl]-2-(hydroxymethyl)phenol.

^c 4-[1-Hydroxy-2-(6-phenethoxyhexylamino)ethyl]-2-(hydroxymethyl)phenol.

^d 4-{1-Hydroxy-2-[6-(3-phenylpropoxy)hexylamino]ethyl}-2-(hydroxymethyl)phenol.

^e 4-{1-Hydroxy-2-[6-(4-phenylbutan-2-yloxy)hexylamino]ethyl}-2-(hydroxymethyl)phenol.

^f This is a counter ion of salmeterol that is included in this table for identification only. It is not to be reported for the drug product or to be included in the total degradation products.

^g 4-{1-Hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}-2-methylphenol.

^h 6 α ,9 α -Difluoro-11 β -hydroxy-16 α -methyl-3-oxo-17 α -propionyloxyandrosta-1,4-diene-17 β -carbodithioic acid.

ⁱ 4-{1-Hydroxy-2-[(2-hydroxy-5-{1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}benzyl)[6-(4-phenylbutoxy)hexyl]amino]ethyl}-2-(hydroxymethyl)phenol.

6 α , 9 α -Difluoro-11 β ,17 α -dihydroxy-16 α -methyl-3-oxoandrosta-1,4-diene-17 β -carboxylic acid 6 α , 9 α -difluoro-17 β -(fluoromethylthio)carbonyl-11 β -hydroxy-16 α -methyl-3-oxoandrosta-1,4-diene-17 β -yl ester.

SPECIFIC TESTS

- **Microbial Enumeration Tests** $\langle 61 \rangle$ and **Tests For Specified Microorganisms** $\langle 62 \rangle$: The total aerobic microbial count does not exceed 10^1 cfu/g of powder. The total aerobic yeasts and molds count does not exceed 10^1 cfu/g of formulation. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.
- **Foreign Particulate Matter**

General chapter *Particulate Matter in Injections* $\langle 788 \rangle$ describes details of the test apparatus to be used for the determination of particulate matter using a microscopic particle count test methodology. Samples should be carefully prepared to avoid environmental contamination, and testing should be performed with suitable controls, including the appropriate use of blank determinations.

Diluent: Methanol and water (65:35) passed through a filter of 0.45- μ m pore size

Filter: Mixed cellulose and ester filter; 25-mm diameter and 0.45- μ m pore size

Sample solution: Transfer contents of NLT 8 unit doses to a suitable container. Dissolve in 75 mL of *Diluent*.

Analysis

Sample: *Sample solution*

Pass the *Sample solution* through the filter and allow the filter to dry under conditions that will limit particulate contamination. Using a microscopic particle count test method, enumerate the number of particles present in the *Sample solution*. Calculate the total number of particles per actuation by the formula:

$$\text{Result} = (N_{<10} + N_{10-100} + N_{>100})/8$$

$N_{<10}$ = total number of particles <10 μ m present in the *Sample solution*

N_{10-100} = total number of particles between 10 and 100 μ m present in the *Sample solution*

$N_{>100}$ = total number of particles >100 μ m present in the *Sample solution*

Acceptance criteria: See *Table 5*.

Table 5

Particle Size Range (μ m)	Number of Particles/Dose (NMT)
<10	200
10–100	100
>100	10
Total	300

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in non-reactive, light-resistant Inhalation Powder containers with metered valves fitted with a dose counter and provided with oral inhalation actuators. Avoid exposure to heat. Store at controlled room temperature.
- **USP Reference Standards** $\langle 11 \rangle$
 - USP Fluticasone Propionate RS
 - USP Fluticasone Propionate Related Compound D RS
 - S-Methyl 6α , 9α -difluoro- 11β -hydroxy- 16α -methyl-3-oxo-17-propionyloxyandrost-1,4-diene- 17α -carbothioate.
 - $C_{25}H_{32}F_2O_5S$ 482.58
 - USP Fluticasone Propionate Related Compound J RS
 - 6α , 9α -Difluoro- 11β , 17α -dihydroxy- 16α -methyl-3-oxoandrost-1,4-diene- 17β -carboxylic acid.
 - $C_{21}H_{26}F_2O_5$ 396.42
 - USP Salmeterol Related Compound H RS
 - 1-Hydroxy-4-[2-hydroxy-5-(1-hydroxy-2-[[6-(4-phenylbutoxy)hexyl]amino}ethyl)benzyl]-2-naphthoic acid.
 - $C_{36}H_{43}NO_6$ 585.73
 - USP Salmeterol Xinafoate RS

▲USP39

BRIEFING

Hard Gelatin Capsule Shell. It is proposed to add this new monograph for empty Hard Gelatin Capsule Shell. Comments and suggestions should be sent to Margareth Marques, Ph.D., at mrm@usp.org not later than March 31, 2015.

(GCDF: M. Marques.)

Correspondence Number—C153576

Comment deadline: March 31, 2015

Add the following:

▲Hard Gelatin Capsule Shell

DEFINITION

Hard Gelatin Capsule Shell consists of two overlapping pieces (cap and body). One end of each piece is rounded and closed while the other is open. The cap overlaps the body and maintains a tight closure. It is composed of gelatin, water, and additives such as plasticizers, surfactants, dispersing agents, flavoring agents, antimicrobial agents, and sweeteners. It may contain opacifiers, colorants, and/or processing aids. It may be externally coated.

IDENTIFICATION

- **A.**

Sample solution: Dissolve an amount of Hard Gelatin Capsule Shell equivalent to 1 g in 100 mL of carbon dioxide free water at about 55° . Let the solution stand for a period of time to allow any colorant present to settle to the bottom of the vessel.

Analysis: To 2 mL of the supernatant of the *Sample solution* add 0.05 mL of a 125-g/L solution of copper sulfate pentahydrate. Mix, and add 0.5 mL of an 85-g/L solution of

sodium hydroxide.

Acceptance criteria: A violet color is produced.

- **B.**

Picric acid solution: 1% (w/v) solution of picric acid in hot water

Tannic acid solution: 10% (w/v) solution of tannic acid in water. Prepare immediately before use.

Analysis: Boil 1 Hard Gelatin Capsule Shell with 20 mL of water. Allow to cool and centrifuge. To 5 mL of the lukewarm supernatant, add 1 mL of *Picric acid solution*. To another 5 mL of the lukewarm supernatant, add 1 mL of *Tannic acid solution*.

Acceptance criteria: A precipitate is produced in each case.

SPECIFIC TESTS

- **Loss on Drying** 〈 731 〉

Sample: 1–2 g

Analysis: Dry the *Sample* in an oven at $105 \pm 1^\circ$ for 4 h.

Acceptance criteria: 13.0%–16.0%

- **Limit of Chromium**

Analysis: Atomic absorption spectrometry, standard addition method, or any other validated procedure

Acceptance criteria: NMT 10 ppm

- **Disintegration** 〈 701 〉

Analysis: Fill each Hard Gelatin Capsule Shell body to capacity with lactose or other suitable material. Place the cap onto the body and press the cap and body together to lock. Place the Hard Gelatin Capsule Shell in the basket, and add disks.

Medium: Water

Acceptance criteria: NMT 15 min

- **Residual Solvents** 〈 467 〉: Meets the requirements

- **Microbial Enumeration Tests** 〈 61 〉 and **Tests for Specified Microorganisms** 〈 62 〉: The total bacteria count does not exceed 10^3 cfu/g, and the total yeast and molds count does not exceed 10^2 cfu/g. The test for *Escherichia coli* is negative in 1 g.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store in tightly closed containers at 15° – 30° and a relative humidity of 35%–65%. Avoid light exposure. ▲USP39

BRIEFING

Hydromorphone Hydrochloride, USP 38 page 3799. On the basis of comments received, it is proposed to make the following changes:

1. Revise the *Definition* and *Acceptance criteria* in the *Assay* from 98.0%–101.0% to 98.0%–102.0%, which is typical for a chromatographic assay.
2. Replace *Identification* test B with an HPLC retention time agreement based on the proposed chromatographic procedure in the *Assay*.
3. Replace the titration procedure in the *Assay* with an HPLC procedure validated with the method in *Organic Impurities, Procedure 2*. The proposed liquid chromatography

procedure is based on analysis performed with the Symmetry C8 brand of L7 column. The typical retention time for hydromorphone is about 8.3 min.

4. Delete the test for *Sulfate*; the remaining tests in the revised monograph are sufficient to ensure quality and purity.
5. Revise USP Morphine RS to USP Morphine Sulfate RS in *Organic Impurities, Procedure 2*, and add USP Morphine Sulfate RS to the *USP Reference Standards* section.
6. Add the *Labeling* statement.
7. Add chemical information for USP Hydromorphone Related Compound A RS in the *USP Reference Standards* section.

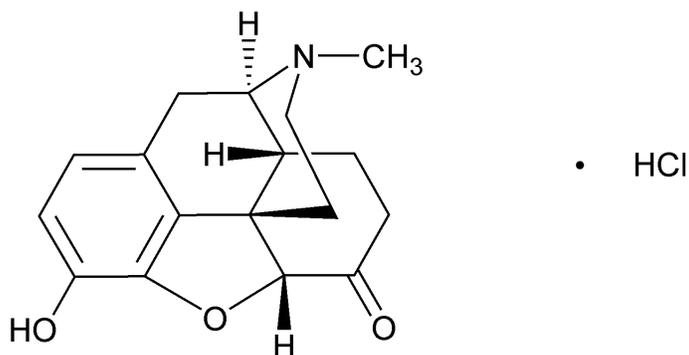
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: H. Cai.)

Correspondence Number—C135853

Comment deadline: March 31, 2015

Hydromorphone Hydrochloride



$C_{17}H_{19}NO_3 \cdot HCl$ 321.80

Morphinan-6-one, 4,5-epoxy-3-hydroxy-17-methyl-, hydrochloride, (5 α)-;
4,5 α -Epoxy-3-hydroxy-17-methylmorphinan-6-one hydrochloride [71-68-1].

DEFINITION

Change to read:

~~Hydromorphone Hydrochloride, dried at 105° for 2 h, contains NLT 98.0% and NMT 101.0% of hydromorphone hydrochloride ($C_{17}H_{19}NO_3 \cdot HCl$)~~

▲Hydromorphone Hydrochloride contains NLT 98.0% and NMT 102.0% of hydromorphone hydrochloride ($C_{17}H_{19}NO_3 \cdot HCl$), on the dried basis. ▲*USP39*

IDENTIFICATION

- **A. Infrared Absorption** <197K>

Delete the following:

- ▲• **B. Ultraviolet Absorption** <197U>

Sample solution: ~~100 µg/mL~~

Analytical wavelength: 280 nm

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%. ▲*USP39*

Add the following:

▲● **B.** The retention time of the hydromorphone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP39*

● **C. Identification Tests—General, Chloride** 〈 191 〉

Sample solution: 1 in 20

Acceptance criteria: Meets the requirements

ASSAY

Change to read:

● **Procedure**

Sample: 225 mg, Previously dried

Analysis: Transfer the *Sample* to a 250 mL conical flask. Dissolve in 80 mL of glacial acetic acid, warming, if necessary. Cool, and add 5 mL of acetic anhydride and 10 mL of mercuric acetate TS. Add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction (see *Titrimetry* 〈 541 〉). Each mL of 0.1 N perchloric acid is equivalent to 32.18 mg of $C_{17}H_{19}NO_3 \cdot HCl$.

Acceptance criteria: 98.0%–101.0%, dried at 105° for 2 h

▲**Diluent:** 1% (v/v) Phosphoric acid in water

Solution A: Mix 3520 mL of water, 18.40 g of monobasic ammonium phosphate, and 4.32 g (on the anhydrous basis) of sodium 1-octanesulfonate. Add 4.0 mL of triethylamine, adjust with phosphoric acid to a pH of 2.90, and add 480 mL of acetonitrile.

Solution B: Acetonitrile and water (160:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	80	20
21	100	0
30	100	0

Standard solution: 3 mg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*

Sample solution: 3 mg/mL of Hydromorphone Hydrochloride, previously dried, in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 15-cm; 5-μm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NLT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of hydromorphone hydrochloride (C₁₇H₁₉NO₃·HCl) in the portion of Hydromorphone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of hydromorphone from the *Sample solution*

r_S

= peak response of hydromorphone from the *Standard solution*

C_S

= concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= concentration of Hydromorphone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ▲*USP39*

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.3%

Delete the following:

▲• **Sulfate**

Sample solution: 100 mg in 5 mL of water

Analysis: To the *Sample solution* add 0.5 mL of 3 N hydrochloric acid and 1 mL of barium chloride TS.

Acceptance criteria: No turbidity is produced. ▲*USP39*

Change to read:

- **Organic Impurities**

If (5α)-7-[(5α)-3,6-dihydroxy-17-methyl-4,5-epoxymorphinan-6-yl]-3-hydroxy-17-methyl-4,5-epoxymorphinan-6-one (hydromorphone aldol dimer) or (5α)-3-hydroxy-8-[(5α)

)-3-hydroxy-17-methyl-6-oxo-4,5-epoxymorphinan-7-yl]-17-methyl-4,5-epoxymorphinan-6-one (7,8'-bishydromorphone) are potential impurities, *Procedure 2* is recommended.

Procedure 1

Solution A: 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in 1000 mL of methanol and water (1:9). Add 1.0 mL of triethylamine and adjust with phosphoric acid to a pH of 2.5 ± 0.1 .

Solution B: 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in 1000 mL of methanol and water (1:1). Add 1.0 mL of triethylamine and adjust with phosphoric acid to a pH of 2.5 ± 0.1 .

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	94	6
25	94	6
40	20	80
70	20	80
75	94	6
90	94	6

Diluent: Phosphoric acid and water (1:1000)

System suitability solution: 0.8 mg/mL each of USP Hydromorphone Hydrochloride RS and USP Hydromorphone Related Compound A RS in *Diluent*. The solution should be kept in a cool place protected from light.

Standard solution: 4 µg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*

Sample solution: 0.8 mg/mL of Hydromorphone Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* 〈621〉, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 45°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between hydromorphone related compound A and hydromorphone peaks, *System suitability solution*

Tailing factor: NMT 1.5 for the hydromorphone peak, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Diluent*, *Standard solution*, and *Sample solution*

Calculate the percentage of any specified or unspecified impurity in the portion of Hydromorphone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of individual specified or unspecified impurity from the *Sample solution*

r_S = peak response of hydromorphone from the *Standard solution*

C_S = concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Hydromorphone Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard peaks corresponding to those obtained from the *Diluent*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
8-Hydroxyhydromorphone ^a	0.50	1.0	0.15
Dihydromorphone (DHM) ^b	0.61	1.0	0.5
Morphine ^c	0.65	1.8	0.15
Hydromorphone <i>N</i> -oxide ^d	0.79	1.0	0.15
Hydromorphone related compound A ^e	0.93	1.4	0.1
Hydromorphone	1.0	—	—
8,14-Dihydrooripavine ^f	1.66	1.0	0.15
6 β -Tetrahydrooripavine ^f	1.71	1.0	0.15
2,2'-Bis hydromorphone ^g	2.02	1.7	0.15
Individual unspecified impurities	—	1.0	0.1
Total impurities	—	—	1.0

a 4,5 α -Epoxy-17-methylmorphinan-3,8-diol-6-one.
b 4,5 α -Epoxy-17-methylmorphinan-3,6 α -diol.
c 7,8-Didehydro-4,5 α -epoxy-17-methylmorphinan-3,6 α -diol.
d 4,5 α -Epoxy-3-hydroxy-17-methylmorphinan-6-one *N*-oxide.
e 7,8-Didehydro-4,5 α -epoxy-3-hydroxy-17-methylmorphinan-6-one.
f 8,14-Dihydrooripavine and 6 β -tetrahydrooripavine are process impurities from another process and are controlled only if present.
g (5 β)-3-Hydroxy-2-[(5 α)-3-hydroxy-17-methyl-6-oxo-4,5-epoxymorphinan-2-yl]-17-methyl-4,5-epoxymorphinan-6-one.

Procedure 2

Diluent: Phosphoric acid and water (1:100)

Solution A: Mix 3520 mL of water, 18.40 g of monobasic ammonium phosphate, and 4.32 g of sodium 1-octanesulfonate. Add 4.0 mL of triethylamine, adjust with phosphoric acid to a pH of 2.90, and add 480 mL of acetonitrile.

Solution B: Acetonitrile and water (160:40)

Mobile phase: See *Table 1*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	80	20
21	100	0
30	100	0

▲Solution A, Solution B, Mobile phase, Diluent, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*. ▲USP39

System suitability solution: 0.15 mg/mL of USP Hydromorphone Hydrochloride RS and 0.1 mg/mL of USP Morphine RS

▲USP Morphine Sulfate RS ▲USP39

in *Diluent*. The solution should be kept in a cool place protected from light.

Standard solution: 15 µg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*

Sample solution: 3 mg/mL of Hydromorphone Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9 mm × 15 cm; 5 µm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: 30 min

▲ ▲USP39

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between morphine and hydromorphone peaks, *System suitability solution*

Tailing factor: NMT 1.5 for the hydromorphone peak, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Diluent*, *Standard solution*, and *Sample solution*

Calculate the percentage of any specified or unspecified impurity in the portion of Hydromorphone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of individual specified or unspecified impurity from the *Sample solution*

r_S peak response of hydromorphone from the *Standard solution*

C_S concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U concentration of Hydromorphone Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see Table 4)

Acceptance criteria: See Table 4. Disregard peaks corresponding to those obtained from the Diluent.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dihydromorphine (DHM) ^a	0.63	1.0	0.5
Morphine ^b	0.70	1.0	0.5
Hydromorphone	1.0	—	—
2,2'-Bis hydro morphone ^c	1.82	2.0	0.5
Hydromorphone hydrochloride aldol dimer ^d	2.12	1.0	0.5
7,8-Bis hydro morphone ^e	2.32	1.0	0.5
Individual unspecified impurities	—	1.0	0.10
Total impurities	—	—	2.0

a 4,5 α -Epoxy-17-methylmorphinan-3,6 α -diol.
b 7,8-Didehydro-4,5 α -epoxy-17-methylmorphinan-3,6 α -diol.
c (5 β)-3-Hydroxy-2-[(5 α)-3-hydroxy-17-methyl-6-oxo-4,5-epoxymorphinan-2-yl]-17-methyl-4,5-epoxymorphinan-6-one.
d (5 α)-7-[(5 α)-3,6-Dihydroxy-17-methyl-4,5-epoxymorphinan-6-yl]-3-hydroxy-17-methyl-4,5-epoxymorphinan-6-one.
e (5 α)-3-Hydroxy-8-[(5 α)-3-hydroxy-17-methyl-6-oxo-4,5-epoxymorphinan-7-yl]-17-methyl-4,5-epoxymorphinan-6-one.

SPECIFIC TESTS

- **Optical Rotation**, *Specific Rotation* $\langle 781S \rangle$
Sample solution: 50 mg/mL
Acceptance criteria: Between -136° and -139°
- **Acidity**
Sample: 300 mg
Analysis: Dissolve the *Sample* in 10 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide VS.
Acceptance criteria: NMT 0.30 mL is required to produce a yellow color.
- **Loss on Drying** $\langle 731 \rangle$
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at 25° , excursions permitted between 15° and 30° .

Add the following:

- ▲● **Labeling:** The labeling states with which *Organic Impurities* procedure the article complies, if other than *Procedure 1*. ▲*USP39*

Change to read:

- **USP Reference Standards** (11)

USP Hydromorphone Hydrochloride RS

USP Hydromorphone Related Compound A RS

▲7,8-Didehydro-4,5 α -epoxy-3-hydroxy-17-methylmorphinan-6-one hydrochloride.

C₁₇H₁₇NO₃·HCl 319.78

USP Morphine Sulfate RS

▲*USP39*

BRIEFING

Hydroxyzine Hydrochloride, *USP 38* page 3812. On the basis of comments received in *PF 39(5)* [Sep.–Oct. 2013] from the FDA and USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Replace the liquid chromatographic procedure in the test for *Organic Impurities* with a more specific chromatographic procedure that is capable of identifying 4-chlorobenzophenone and add a corresponding limit for 4-chlorobenzophenone based on ICH guidelines. The liquid chromatographic procedure was validated using the Acquity UPLC HSS C18 SB brand of L1 column. The typical retention time of hydroxyzine is about 14.5 min.
2. Replace the current HPLC procedure in the *Assay* with a chromatographic procedure using the same parameters in the proposed test for *Organic Impurities*.
3. Add USP 4-Chlorobenzophenone RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

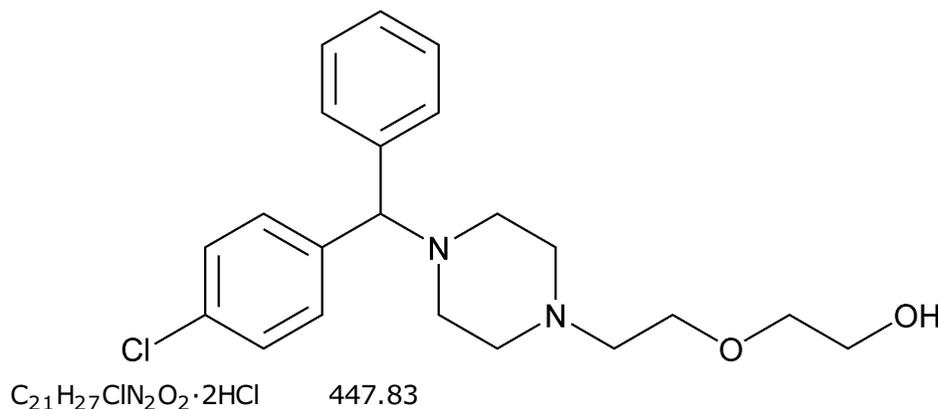
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R.-H. Yeh, D. Min.)

Correspondence Number—C143445

Comment deadline: March 31, 2015

Hydroxyzine Hydrochloride



Ethanol, 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]-, dihydrochloride, (±)-
;
(±)-2-[2-[4-(*p*-Chloro- α -phenylbenzyl)-1-piperazinyl]ethoxy]ethanol dihydrochloride [2192-20-3].

DEFINITION

Hydroxyzine Hydrochloride contains NLT 98.0% and NMT 102.0% of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.**
Sample solution: 2.5 mg/mL of Hydroxyzine Hydrochloride in water
Analysis: To 10 mL of *Sample solution* add 2 drops of nitric acid and 1 mL of silver nitrate TS.
Acceptance criteria: A curdy, white precipitate, insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, separates (presence of chloride).

ASSAY

Change to read:

- **Procedure**

~~**Mobile phase:** Acetonitrile and 0.12 N sulfuric acid (90:10)~~

~~**System suitability solution:** 3.6 μ g/mL each of USP Hydroxyzine Hydrochloride RS and USP Hydroxyzine Related Compound A RS in *Mobile phase*~~

~~**Standard solution:** 0.3 mg/mL of USP Hydroxyzine Hydrochloride RS in *Mobile phase*~~

~~**Sample solution:** 0.3 mg/mL of Hydroxyzine Hydrochloride in *Mobile phase*~~

~~**Chromatographic system**~~

~~(See *Chromatography* (621), *System Suitability*.)~~

~~**Mode:** LC~~

~~**Detector:** UV 230 nm~~

~~**Column:** 4.6 mm \times 25 cm, packing L3~~

~~**Flow rate:** 1 mL/min~~

~~**Injection volume:** 20 μ L~~

~~**Run time:** NLT 1.8 times the retention time of the hydroxyzine peak~~

~~**System suitability**~~

~~**Samples:** *System suitability solution* and *Standard solution*~~

~~[Note—The relative retention times for hydroxyzine related compound A and hydroxyzine are about 0.9 and 1.0, respectively.]~~

~~**Suitability requirements**~~

~~**Resolution:** NLT 1.5 between hydroxyzine related compound A and hydroxyzine, *System suitability solution*~~

~~**Relative standard deviation:** NMT 2.0%, *Standard solution*~~

- ▲**Solution A:** Trifluoroacetic acid and water (0.1: 99.9)
Solution B: Trifluoroacetic acid and acetonitrile (0.5: 99.5)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
12	55	45
16	55	45
21	20	80
25	20	80
26	90	10
30	90	10

Diluent: Acetonitrile and water (30:70)

Standard solution: 0.05 mg/mL of USP Hydroxyzine Hydrochloride RS in *Diluent*

Sample solution: 0.05 mg/mL of Hydroxyzine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 2.1-mm × 15-cm; 1.8-μm packing L1

Flow rate: 0.3 mL/min

Injection volume: 2 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

▲USP39

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) in the portion of Hydroxyzine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydroxyzine from the *Sample solution*

r_S = peak response of hydroxyzine from the *Standard solution*

C_S = concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Hydroxyzine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.5%

Delete the following:

- **Heavy Metals, Method II** 〈 231 〉: NMT 20 ppm (Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

~~Mobile phase, System suitability solution, Chromatographic system, and System suitability:~~ Proceed as directed in the *Assay*.

~~Standard solution:~~ 1.8 µg/mL of USP Hydroxyzine Hydrochloride RS in *Mobile phase*

~~Sample solution:~~ 600 µg/mL of Hydroxyzine Hydrochloride in *Mobile phase*

~~**Analysis**~~

~~Samples:~~ *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Hydroxyzine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

~~r_U~~ peak response of each impurity from the *Sample solution*

~~r_S~~ peak response of hydroxyzine from the *Standard solution*

~~C_S~~ concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* (µg/mL)

~~C_U~~ concentration of Hydroxyzine Hydrochloride in the *Sample solution* (µg/mL)

~~**Acceptance criteria**~~

~~**Any individual impurity:**~~ NMT 0.3%

~~**Total impurities:**~~ NMT 1.5%

▲Solution A, Solution B, Mobile phase, and Diluent: Proceed as directed in the *Assay*.

Standard solution: 1.5 µg/mL each of USP Hydroxyzine Hydrochloride RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS in *Diluent*

Sample solution: 500 µg/mL of Hydroxyzine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detectors

Declozixine, hydroxyzine related compound A, and hydroxyzine: UV 230 nm

4-Chlorobenzophenone: UV 254 nm

Column: 2.1-mm × 15-cm; 1.8-µm packing L1

Flow rate: 0.3 mL/min

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between peaks due to hydroxyzine related compound A and hydroxyzine

Relative standard deviation: NMT 3.0% for hydroxyzine related compound A, hydroxyzine, and 4-chlorobenzophenone

Analysis

Samples: *Standard solution* and *Sample solution*

For impurities detected at UV 230 nm

Calculate the percentage of hydroxyzine related compound A in the portion of Hydroxyzine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of hydroxyzine related compound A from the *Sample solution*

r_S

= peak response of hydroxyzine related compound A from the *Standard solution*

C_S

= concentration of USP Hydroxyzine Related Compound A RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= concentration of Hydroxyzine Hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

Calculate the percentage of declozine or any individual unspecified impurity in the portion of Hydroxyzine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U

= peak response of declozine or any other individual impurity from the *Sample solution*

r_S

= peak response of hydroxyzine from the *Standard solution*

C_S

= concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= concentration of Hydroxyzine Hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

F

= relative response factor (see *Table 2*)

For 4-chlorobenzophenone detected at UV 254 nm

Calculate the percentage of 4-chlorobenzophenone in the portion of Hydroxyzine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of 4-chlorobenzophenone from the *Sample solution*

r_S

= peak response of 4-chlorobenzophenone from the *Standard solution*

C_S

= concentration of USP 4-Chlorobenzophenone RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= concentration of Hydroxyzine Hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.03%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Decloxizine ^a	0.87	0.68	0.3
Hydroxyzine related compound A	0.96	—	0.3
Hydroxyzine	1.0	—	—
4-Chlorobenzophenone	1.4	—	0.10
Any individual unspecified impurity	—	1.0	0.3
Total impurities	—	—	1.5

^a 2-[2-(4-Benzhydrylpiperazin-1-yl)ethoxy]ethanol, also known as 2-{2-[4-(diphenylmethyl)piperazin-1-yl]ethoxy}ethano'.

▲USP39

SPECIFIC TESTS

- **Water Determination** (921): NMT 5.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature. Protect from light.

Change to read:

- **USP Reference Standards** (11)

▲USP 4-Chlorobenzophenone RS

4-Chlorobenzophenone.

C₁₃H₉ClO 216.66▲*USP39*

USP Hydroxyzine Hydrochloride RS

USP Hydroxyzine Related Compound A RS

1-[(4-Chlorophenyl)phenylmethyl]piperazine.

C₁₇H₁₉ClN₂ 286.80

BRIEFING

Hydroxyzine Hydrochloride Injection, *USP 38* page 3813 and *PF 39(5)* [Sept.–Oct. 2013]. Based on USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Replace the liquid chromatographic procedures in the test for *Limit of 4-Chlorobenzophenone* with a more specific chromatographic procedure that is capable of analyzing 4-chlorobenzophenone and other organic impurities. The proposed procedure in the test for *Organic Impurities* was validated using the Acquity UPLC HSS C18 SB brand of L1 column. The typical retention time of hydroxyzine is about 14.5 min.
2. Replace the current *Assay* with a chromatographic procedure using the same parameters in the proposed test for *Organic Impurities*.
3. Add USP Hydroxyzine Related Compound A RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R.-H. Yeh, D. Min.)

Correspondence Number—C145626

Comment deadline: March 31, 2015

Hydroxyzine Hydrochloride Injection

DEFINITION

Hydroxyzine Hydrochloride Injection is a sterile solution of Hydroxyzine Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydroxyzine hydrochloride (C₂₁H₂₇ClN₂O₂·2HCl).

IDENTIFICATION

• **A.**

Standard solution: 20 µg/mL of USP Hydroxyzine Hydrochloride RS in 0.1 N hydrochloric acid

Sample solution: Nominally 20 µg/mL of hydroxyzine hydrochloride from Injection in 0.1 N hydrochloric acid

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, concomitantly measured.

Add the following:

- ▲● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the hydroxyzine peak of the *Standard solution*, as obtained in the *Assay*. ▲*USP38*

ASSAY

Change to read:

- **Procedure**

Protect the *Standard solution* and the *Sample solution* from light.

Buffer: ~~2 g/L of dibasic potassium phosphate and 8 g/L of monobasic potassium phosphate adjusted with 10 N potassium hydroxide to a pH of 6.6~~

Mobile phase: ~~Methanol and Buffer (65:35)~~

Standard solution: 0.25 mg/mL of USP Hydroxyzine Hydrochloride RS and 0.5 µg/mL of

▲USP 4-Chlorobenzophenone RS ▲*USP38*

in *Mobile phase*

Sample solution: Nominally 0.25 mg/mL of hydroxyzine hydrochloride from Injection in *Mobile phase*

Chromatographic system

(See *Chromatography* ~~621~~, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for 4-chlorobenzophenone and hydroxyzine are 0.75 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the 4-chlorobenzophenone and hydroxyzine peaks

Tailing factor: NMT 2.5 for the ~~4-chlorobenzophenone and hydroxyzine peaks~~

Relative standard deviation: NMT 2.0%

▲for the hydroxyzine peak ▲*USP38*

▲**Solution A:** Trifluoroacetic acid and water (0.1: 99.9)

Solution B: Trifluoroacetic acid and acetonitrile (0.5: 99.5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
12	55	45

16	55	45
21	20	80
25	20	80
26	90	10
30	90	10

Diluent: Acetonitrile and water (30:70)

Standard solution: 0.05 mg/mL of USP Hydroxyzine Hydrochloride RS in *Diluent*

Sample stock solution: Nominally 0.5 mg/mL of hydroxyzine hydrochloride from Injection in *Diluent* prepared as follows. Transfer a portion of the Injection equivalent to 50 mg of hydroxyzine hydrochloride to a 100-mL volumetric flask, dissolve, and dilute with *Diluent* to volume.

Sample solution: Nominally 0.05 mg/mL of hydroxyzine hydrochloride in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 2.1-mm × 15-cm; 1.8-μm packing L1

Flow rate: 0.3 mL/min

Injection volume: 2 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

▲USP39

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of hydroxyzine from the *Sample solution*

r_S peak response of hydroxyzine from the *Standard solution*

C_S concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of hydroxyzine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Delete the following:

▲● Limit of 4-Chlorobenzophenone

Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis**Samples:** *Standard solution* and *Sample solution*

▲
 Calculate the percentage of 4-chlorobenzophenone in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of 4-chlorobenzophenone in the *Sample solution* r_S = peak response of 4-chlorobenzophenone in the *Standard solution* C_S = concentration of USP 4-Chlorobenzophenone RS in the *Standard solution* (mg/mL) C_U = nominal concentration of hydroxyzine hydrochloride in the *Sample solution* (mg/mL)

▲USP38

Acceptance criteria: NMT 0.2% ▲USP39**Add the following:****▲● Organic Impurities****Solution A, Solution B, Mobile phase, and Diluent:** Proceed as directed in the *Assay*.**Standard solution:** 1.0 µg/mL each of USP Hydroxyzine Hydrochloride RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS in *Diluent***Sample solution:** Nominally 500 µg/mL of hydroxyzine hydrochloride from Injection in *Diluent* prepared as follows. Transfer a suitable portion of the Injection to a suitable volumetric flask and add *Diluent* to 80% of the final volumetric flask volume. Mix well and dilute with *Diluent* to volume.**Chromatographic system**(See *Chromatography* { 621 }, *System Suitability*.)**Mode:** LC**Detectors****Hydroxyzine related compound A and hydroxyzine:** UV 230 nm**4-Chlorobenzophenone:** UV 254 nm**Column:** 2.1-mm × 15-cm; 1.8-µm packing L1**Flow rate:** 0.3 mL/min**Injection volume:** 2 µL**System suitability****Sample:** *Standard solution*[Note—See *Table 2* for the relative retention times.]**Suitability requirements****Resolution:** NLT 5.0 between hydroxyzine related compound A and hydroxyzine**Relative standard deviation:** NMT 3.0% for hydroxyzine related compound A, hydroxyzine, and 4-chlorobenzophenone

Analysis**Samples:** *Standard solution and Sample solution***For impurities detected at UV 230 nm**

Calculate the percentage of any individual unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak response of each degradation product from the *Sample solution* r_S peak response of hydroxyzine from the *Standard solution* C_S concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* ($\mu\text{g/mL}$) C_U nominal concentration of hydroxyzine hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)**For 4-chlorobenzophenone detected at UV 254 nm**

Calculate the percentage of 4-chlorobenzophenone in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak response of 4-chlorobenzophenone from the *Sample solution* r_S peak response of 4-chlorobenzophenone from the *Standard solution* C_S concentration of USP 4-Chlorobenzophenone RS in the *Standard solution* ($\mu\text{g/mL}$) C_U nominal concentration of hydroxyzine hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)**Acceptance criteria:** See *Table 2*. Disregard any peak below 0.03%.**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Decloxizine ^a	0.87	—
Hydroxyzine related compound A ^a	0.95	—
Hydroxyzine	1.0	—
4-Chlorobenzophenone	1.4	0.2
Any individual unspecified degradation product	—	0.3
Total degradation products	—	0.5

^a These are process impurities that are controlled in the drug substance. They are not to be reported or included in the total degradation products.

▲USP39

SPECIFIC TESTS

- **pH** (791): 3.5–6.0
- **Bacterial Endotoxins Test** (85): NMT 3.6 USP Endotoxin Units/mg of hydroxyzine hydrochloride
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* (1)

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, protected from light.

▲Store at controlled room temperature. ▲*USP38*

Change to read:

- **USP Reference Standards** { 11 }

▲USP 4-Chlorobenzophenone RS

$C_{13}H_9ClO$ 216.66 ▲*USP38*

USP Endotoxin RS

USP Hydroxyzine Hydrochloride RS

▲USP Hydroxyzine Related Compound A RS

1-[(4-Chlorophenyl)phenylmethyl]piperazine.

$C_{17}H_{19}ClN_2$ 286.80 ▲*USP39*

BRIEFING

Hydroxyzine Hydrochloride Tablets, *USP 38* page 3815. As part of the USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Add a stability-indicating liquid chromatographic procedure as a test for *Organic Impurities* and replace the existing *Assay* with a similar procedure. These procedures were validated using the Acquity UPLC HSS C18 SB brand of L1 column. The typical retention time for hydroxyzine is about 14.5 min.
2. Replace *Identification* test *A* that is based on the TLC procedure with a test based on the retention time agreement using the proposed *Assay*.
3. Add *Identification* test *B* based on the UV spectral agreement for the main peak from the proposed *Assay*.
4. Include a storage requirement to the *Packaging and Storage* section based on the information for an approved drug product.
5. Add two new Reference Standards to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R.-H. Yeh, D. Min.)

Correspondence Number—C117376

Comment deadline: March 31, 2015

Hydroxyzine Hydrochloride Tablets**DEFINITION**

Hydroxyzine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$).

IDENTIFICATION

Delete the following:

▲• A.

Standard solution: 2 mg/mL of USP Hydroxyzine Hydrochloride RS in methanol

Sample solution: Nominally 2 mg/mL of hydroxyzine hydrochloride in methanol prepared as follows. Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of hydroxyzine hydrochloride, with 50 mL of methanol, and filter. Use the filtered solution.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: Thin-layer chromatographic plate, coated with a 0.25-mm layer of chromatographic silica gel, and dried in air for 30 min, followed by drying under vacuum at 140 ° for 30 min.

Application volume: 100 µL

Developing solvent system: Toluene, alcohol, and ammonium hydroxide (150:95:1)

Spray reagent: Potassium iodoplatinate TS

Analysis

Samples: *Standard solution and Sample solution*

Apply the solutions on the chromatographic plate, and allow the spots to dry. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots by lightly spraying with *Spray reagent*.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

▲USP39

Add the following:

▲• **A.** The retention time of the main peak of the *Sample solution* corresponds to that of the hydroxyzine peak of the *Standard solution*, as obtained in the Assay. ▲USP39

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ▲USP39

ASSAY

Change to read:

• Procedure

Solution A: 6.8 g/L of monobasic potassium phosphate in water

Mobile phase: ~~Methanol and Solution A (50:50). Pass through a polytef membrane filter of NMT 5- μ m pore size.~~

Standard solution: ~~100 μ g/mL of USP Hydroxyzine Hydrochloride RS in methanol~~

Sample stock solution: ~~Place 20 Tablets in a high-speed blender jar containing 400.0 mL of methanol, and blend for 5 min. The Tablets are completely disintegrated. Allow to settle, and pass a portion of the supernatant through a polytef membrane filter of NMT 1- μ m pore size.~~

Sample solution: ~~Nominally 100 μ g/mL of hydroxyzine hydrochloride from the Sample stock solution in methanol~~

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: ~~LC~~

Detector: ~~UV 232 nm~~

Column: ~~4.6 mm \times 25 cm; packing L9~~

Flow rate: ~~2.0 mL/min~~

Injection volume: ~~20 μ L~~

System suitability

Sample: ~~Standard solution~~

Suitability requirements

Relative standard deviation: ~~NMT 2.5%~~

▲Solution A: Trifluoroacetic acid and water (0.1: 99.9)

Solution B: Trifluoroacetic acid and acetonitrile (0.5: 99.5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
12	55	45
16	55	45
21	20	80
25	20	80
26	90	10
30	90	10

Diluent: Acetonitrile and water (30:70)

Standard solution: 0.05 mg/mL of USP Hydroxyzine Hydrochloride RS in *Diluent*

Sample stock solution: Nominally 0.5 mg/mL of hydroxyzine hydrochloride from Tablets in *Diluent* prepared as follows. Transfer a portion of finely powdered Tablets (NLT 10), equivalent to 50 mg of hydroxyzine hydrochloride, to a 100-mL volumetric flask and add 80 mL of *Diluent*. Sonicate for 30 min to dissolve and dilute with *Diluent* to volume. Centrifuge the solution and use the supernatant.

[Note—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]

Sample solution: Nominally 0.05 mg/mL of hydroxyzine hydrochloride from the *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 230 nm. For *Identification* test B, use a diode array detector in the range of 200–400 nm.

Column: 2.1-mm × 15-cm; 1.8-μm packing L1

Flow rate: 0.3 mL/min

Injection volume: 2 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

▲USP39

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of hydroxyzine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

● **Dissolution** 〈 711 〉

Test 1

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: USP Hydroxyzine Hydrochloride RS in *Medium*

Sample solution: Use a filtered portion of the solution under test. Dilute with *Medium*, if necessary.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 230 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) dissolved from the UV absorbances.

Tolerances: NLT 75% (Q) of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Water; 250 mL

Apparatus 3: 30 dips/min

Time: 45 min

Standard solution: USP Hydroxyzine Hydrochloride RS in *Medium*

Sample solution: Use a filtered portion of the solution under test. Dilute with *Medium*, if necessary.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 230 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) dissolved from the UV absorbances.

Tolerances: NLT 75% (Q) of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) is dissolved.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

Add the following:

▲• **Organic Impurities**

Solution A, Solution B, Mobile phase, and Diluent: Proceed as directed in the *Assay*.

Standard stock solution: 25.0 µg/mL each of USP Hydroxyzine Hydrochloride RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS in *Diluent*

Standard solution: 1.0 µg/mL each of USP Hydroxyzine Hydrochloride RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS from the *Standard stock solution* in *Diluent*

Sample solution: Nominally 500 µg/mL of hydroxyzine hydrochloride from Tablets prepared as follows. Transfer a portion of finely powdered Tablets (NLT 10), equivalent to 50 mg of hydroxyzine hydrochloride, to a 100-mL volumetric flask. Add 80 mL of *Diluent*. Sonicate 30 min to dissolve and dilute with *Diluent* to volume. Centrifuge the solution and use the supernatant.

[Note—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detectors

Hydroxyzine and hydroxyzine related compound A: UV 230 nm

4-Chlorobenzophenone: UV 254 nm

Column: 2.1-mm × 15-cm; 1.8-µm packing L1

Flow rate: 0.3 mL/min

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between hydroxyzine related compound A and hydroxyzine

Relative standard deviation: NMT 3.0% each for hydroxyzine related compound A, hydroxyzine, and 4-chlorobenzophenone

Analysis

Samples: *Standard solution* and *Sample solution*

For impurities detected at UV 230 nm

Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each unspecified degradation product from the *Sample solution*

r_S peak response of hydroxyzine from the *Standard solution*

C_S concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U nominal concentration of hydroxyzine hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

For 4-Chlorobenzophenone detected at UV 254 nm

Calculate the percentage of 4-chlorobenzophenone in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of 4-chlorobenzophenone from the *Sample solution*

r_S peak response of 4-chlorobenzophenone from the *Standard solution*

C_S concentration of USP 4-Chlorobenzophenone RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U nominal concentration of hydroxyzine hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Declocixine ^a	0.87	—
Hydroxyzine related compound A ^a	0.96	—
Hydroxyzine	1.0	—
4-Chlorobenzophenone	1.4	0.2
Any individual unspecified degradation product	—	0.3
Total degradation products	—	0.5

^a These are process impurities that are controlled in the drug substance. They are not to be reported or included in the total degradation products.

▲USP39

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers.

▲Store at controlled room temperature. ▲*USP39*

- **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

- **USP Reference Standards** 〈 11 〉

▲USP 4-Chlorobenzophenone RS

4-Chlorobenzophenone.

C₁₃H₉ClO 216.66 ▲*USP39*

USP Hydroxyzine Hydrochloride RS

▲USP Hydroxyzine Related Compound A RS

1-[(4-Chlorophenyl)phenylmethyl]piperazine.

C₁₇H₁₉ClN₂ 286.80 ▲*USP39*

BRIEFING

Ibuprofen Oral Suspension, *USP 38* page 3828. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Revise *Identification* test *A* and *Identification* test *B*, based on the UV spectra and retention time agreement as obtained in the *Assay*, to eliminate the use of chloroform due to safety concerns.
2. Replace the *Assay* procedure with a new HPLC procedure to eliminate the use of internal standard. The proposed liquid chromatography is based on analysis using the Spherisorb S5C8 brand of L7 column. The typical retention time for ibuprofen is about 10.5 min.
3. Delete the test for *Limit of Ibuprofen Related Compound C* and add a single HPLC procedure for *Organic Impurities*, in which the *Mobile phase* and *Chromatographic system* are the same as those in the proposed *Procedure* in the *Assay*.
4. Include USP Benzoic Acid RS and USP Ibuprofen Related Compound J RS in the *USP Reference Standards* section and add the chemical information for USP Ibuprofen Related Compound C RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: H. Cai.)

Correspondence Number—C137672

Comment deadline: March 31, 2015

Ibuprofen Oral Suspension

DEFINITION

Ibuprofen Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$).

IDENTIFICATION

Change to read:

• A.

~~**Standard solution:** 20 mg/mL of USP Ibuprofen RS in chloroform~~

~~**Sample solution:** Transfer a volume of Oral Suspension, nominally equivalent to 200 mg of ibuprofen, to a separator containing 10 mL of chloroform, and shake for about 1 min. Allow the layers to separate, and pass the lower chloroform layer through a filter containing about 2 g of anhydrous sodium sulfate. Use the filtrate.~~

~~[Note—Retain a portion of this solution for use in *Identification* test B.]~~

~~**Chromatographic system**~~

~~(See *Chromatography* ~~621~~, *Thin-Layer Chromatography*.)~~

~~**Adsorbent:** 0.25-mm layer of chromatographic silica gel, previously activated by heating at 105° for 30 min~~

~~**Application volume:** 10 µL~~

~~**Developing solvent system:** *n*-hexane, butyl acetate, and glacial acetic acid (17:3:1)~~

~~**Analysis**~~

~~**Samples** *Standard solution* and *Sample solution*~~

~~Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and dry in a current of cool air. Examine the chromatograms under short-wavelength UV light.~~

~~**Acceptance criteria:** The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.~~

▲The UV absorption spectra of the ibuprofen peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*. ▲USP39

Delete the following:

▲• ~~**B. Infrared Absorption**~~ ~~197K~~

~~**Standard and Sample:** Evaporate about 20 drops of the *Standard solution* and the *Sample solution* retained from *Identification* test A to dryness in a current of air without heating.~~

~~**Acceptance criteria:** Meets the requirements. ▲USP39~~

Add the following:

▲• **B.** The retention time of the ibuprofen peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP39

ASSAY

Change to read:● **Procedure**

Buffer: Dilute 0.7 mL of phosphoric acid with water to obtain 1000 mL of 0.01 M phosphoric acid.

Mobile phase: Acetonitrile and *Buffer* (37:63)

Diluent: Acetonitrile and water (1:1)

Internal standard solution: 3.2 mg/mL of benzophenone in acetonitrile

Standard stock solution: 1.2 mg/mL of USP Ibuprofen RS in *Diluent*

Standard solution: Transfer 20.0 mL of the *Standard stock solution* and 5.0 mL of the *Internal standard solution* to a 50 mL volumetric flask, dilute with acetonitrile to volume, mix, and filter. This solution contains about 0.48 mg of ibuprofen per mL.

Density: Using a tared 50 mL volumetric flask, weigh 50 mL of Oral Suspension that has previously been well shaken to ensure homogeneity, allow to stand until the entrapped air has risen, and invert carefully just prior to transferring it to the volumetric flask. From the observed weight of 50 mL of the Oral Suspension, calculate the density of the Oral Suspension in g/mL.

Sample stock solution: Nominally 1.2 mg/mL of ibuprofen prepared as follows. Transfer a portion of Oral Suspension to a suitable volumetric flask, and dilute with *Diluent* to volume.

[Note—Retain a portion of this solution for use in *Limit of Ibuprofen Related Compound C* below.]

Sample solution: Transfer 20.0 mL of the *Sample stock solution* and 5.0 mL of the *Internal standard solution* to a second 50 mL volumetric flask, dilute with acetonitrile to volume, mix, and filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6 mm × 15 cm; 5 µm packing L7

Flow rate: 2 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for benzophenone and ibuprofen are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between benzophenone and ibuprofen

Relative standard deviation: NMT 2.0%

Tailing factor: NMT 2.0

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

~~r_U~~ ratio of the ibuprofen peak area to the benzophenone peak area from the *Sample solution*

~~r_S~~ ratio of the ibuprofen peak area to the benzophenone peak area from the *Standard solution*

~~C_S~~ concentration of USP Ibuprofen RS in the *Standard solution* (mg/mL)

~~C_U~~ nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

▲Mobile phase:

Dissolve 1.52 g of monobasic potassium phosphate in 560 mL of water. Adjust with phosphoric acid to a pH of 2.05 if necessary. Add 440 mL of tetrahydrofuran and mix.

Diluent: Methanol and water (1:1)

Standard solution: 0.4 mg/mL of USP Ibuprofen RS in *Diluent*

Sample solution: Nominally 0.4 mg/mL of ibuprofen in *Diluent* prepared as follows.

Transfer a suitable amount of Oral Suspension to a suitable volumetric flask. Add about 60% final volume of *Diluent*. Sonicate for 45 min. Cool and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detectors

Assay: UV 254 nm

Identification test A: Diode array UV 200–400 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.0 mL/min

Injection volume: 25 µL

Run time: NLT 1.9 times the retention time of ibuprofen

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 3.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of ibuprofen from the *Sample solution*

r_S

= peak response of ibuprofen from the *Standard solution*

C_S

= concentration of USP Ibuprofen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

▲USP39

Acceptance criteria: 90.0%–110.0%**PERFORMANCE TESTS**● **Dissolution** 〈 711 〉**Medium:** pH 7.2 phosphate buffer (see *Buffers in Reagents, Indicators, and Solutions*); 900 mL**Apparatus 2:** 50 rpm**Time:** 60 min**Buffer:** Dilute 0.7 mL of phosphoric acid with water to obtain 1000 mL of 0.01 M phosphoric acid.**Mobile phase:** Acetonitrile and *Buffer* (37:63)**Internal standard solution:** 0.3 mg/mL of benzophenone in acetonitrile**Standard stock solution:** Dissolve a quantity of USP Ibuprofen RS in *Medium* to obtain a solution having a known concentration of 0.011J mg/mL, J being the labeled amount of ibuprofen in the Oral Suspension, in mg/mL.**Standard solution:** *Internal standard solution* and *Standard stock solution* (1:1), passed through a suitable filter of 0.5- μ m or finer pore size**Sample stock solution:** Filter a portion of the solution under test.**Sample solution:** *Internal standard solution* and *Sample stock solution* (1:1), passed through a suitable filter of 0.5- μ m or finer pore size**Density:** Using a tared 50-mL volumetric flask, weigh 50 mL of Oral Suspension that has been previously well shaken to ensure homogeneity. Allow to stand until the entrapped air has risen, and invert carefully just prior to transferring it to the volumetric flask. From the observed weight of 50 mL of Oral Suspension, calculate the density of Oral Suspension in g/mL.**Chromatographic system**(See *Chromatography* 〈 621 〉, *System Suitability*.)**Mode:** LC**Detector:** UV 220 nm**Column:** 4.6-mm \times 15-cm; 5- μ m packing L7**Flow rate:** 2 mL/min**Injection volume:** 10 μ L**System suitability****Sample:** *Standard solution*

[Note—The relative retention times for benzophenone and ibuprofen are 0.9 and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 1.5 between benzophenone and ibuprofen**Relative standard deviation:** NMT 2.0%**Tailing factor:** NMT 2.0**Analysis****Samples:** *Standard solution* and *Sample solution*

Using an accurately tared syringe, draw about 10 mL of well-mixed Oral Suspension into the syringe, which is connected to tubing, and weigh. [Note—The tubing of the syringe is placed into a zone that is between the surface of the *Medium* and the top of the rotating blade.] Express the Oral Suspension into the *Medium*. Promptly reweigh the syringe and determine the weight, in g, of Oral Suspension added to the *Medium*.

Calculate the percentage of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$) dissolved:

$$\text{Result} = (R_U/R_S) \times C_S \times V \times (D/W_U) \times (1/L) \times 100$$

R_U = peak area ratio of ibuprofen to benzophenone from the *Sample solution*

R_S = peak area ratio of ibuprofen to benzophenone from the *Standard solution*

C_S = concentration of USP Ibuprofen RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

D = density of Oral Suspension (g/mL)

W_U = weight of the portion of Oral Suspension added to the *Medium* (g)

L = label claim (mg/mL)

Tolerances: NLT 80% (Q) of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$) is dissolved.

- **Uniformity of Dosage Units** $\langle 905 \rangle$: Meets the requirements for oral suspension packaged in single-unit containers
- **Deliverable Volume** $\langle 698 \rangle$: Meets the requirements for oral suspension packaged in multiple-unit containers

IMPURITIES

Change to read:

- **Organic Impurities**

- ~~Limit of Ibuprofen Related Compound C~~

~~**Mobile phase and Diluent:** Proceed as directed in the Assay~~

~~**Standard stock solution 1:** 0.5 mg/mL of USP Ibuprofen Related Compound C RS in acetonitrile~~

~~**Standard stock solution 2:** 0.03 mg/mL of USP Ibuprofen Related Compound C RS prepared by diluting *Standard stock solution 1* with *Diluent*~~

~~**Standard solution:** 0.0012 mg/mL of USP Ibuprofen Related Compound C RS prepared as follows. Transfer 2.0 mL of *Standard stock solution 2* to a 50 mL volumetric flask, add 18 mL of *Diluent*, dilute to volume with acetonitrile, and pass through a suitable filter of 0.22 μm pore size.~~

~~**Sample solution:** Transfer 20.0 mL of the *Sample stock solution* prepared in the Assay to a 50 mL volumetric flask, dilute to volume with acetonitrile, and pass through a suitable filter of 0.22 μm pore size.~~

~~**System suitability solution:** Transfer 1.5 mL of the *Standard stock solution 1* and 9 mL of the *Standard stock solution* prepared in the Assay to a 25 mL volumetric flask, dilute with acetonitrile to volume, and pass through a suitable filter of 0.22 μm pore size. This solution contains about 0.03 mg/mL of ibuprofen related compound C and 0.4 mg/mL of ibuprofen.~~

~~**Chromatographic system**~~

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6 mm × 15 cm; 5-μm packing L7

Flow rate: 2 mL/min

Injection volume: 35 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for ibuprofen related compound C and ibuprofen for *System suitability solution* are 1.3 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between ibuprofen related compound C and ibuprofen, *System suitability solution*

Tailing factor: NMT 2.0, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ibuprofen related compound C in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of ibuprofen related compound C from the *Sample solution*

r_S = peak area of ibuprofen related compound C from the *Standard solution*

C_S = concentration of USP Ibuprofen Related Compound C RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.25%

▲ Mobile phase, Diluent, and Sample solution: Proceed as directed in the *Assay*.

System suitability solution: 0.4 mg/mL of USP Ibuprofen RS, 0.004 mg/mL each of USP Ibuprofen Related Compound C RS and USP Ibuprofen Related Compound J RS, and 0.0169 mg/mL of USP Benzoic Acid RS in *Diluent*

Sensitivity solution: 0.0002 mg/mL each of USP Ibuprofen Related Compound J RS and USP Ibuprofen Related Compound C RS in *Diluent*

Standard solution A: 0.0002 mg/mL of USP Ibuprofen RS in *Diluent*

Standard solution B: 0.004 mg/mL each of USP Ibuprofen Related Compound J RS and USP Ibuprofen Related Compound C RS in *Diluent*

Chromatographic system: Proceed as directed in the *Assay*, except for the *Detector*.

Detectors

For the quantitation of unspecified degradation products: UV 220 nm

For the quantitation of ibuprofen related compound C and ibuprofen related compound J: UV 254 nm

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, *Standard solution A*, and *Standard solution B*

Suitability requirements

For the quantitation of unspecified degradation products

Resolution: NLT 2.0 between benzoic acid and ibuprofen related compound J; NLT 2.0 between ibuprofen related compound J and ibuprofen related compound C; NLT 2.0 between ibuprofen related compound C and ibuprofen, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution A*

For the quantitation of ibuprofen related compound J and ibuprofen related compound C

Resolution: NLT 2.0 between benzoic acid and ibuprofen related compound J; NLT 2.0 between ibuprofen related compound J and ibuprofen related compound C; NLT 2.0 between ibuprofen related compound C and ibuprofen, *System suitability solution*

Signal-to-noise ratio: NLT 10 for ibuprofen related compound J and ibuprofen related compound C, *Sensitivity solution*

Relative standard deviation: NMT 2.0% for ibuprofen related compound J and ibuprofen related compound C, *Standard solution B*

Analysis

Samples: *Standard solution A, Standard solution B, and Sample solution*

Calculate the percentage of ibuprofen related compound J and ibuprofen related compound C in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of ibuprofen related compound J or ibuprofen related compound C from the *Sample solution*

r_S

= peak response of ibuprofen related compound J or ibuprofen related compound C from *Standard solution B*

C_S

= concentration of USP Ibuprofen Related Compound J RS or USP Ibuprofen Related Compound C RS in *Standard solution B* (mg/mL)

C_U

= nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of any individual unspecified degradation product from the *Sample solution*

r_s = peak response of ibuprofen from *Standard solution A* C_s = concentration of USP Ibuprofen RS in *Standard solution A* (mg/mL) C_U = nominal concentration of ibuprofen in the *Sample solution* (mg/mL)**Acceptance criteria:** See *Table 1*. Disregard any peaks less than 0.05%.**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzoic acid ^a	0.33	—
Ibuprofen related compound J	0.48	0.2
Ibuprofen related compound C	0.81	0.25
Ibuprofen	1.00	—
Any unspecified degradation product	—	0.2
Total degradation products	—	0.9
a Not included in the total degradation products.		

▲USP39

SPECIFIC TESTS

- pH 〈 791 〉: 3.6–4.6

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, and store at controlled room temperature.

Change to read:

- **USP Reference Standards** 〈 11 〉

▲USP Benzoic Acid RS▲USP39

USP Ibuprofen RS USP Ibuprofen Related Compound C RS

▲4-Isobutylacetophenone.

 $C_{12}H_{16}O$ 176.25

USP Ibuprofen Related Compound J RS

2-(4-Isobutyrylphenyl)propanoic acid.

 $C_{13}H_{16}O_3$ 220.26

▲USP39

Ibuprofen Tablets, *USP 38* page 3829. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Revise *Identification* test *A* based on the retention time agreement as obtained in the *Assay*, to eliminate the use of chloroform due to safety concerns.
2. Revise *Identification* test *B* based on the proposed *Procedure* in the *Assay*.
3. Replace the *Assay* procedure with a new HPLC procedure to eliminate the use of the internal standard. The proposed liquid chromatography is based on analysis using the Zorbax ODS brand of L1 packing. The typical retention time for ibuprofen is about 4.5 min.
4. Delete the test for *Limit of Ibuprofen Related Compound C* and add a single HPLC procedure in the test for *Organic Impurities*, in which *Mobile phase* and *Chromatographic system* are the same as those in the proposed *Procedure* in the *Assay*.
5. Delete the test for *Water Determination* to allow flexibility.
6. Include USP Ibuprofen Related Compound J RS in the *USP Reference Standards* section and add chemical information for USP Ibuprofen Related Compound C RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: H. Cai.)

Correspondence Number—C124209

Comment deadline: March 31, 2015

Ibuprofen Tablets

DEFINITION

Ibuprofen Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$).

IDENTIFICATION

Change to read:

- **A.**

~~**Sample:** Grind 1 Tablet to a fine powder in a mortar, add about 5 mL of chloroform, and swirl.~~

~~**Analysis:** Filter the *Sample*, and evaporate the filtrate with the aid of a stream of nitrogen to dryness.~~

~~**Acceptance criteria:** The IR absorption spectrum of a mineral oil dispersion of the residue exhibits maxima only at the same wavelengths as that of a similar preparation of USP Ibuprofen RS.~~

▲The UV absorption spectra of the ibuprofen peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*. ▲*USP39*

Change to read:

- **B.** The relative retention time of the ibuprofen peak, relative to the internal standard, from the *Sample solution* corresponds to that of the USP Ibuprofen RS, as obtained in the *Assay*.
- ▲ The retention time of the ibuprofen peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP39*

ASSAY

Change to read:

- **Procedure**

Mobile phase: Dissolve 4.0 g of chloroacetic acid in 400 mL of water, and adjust with ammonium hydroxide to a pH of 3.0. Add 600 mL of acetonitrile, filter and degas

Internal standard solution: 0.35 mg/mL of valerophenone in *Mobile phase*

Standard solution: 12 mg/mL of USP Ibuprofen RS in *Internal standard solution*

Ibuprofen related compound C standard stock solution: 0.6 mg/mL of USP Ibuprofen Related Compound C RS in acetonitrile

Ibuprofen related compound C standard solution: Dilute *Ibuprofen related compound C standard stock solution* with *Internal standard solution* to obtain a solution containing 0.012 mg/mL of ibuprofen related compound C

Sample solution: Nominally 12 mg/mL of ibuprofen prepared as follows. Transfer a portion nominally equivalent to 1200 mg of ibuprofen from NLT 20 finely powdered to a suitable container, add 100.0 mL of *Internal standard solution*, and shake for 10 min. [Note—Where the Tablets are coated, place an accurately counted number of Tablets, nominally equivalent to NLT 1200 mg of ibuprofen, in a container, add a volume of *Internal standard solution*, sufficient to obtain a *Sample solution* containing 12 mg/mL of ibuprofen, and about 15 glass beads, and then shake until the Tablets are completely disintegrated.] Centrifuge a portion of the suspension and use the clear supernatant.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6 mm × 25 cm column; packing L1

Flow rate: 2 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution* and *Ibuprofen related compound C standard solution*

[Note—The relative retention times for ibuprofen, valerophenone and ibuprofen related compound C are 0.75, 1.0 and 1.2 respectively.]

Suitability requirements

Tailing factor: NMT 2.5 for ibuprofen, valerophenone and ibuprofen related compound C, *Standard solution* and *Ibuprofen related compound C standard solution*

Resolution: NLT 2.5 between ibuprofen and the valerophenone, *Standard solution*; NLT 2.5 between valerophenone and ibuprofen related compound C, *Ibuprofen related compound C standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution* and *Ibuprofen related*

compound C standard solution

Analysis

Samples: *Standard solution, Sample solution, and Ibuprofen related compound C standard solution*

Calculate the percentage of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U ratio of the ibuprofen peak response to the valerophenone peak response from the *Sample solution*

R_S ratio of the ibuprofen peak response to the valerophenone peak response from the *Standard solution*

C_S concentration of USP Ibuprofen RS in the *Standard solution* (mg/mL)

C_U nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

▲Mobile phase:

Dissolve 4.0 g of chloroacetic acid in 400 mL of water, adjust with ammonium hydroxide to a pH of 3.0 if necessary, add 600 mL of acetonitrile, and mix.

Standard solution: 10.0 mg/mL of USP Ibuprofen RS in *Mobile phase*

Sample solution: Nominally 10.0 mg/mL of ibuprofen prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask and add about 50% final volume of *Mobile phase*. Shake on a flat bed shaker for at least 60 min or until the Tablets are disintegrated. Dilute with *Mobile phase* to volume. Centrifuge a portion of the solution at about 3000 rpm for about 10 min or until a clear supernatant is obtained. Use the supernatant for analysis.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

[Note—It is suggested to use the mixture of methanol and water (90:10) for needle wash.]

Mode: LC

Detectors

Assay: UV 254 nm

Identification test A: Diode array UV 200–400 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 2.0 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of ibuprofen from the *Sample solution*

 r_S

= peak response of ibuprofen from the *Standard solution*

 C_S

= concentration of USP Ibuprofen RS in the *Standard solution* (mg/mL)

 C_U

= nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

▲USP39

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution 〈 711 〉

Medium: pH 7.2 phosphate buffer (see *Buffers in Reagents, Indicators, and Solutions*); 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Standard solution: A known concentration of USP Ibuprofen RS in *Medium*

Sample solution: Filter a portion of the solution under test, and suitably dilute with *Medium* if necessary.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 221 nm

Analysis: Determine the amount of ibuprofen ($C_{13}H_{18}O_2$) dissolved by comparing the UV absorbance of the *Sample solution* with that of the *Standard solution*. [Note—Where the Tablets are labeled as gelatin-coated, determine the amount of ibuprofen ($C_{13}H_{18}O_2$) dissolved from the UV absorbance at the wavelength of maximum absorbance at about 266 nm, from which is subtracted the absorbance at 280 nm, in comparison with the *Standard solution*, similarly measured.]

Tolerances: NLT 80% (Q) of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$) is dissolved.

• Uniformity of Dosage Units 〈 905 〉: Meet the requirements

IMPURITIES

Change to read:

• Organic Impurities

• ~~Limit of Ibuprofen-Related Compound C Analysis~~

~~Use the chromatograms of the *Sample solution* and the *Ibuprofen-related compound C standard solution*, obtained as directed in the *Assay*. Calculate the percentage of~~

ibuprofen-related compound ($C_{12}H_{16}O$) in portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U ratio of the ibuprofen-related compound C peak response to the valerophenone peak response from the *Sample solution*

R_S ratio of the ibuprofen-related compound C peak response to the valerophenone peak response from the *Standard solution*

C_S concentration of USP Ibuprofen Related Compound C RS in the *Ibuprofen-related compound C standard solution* (mg/mL)

C_U nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.25%

▲ Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Sensitivity solution: 0.005 mg/mL of USP Ibuprofen RS in *Mobile phase*

System suitability solution: 10.0 mg/mL of USP Ibuprofen RS and 0.01 mg/mL each of USP Ibuprofen Related Compound C RS and USP Ibuprofen Related Compound J RS in *Mobile phase*

Standard solution: 0.02 mg/mL of USP Ibuprofen RS and 0.01 mg/mL each of USP Ibuprofen Related C RS and USP Ibuprofen Related Compound J RS in *Mobile phase*

System suitability

Samples: *Sensitivity solution, System suitability solution, and Standard solution*

Suitability requirements

Resolution: NLT 2.5 between ibuprofen related compound J and ibuprofen; NLT 2.5 between ibuprofen and ibuprofen related compound C, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Relative standard deviation: NMT 6.0% for ibuprofen related compound J, ibuprofen, and ibuprofen related compound C, *Standard solution*

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of ibuprofen related compound J and ibuprofen related compound C in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of ibuprofen related compound J or ibuprofen related compound C from the *Sample solution*

r_S

= peak response of ibuprofen related compound J or ibuprofen related compound C from the *Standard solution*

C_S

= concentration of USP Ibuprofen Related Compound J RS or USP Ibuprofen Related Compound C RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of any individual unspecified degradation product from the *Sample solution*

r_S

= peak response of ibuprofen from the *Standard solution*

C_S

= concentration of USP Ibuprofen RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ibuprofen related compound J	0.47	0.2
Ibuprofen	1.00	—
Ibuprofen related compound C	1.62	0.1
Any unspecified degradation product	—	0.2
Total degradation products	—	1.5

▲USP39

SPECIFIC TESTS

Delete the following:

▲● ~~Water Determination, Method I (921): NMT 5.0%~~

~~[Note—Tablets labeled as gelatin-coated are exempt from this requirement.]~~

▲USP39

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **Labeling:** Where the Tablets are gelatin-coated, the label so states.

Change to read:● **USP Reference Standards** (11)USP Ibuprofen RS USP Ibuprofen Related Compound C RS

▲4-Isobutylacetophenone.

 $C_{12}H_{16}O$ 176.25

USP Ibuprofen Related Compound J RS

2-(4-Isobutylphenyl)propanoic acid.

 $C_{13}H_{16}O_3$ 220.26

▲USP39

BRIEFING

Iodixanol, *USP 38* page 3901 and *PF 39(3)* [May–June 2013]. On the basis of comments received, it is proposed to cancel the proposal that appeared in *PF 39(3)* and republish the monograph proposal with the following changes:

1. Revise *Identification* test *B* with a retention time match based on the test for *Limit of Iodixanol Related Compound E and Iodixanol Impurity H*.
2. Delete *Identification* test *C* because it generates iodine vapors, which are a potential safety hazard because iodine vapors can irritate the nose and eyes.
3. Revise the *Assay* to include a formula for calculation of the content of iodixanol.
4. Revise the *Limit of Free Iodide* test to include a formula for calculation of iodide.
5. Delete the *Limit of Calcium* test because the manufacturing history indicates that no detectable levels of calcium have been observed.
6. Delete the *Free Iodine* test because the manufacturing history indicates that no free iodine has been observed.
7. Revise the *Limit of Free Aromatic Amine* test to be consistent with the procedure in *European Pharmacopoeia 8.4*.
8. Replace the *Limit of Methanol, Isopropyl Alcohol, and Methoxyethanol* test with a modified gas chromatographic procedure for quantifying 2-methoxyethanol. The procedure is based on analyses performed with the JW Scientific (Agilent Technologies) DB-Wax brand of G16 column. The retention time of 2-methoxyethanol is about 8 min.
9. Rename *Organic Impurities, Procedure 1* as *Organic Impurities*. Revise the test to be consistent with the *Related Substances* test in *European Pharmacopoeia 8.4*. The procedure is based on validations performed using the Hypersil-ODS brand of L1 column. Under chromatographic conditions, the first major peak of iodixanol elutes at 28.5 min.
10. Replace *Organic Impurities, Procedure 2* with the *Limit of Iodixanol Related Compound E and Iodixanol Impurity H* test. The new test is consistent with the *Impurities E and H* test in *European Pharmacopoeia 8.4*. This procedure is based on validations performed with the Supelcosil-NH2 brand of L8 column. The Zorbax-NH2 brand of L8 column is also suitable for this analysis. Under chromatographic conditions, iodixanol related compound E and iodixanol impurity H elute at about 12 and 22 min, respectively.
11. Delete the redundant *Microbial Enumeration Tests*, because it needs to be performed in the finished formulation, regardless of whether the drug substance is tested for

microbial limits.

12. Delete the *Optical Rotation* test, which does not add value.
13. Revise the existing storage condition in *Packing and Storage* to room temperature, based on the stability data.

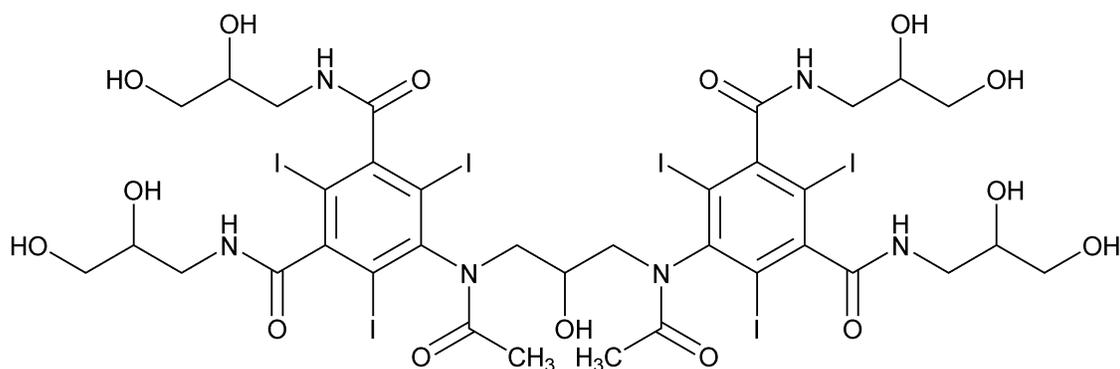
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R. Ravichandran.)

Correspondence Number—C135452

Comment deadline: March 31, 2015

Iodixanol



$C_{35}H_{44}I_6N_6O_{15}$ 1550.18

1,3-Benzenedicarboxamide, 5,5'-[(2-hydroxy-1,3-propanediyl)bis(acetylimino)]bis[*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-];
5,5'-[(2-Hydroxytrimethylene)bis(acetylimino)]bis[*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamide] [92339-11-2].

DEFINITION

Iodixanol contains NLT 98.6% and NMT 101.0% of iodixanol ($C_{35}H_{44}I_6N_6O_{15}$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈 197K 〉

Change to read:

- **B.** The retention times of the two principal peaks from the *Sample solution* correspond to those from *Standard solution B*, as obtained in *Limit of Iodixanol Related Compound E and Iodixanol impurity H*.

▲The retention times of the three principal peaks of the *Sample solution* correspond to those of the *Identification solution*, as obtained in the test for *Limit of Iodixanol Related Compound E and Iodixanol Impurity H*. ▲*USP39*

[Note—A third isomer may appear as a minor peak.]

Delete the following:

▲● **C. Procedure**

Sample: 0.5 g

Analysis: Heat the *Sample* in a crucible.

Acceptance criteria: Violet vapors are evolved. ▲USP39

ASSAY

Change to read:

● **Procedure**

Analysis: ~~Titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 25.84 mg of $C_{35}H_{44}I_6N_6O_{15}$.~~

▲**Sample solution:** Transfer 500 mg of Iodixanol to a glass-stoppered, 125-mL conical flask. Add 25 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc. Connect the flask to a reflux condenser, and reflux for 1 h. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and pass the mixture through a filter. Rinse the flask and the filter thoroughly with small portions of water, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* 〈 541 〉.)

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Potentiometric

Analysis

Sample: *Sample solution*

Titrate the *Sample solution* with the *Titrant*.

Calculate the percentage of iodixanol ($C_{35}H_{44}I_6N_6O_{15}$) in the portion of Iodixanol taken:

$$\text{Result} = [(V \times N \times F)/W] \times 100$$

V

= sample titrant volume (mL)

N

= *Titrant* normality (meq/mL)

F

= equivalent weight of iodixanol, 258.4 mg/meq

W

= weight of iodixanol (mg)

▲USP39

Acceptance criteria: 98.6%–101.0% on the anhydrous basis

IMPURITIES

Delete the following:

- **Heavy Metals, Method I** (231)
: NMT 10 ppm (Official 1-Dec-2015)

Change to read:

- **Limit of Free Iodide** ~~Transfer 5.0 g to a suitable container, add about 30 mL of water, and titrate with 0.001 N silver nitrate VS, determining the endpoint potentiometrically. Each mL of 0.001 N silver nitrate is equivalent to 126.9 µg of iodine. Not more than 0.39 mL of 0.001 N silver nitrate is required: not more than 10 µg of iodide per g is found.~~

▲ **Sample solution:** 5 g of Iodixanol in 30 mL of water

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.001 N silver nitrate VS

Endpoint detection: Potentiometric

Analysis: Calculate the percentage of free iodide in the portion of Iodixanol taken:

$$\text{Result} = [(V \times N \times F)/W] \times 100$$

V

= sample titrant volume (mL)

N

= Titrant normality (meq/mL)

F

= equivalent weight of iodide, 0.1269 mg/meq

W

= weight of iodixanol (mg)

Acceptance criteria: NMT 0.001% ▲ *USP39*

Delete the following:

▲ **Limit of Calcium**

Internal standard solution: 0.2 mg/mL of scandium from suitable amount of scandium oxide in water

Blank solution: 0.02 mg/mL of scandium from *Internal standard solution* in water

Standard stock solution: 10 µg/mL of calcium in water

Standard solution A: 0.1 µg/mL of calcium and 0.02 mg/mL of scandium from *Standard stock solution*, *Internal standard solution*, and water

Standard solution B: 0.5 µg/mL of calcium and 0.02 mg/mL of scandium from *Standard stock solution*, *Internal standard solution*, and water

Standard solution C: ~~1 µg/mL of calcium and 0.02 mg/mL of scandium from *Standard stock solution*, *Internal standard solution*, and water~~

Standard solution D: ~~2 µg/mL of calcium and 0.02 mg/mL of scandium from *Standard stock solution*, *Internal standard solution*, and water~~

Sample solution: ~~Transfer about 2 g of Iodixanol, accurately weighed, to a 20-mL volumetric flask, add about 10 mL of water, and mix. Add 2.0 mL of *Internal standard solution*, dilute with water to volume, and mix.~~

Instrumental conditions

Mode: ~~Atomic absorption spectrophotometry~~

Analytical wavelength: ~~393.366 nm, calcium emission line, and 361.38 nm, scandium emission line~~

Analysis

Samples: ~~*Blank solution*, *Standard solutions*, and *Sample solution*~~

~~Determine the absorbances of each *Standard solution* and the *Sample solution* against the *Blank solution*. Plot a standard curve of the ratio of the calcium absorption to the scandium absorption versus the respective calcium concentrations. From the graph so obtained, determine the calcium concentration (C) in µg/mL, in the *Sample solution*.~~

~~Calculate the content of calcium, in µg/g, in the portion of Iodixanol taken:~~

$$\text{Result} = C \times (V/W)$$

~~C~~ as obtained above

~~V~~ volume of the *Sample solution* (mL)

~~W~~ weight of Iodixanol taken to prepare the *Sample solution* (g)

Acceptance criteria: ~~NMT 5 µg/g~~▲*USP39*

Delete the following:

▲● **Free Iodine**

Sample solution: ~~20 mg/mL in toluene, water, and 2 N sulfuric acid (5:20:5)~~

~~{Note—Shake vigorously, and allow the phases to separate. }~~

Acceptance criteria: ~~The toluene layer shows no red or pink color.~~▲*USP39*

● **Limit of Ionic Compounds**

[Note—Rinse all glassware with water.]

Standard solution: 4 µg/mL of sodium chloride in water

Sample solution: 2 g of Iodixanol in 100 mL of water

Acceptance criteria: The specific conductance in the *Sample solution* is NMT that of the *Standard solution* (equivalent to NMT 0.02% of ionic compounds, as sodium chloride).

Change to read:

● **Limit of Free Aromatic Amine**

Solution A: 3 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in a mixture of propylene glycol and water (70:30)

Blank solution: Add 15 mL of water to a 25-mL volumetric flask.

Standard stock solution: 10 µg/mL of USP Iohexol Related Compound B RS in water

Standard solution: Transfer 10.0 mL of the *Standard stock solution* and 5 mL of water to a 25-mL volumetric flask.

Sample solution: Transfer 200 mg of Iodixanol to a 25-mL volumetric flask, and add 15 mL of water.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 495 nm

Cell: 5 cm

Analysis

Samples: *Blank solution*, *Standard solution*, and *Sample solution*

Treat the *Samples* as follows. Place the flask in an ice bath for 5 min. Add 1.5 mL of 6 N hydrochloric acid, and mix by swirling. Add 1.0 mL of sodium nitrite solution (20 mg/mL), and allow to stand in the ice bath for 4 min. Remove the flask from the ice bath, add 1.0 mL of sulfamic acid solution (40 mg/mL), and swirl gently until gas evolution ceases.

▲ [**Caution**—Considerable pressure is produced.]▲*USP39*

Add 1.0 mL of *Solution A*, dilute with water to volume, and allow to stand for 5 min. Transfer the solution obtained from the *Sample solution* and the solution obtained from the *Standard solution* to separate color comparison tubes. The solution obtained from the *Sample solution* is lighter than the solution obtained from the *Standard solution*: NMT 0.05% is found. If the solution obtained from the *Sample solution* is the same color or darker than the solution obtained from the *Standard solution*, determine the absorbances of the solutions. Use the solution obtained from the *Blank solution* to zero the spectrophotometer.

Calculate the percentage of free aromatic amine in the portion of Iodixanol taken:

$$\text{Result} = (C_S/C_U) [(A_U - A_B)/(A_S - A_B)]$$

C_S = concentration of USP Iohexol Related Compound B RS in the *Standard solution* ($\mu\text{g}/\text{mL}$)

C_U = weight of Iodixanol taken to prepare the *Sample solution* (mg)

A_U = absorbance of the final solution from the *Sample solution*

A_B = absorbance of the final solution from the *Blank solution*

A_S = absorbance of the final solution from the *Standard solution*

Acceptance criteria: NMT 0.05%

▲ Measure the absorbance of the *Standard solution* and the *Sample solution* against the *Blank solution*.

Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (NMT 0.05% of free aromatic amine).▲*USP39*

Delete the following:

▲ **Limit of Methanol, Isopropyl Alcohol, and Methoxyethanol**

Internal standard stock solution: 1 mg/mL of secondary butyl alcohol in water

Internal standard solution: 0.01 mg/mL of secondary butyl alcohol from *Internal standard stock solution* in water

Standard stock solution: 0.05 mg/mL of methanol, and 0.1 mg/mL each of isopropyl alcohol and methoxyethanol in water

Standard solution: 0.005 mg/mL of methanol, 0.01 mg/mL of isopropyl alcohol, 0.01 mg/mL of methoxyethanol and 0.01 mg/mL of butyl alcohol from *Standard stock solution* and *Internal standard stock solution* in water. Transfer 1 mL to a headspace vial.

Sample solution: Transfer 250 mg of Iodixanol and 1 mL of the *Internal standard solution* to a headspace vial.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC equipped with a headspace injector

Column: 0.54 mm × 30 m capillary column coated with a 1 µm layer of phase G16

Temperature

Autosampler: 105 °

Needle and transfer line: 130–140 °

Injector: 150 °

Detector: 200 °

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	3
40	8	100	1

Carrier gas: Helium

Flow rate: 11 mL/min

Injection volume: 1 mL

System suitability

Sample: *Standard solution*

[Note—Chromatograph using a heated, gas-tight syringe.]

Suitability requirements

[Note—The elution order is methanol, isopropyl alcohol, secondary butyl alcohol, and methoxyethanol.]

Resolution: NLT 1.0 between methanol and isopropyl alcohol

Relative standard deviation: NMT 5% for methanol and isopropyl alcohol, and NMT 10% for methoxyethanol, determined from the peak area ratios

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—Chromatograph using a heated, gas-tight syringe.]

Calculate the concentration of methanol, isopropyl alcohol, and methoxyethanol, in µg/g, in the portion of Iodixanol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U)$$

r_U = peak response of the analyte to peak response from the internal standard from the *Sample solution*

r_S = peak response of the analyte to peak response from the internal standard from the *Standard solution*

~~C_s~~ concentration of the relevant analyte in the *Standard solution* ($\mu\text{g/mL}$)

~~C_g~~ concentration of the *Sample solution* (g/mL)

Acceptance criteria

Individual impurities: NMT 50 $\mu\text{g/g}$ each of methanol, isopropyl alcohol, or methoxyethanol

▲USP39

Add the following:

▲● Limit of 2-Methoxyethanol

Internal standard solution: 0.01 mg/mL of secondary butyl alcohol in water

Standard stock solution: 0.005 mg/mL of methanol and 0.01 mg each of isopropyl alcohol, secondary butyl alcohol, and 2-methoxyethanol in *Internal standard solution*

Standard solution: Transfer about 0.25 g of USP Iodixanol RS and 1.0 mL of *Standard stock solution* to a headspace vial and seal the vial with a septum and crimp cap.

Sample solution: Transfer about 0.25 g of Iodixanol and 1.0 mL of *Internal standard solution* to a headspace vial and seal the vial with a septum and crimp cap.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC with suitable headspace autosampler

Detector: Flame ionization

Column: 0.53-mm \times 30-m fused-silica; coated with a 1- μm phase G16

Temperatures

Autosampler: 105 $^{\circ}$

Needle: 130 $^{\circ}$ –140 $^{\circ}$

Injection port: 150 $^{\circ}$

Detector: 200 $^{\circ}$

Column: See *Table 1*.

Table 1

Initial Temperature ($^{\circ}$)	Temperature Ramp ($^{\circ}/\text{min}$)	Final Temperature ($^{\circ}$)	Hold Time at Final Temperature (min)
40	—	40	3
40	8	100	1

Carrier gas: Helium

Flow rate: 11 mL/min

Injection volume: 1 mL of the headspace

System suitability

Sample: *Standard solution*

[Note—The typical relative retention times for methanol, isopropyl alcohol, secondary butyl alcohol, and 2-methoxyethanol are 0.5, 0.6, 1.0 and 1.9 respectively.]

Suitability requirements

Resolution: NLT 1.0 between methanol and isopropyl alcohol

Relative standard deviation: NMT 10.0% for the ratio of 2-methoxyethanol to internal standard

Analysis**Samples:** *Standard solution* and *Sample solution*

Calculate the amount of 2-methoxyethanol in the portion of Iodixanol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

 R_U peak response ratio of 2-methoxyethanol to the internal standard from the *Sample solution*
 R_S peak response ratio of 2-methoxyethanol to the internal standard from the *Standard solution*
 C_S concentration of 2-methoxyethanol in the *Standard solution* ($\mu\text{g/mL}$)

 C_U concentration of Iodixanol in *Sample solution* (g/mL)
Acceptance criteria: NMT 10 $\mu\text{g/g}$ of 2-methoxyethanol▲*USP39***Change to read:**● **Organic Impurities Procedure 1****Solution A:** Acetonitrile and water (50:50)**Solution B:** Water**Blank solution:** Water**Mobile phase:** See *Table 2*.**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	6	94
30	20	80
70	100	0
80	100	0
81	6	94
90	6	94

Standard stock solution A: 12.5 mg/mL of anhydrous Iodixanol from USP Iodixanol RS in water**Standard stock solution B:** 0.25 mg/mL of anhydrous iodixanol related compound C from USP Iodixanol Related Compound C RS in water**Standard solution A:** 2.5 mg/mL of *Standard stock solution A* in water**Standard solution B:** *Standard stock solution A*, *Standard stock solution B*, *Standard stock solution C*, and water (5:2.5:2.5:15)**Standard stock solution C:** 0.025 mg/mL of anhydrous iodixanol related compound D from USP Iodixanol Related Compound D RS in water**Sample solution A:** 25 mg/mL of anhydrous iodixanol from iodixanol in water**Sample solution B:** 2.5 mg/mL anhydrous iodixanol from *Sample solution A* in water**Sensitivity solution:** 2.5 mg/mL Iodixanol and 0.0125 mg/mL of USP Iodixanol Related Compound C from *Sample solution A* and *Standard stock solution B* in water.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6 mm × 25 cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Blank solution, Standard solution A, Control solution,* and at least three replicates of *Standard solution B*

[Note—The chromatogram from *Standard solution A* exhibits two or three principal unresolved peaks. If the chromatogram exhibits two principal peaks, their relative areas are 60% and 40%. If the chromatogram exhibits three principal peaks, their relative areas are 60%, 38%, and 2%. The chromatogram from *Standard solution B* exhibits two resolved peaks due to iodixanol related compound D that elute before the iodixanol peaks and one iodixanol related compound C peak between the two principal iodixanol peaks. The area of the two iodixanol related compound D peaks is between 0.075% and 0.125% of the total area.]

Suitability requirements

Relative standard deviation: NMT 5% for the sum of the two isomers of iodixanol related compound D, from three injections of *Standard solution B*

Peak-to-valley ratio: NLT 1.3 between iodixanol related compound C and the first major peak of iodixanol, *Standard solution B*

Peak sensitivity: Iodixanol related compound C exhibits a measurable peak, *Sensitivity solution*

Analysis

Samples: *Blank solution, Sample solution A,* and *Sample solution B*

High-low chromatography: Where it is specified to proceed as directed for *High-low chromatography*, for the chromatogram from *Sample solution A*, calculate the percentage of each specified related compound in the portion of Iodixanol taken:

$$\text{Result} = (10X)/(0.1Y + Z)$$

X = peak area for each of the specified related compounds from *Sample solution A*

Y = total area of all the peaks eluted before and after iodixanol from *Sample solution A*; disregarding any peaks due to injection noise or solvent

Z = sum of peak areas of iodixanol and any related compounds which are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

Iohexol: If iohexol is present, it exhibits two peaks, with retention times of 0.37 and 0.39 relative to the main iodixanol peak from *Sample solution A*. Draw a baseline at the height of the baseline from the *Blank solution*. Calculate the total area of the two peaks and the percentage of iohexol in the portion of Iodixanol taken as directed for *High-low chromatography*.

Iodixanol related compound B¹: If iodixanol related compound B is present, it elutes as a single peak with a retention time of 0.34 relative to the main iodixanol peak, in the chromatogram from *Sample solution A*. Draw a baseline at the height of the baseline from the *Blank solution*. Calculate the area of the peak and the percentage of iodixanol related compound B in the portion of Iodixanol taken, as directed for *High-low chromatography*.

Iodixanol related compound C: If iodixanol related compound C is present, only the

first and larger peak, with a retention time of 1.07 relative to the main iodixanol peak, is seen between the two principal iodixanol peaks from *Sample solution A*; the second iodixanol related compound C peak co-elutes with iodixanol. The area of the first and larger peak corresponds to 80% of the total area of iodixanol related compound C. Draw a vertical line through the minimum before the first and larger peak. Draw a horizontal baseline at the minimum after the first and larger peak.

Calculate the percentage of iodixanol related compound C in the portion of Iodixanol taken:

$$\text{Result} = 12.5X_2 / (0.1Y + Z)$$

X_2 = peak area response of iodixanol related compound C

Y = sum of all the peaks eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent

Z = sum of the peak area response of iodixanol and any related compounds which are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

Iodixanol related compound F²: If iodixanol related compound F is present, only the first and smaller peak with a retention time of 0.8 relative to the main iodixanol peak, can be seen in the chromatogram from *Sample solution A*; the second peak co-elutes with iodixanol. The area of the first and smaller peak corresponds to 25% of the total area of iodixanol related compound F. Draw the baseline at the height of the baseline from the *Blank solution*.

Calculate the percentage of iodixanol related compound F in the portion of Iodixanol taken:

$$\text{Result} = 40X_1 / (0.1Y + Z)$$

X_1 = the actual observed area of the peak of iodixanol related compound F from *Sample solution A*

Y = sum of all the peaks eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent

Z = sum of the peak areas of iodixanol and any related compounds which are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

Iodixanol related compound G³: If iodixanol related compound G is present, the second and larger peak, with a retention time of 1.18 relative to the last iodixanol peak, is seen in the chromatogram from *Sample solution A*; the first peak co-elutes with iodixanol. The area of the second peak corresponds to 85% of the total area of iodixanol related compound G. Draw the baseline at the height of the baseline from the *Blank solution*. Calculate the percentage of iodixanol related compound G in the portion of Iodixanol taken:

$$\text{Result} = 10X_2 / \{0.85(0.1Y + Z)\}$$

X_2 = peak area response of iodixanol related compound G

Y = sum of all the peak area responses eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent

Z = the sum of peak areas of iodixanol and any related compounds which are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

Overalkylated related compounds: These compounds elute after iodixanol related compound G, with a retention time greater than 1.18 relative to the last iodixanol peak. Draw the baseline at the height of the baseline from the *Blank solution*, and determine the peak areas.

Calculate the percentage of overalkylated related compounds as directed for *High-low chromatography*.

Unspecified related compounds: Examine the chromatograms from *Sample solution A* and the area of each peak eluting before or after iodixanol, other than those of iodixanol, specified related compounds, specified impurities, and overalkylated related compounds. Draw the baseline at the height of the baseline from the *Blank solution*. Calculate the percentage of the largest of these peaks as directed for *High-low chromatography*.

Other unspecified related compounds: Determine the area of any unspecified peak eluting between those of iodixanol. Draw the baseline between minima, and calculate the percentage as directed for *High-low chromatography*.

Acceptance criteria : See *Table 3* below.

Table 3

Name	Acceptance Criteria, NMT (%)
Iodixanol related compound B	0.2
Iodixanol related compound C	0.4
Iodixanol related compound F	0.2
Iodixanol related compound G	0.2
Iohexol	0.6
Overalkylated related compounds	1.0
Any individual unspecified related compound	0.2
Total unspecified related compounds	0.5

Total related compounds: From each of the chromatograms from *Sample solution A*, calculate the percentage of all related compounds as the sum of the results for the peaks appearing between the two principal iodixanol peaks, and the area percentage obtained as follows:

$$\text{Result} = [100(Y - X_1 - X_3 + X_1/0.25 + X_2/0.8 + X_3/0.85)]/10(0.1Y + Z)$$

Y = sum of all the peaks eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent

X_1 = actual observed area of the peak of iodixanol related compound F from *Sample solution A*

X_2 = peak area response of iodixanol related compound

X_3 = peak area response from iodixanol related compound C

Z = sum of the peak areas of iodixanol and any related compounds which are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

▲Solution A:

Water

Solution B: Acetonitrile and water (50:50)**System suitability solution:** 0.25 mg/mL of USP Iodixanol RS, 0.0025 mg/mL of USP Iodixanol Related Compound C RS, and 0.005 mg/mL of USP Iodixanol Related Compound D RS in water**Sample solution:** 2.5 mg/mL of Iodixanol in water**Mobile phase:** See *Table 2*.**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	94	6
2	94	6
32	80	20
72	0	100
82	0	100

Chromatographic system(See *Chromatography* { 621 }, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Flow rate:** 1 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *System suitability solution*Use the chromatogram from the *System suitability solution* to identify the peaks based on the relative retention times given in *Table 3*.**Suitability requirements****Resolution:** NLT 1.5 between the two peaks due to iodixanol related compound D**Peak-to-valley ratio:** NLT 1.3 between the first iodixanol peak and iodixanol related compound C (first peak)**Analysis****Sample:** *Sample solution*

[Note—If iodixanol related compound C is present, only the first and larger peak with a retention time of 1.04 relative to the main iodixanol peak is seen between the two principal iodixanol peaks; the second iodixanol related compound C peak co-elutes with iodixanol. The area of the first and larger peak corresponds to approximately 80% of the total area of iodixanol related compound C. Determine the peak area of the first peak by drawing a vertical line through the minimum before the peak and a horizontal baseline at the minimum after the peak.]

Calculate the percentage of each impurity in the *Sample solution*:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

 r_U

= peak response of each impurity in the *Sample solution*

r_T

= sum of all peak responses greater than 0.05% of the principal peaks in the *Sample solution*

F

= relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any impurity less than or equal to 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%) (w/w)
Iodixanol related compound D (first peak)	0.8	1.0	Sum of both peaks 0.1% if present
Iodixanol related compound D (second peak)	0.9	1.0	
Iodixanol (first peak)	1.0	—	—
Iodixanol related compound C (first peak)	1.04	0.76	0.4
Overalkylated impurities	1.3–1.7	1.0	(Sum of all) 1.0
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.5

▲USP39

Delete the following:

▲● **Organic Impurities, Procedure 2**

Solution A: Acetonitrile

Solution B: Water

Blank solution: Water

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	85	15
25	66	34

Standard stock solution A: 12.5 mg/mL of USP Iodixanol RS in water

Standard stock solution B: 0.025 mg/mL of USP Iodixanol Related Compound D RS

Standard stock solution C: 2.5 mg/mL of USP Iodixanol Related Compound E RS

Standard solution A: 2.5 mg/mL from *Standard stock solution A* diluted with water

Standard solution B: 2.5 mg/mL of iodixanol and 0.0025 mg/mL of related compound D from *Standard stock solution A* and *Standard stock solution B*, respectively, diluted with water

Standard solution C: 0.25 mg/mL of iodixanol and 0.25 mg/mL of related compound E from *Standard stock solution A* and *Standard stock solution C*, respectively, diluted with water

Sample solution: 2.5 mg/mL of anhydrous iodixanol from iodixanol

Chromatographic system

(See *Chromatography* ~~621~~, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6 mm × 25 cm; 5 μm packing L8

Flow rate: 2.5 mL/min

Injection size: 10 μL

System suitability

Samples: *Standard solution A*, three portions of *Standard solution B*, *Standard solution C*, and *Sample solution*

[Note—The chromatogram from *Standard solution A* exhibits three principal unresolved peaks: the relative areas are 62%, 35%, and 3%; and the retention time of the last iodixanol peak is NMT 14 min. The chromatogram from *Standard solution B* exhibits two partially unresolved peaks due to iodixanol related compound D, with relative retention times of 0.33 and 0.39, that elute before the iodixanol peaks: the peak area of iodixanol related compound D is between 0.075% and 0.125% of the total area. Disregard any peak due to the solvent.]

Relative standard deviation: NMT 5%

Determine the sum of the peak areas of the two isomers of iodixanol related compound D for each of the three chromatograms from *Standard solution B*.

The chromatogram from *Standard solution C* exhibits two unresolved peaks due to iodixanol related compound E, with relative retention times of 0.67 and 0.72, that elute before the iodixanol peaks. Adjust the sensitivity of the amplifier so that the peak heights are between 90% and 100% of full scale of the highest peak.

Resolution: NLT 5.0 between the first and largest iodixanol related compound E peak and the first principal iodixanol peak

Analysis

Samples: *Standard solution A*, three portions of *Standard solution B*, *Standard solution C*, and *Sample solution*

For the first chromatogram from *Standard solution B*, adjust the sensitivity of the amplifier to obtain a peak height of 15% of the first and larger peak that corresponds to iodixanol related compound D. Use this sensitivity setting for the subsequent injections.

Compare the retention times of the peaks from *Standard solution C* to those from the *Sample solution*. Iodixanol related compound E exhibits two peaks, the second of which may partly overlap with another peak; use only the area of the first and larger peak, which corresponds to 60% of the total area of iodixanol related compound E. Draw a baseline at the height of the baseline from the *Blank solution*. Calculate the percentage of iodixanol related compound E by dividing the area from the *Sample solution* by 0.6 and using area percent.

Iodixanol related compound H⁴ appears as a single peak, with a shoulder, on the tail of the iodixanol peak.

Calculate the percentage of iodixanol related compound H by using area percent.

Acceptance criteria: NMT 0.3% of iodixanol related compound E and NMT 0.6% of iodixanol related compound H ▲*USP39*

Add the following:**▲● Limit of Iodixanol Related Compound E and Iodixanol Impurity H****Solution A:** Acetonitrile and water (50:50)**Solution B:** Acetonitrile**Mobile phase:** See *Table 4*.**Table 4**

Time (min)	Solution A (%)	Solution B (%)
0	30	70
2	30	70
27	68	32

System suitability solution: 0.25 mg/mL of USP Iodixanol RS and 0.025 mg/mL of USP Iodixanol Related Compound E RS in water**Identification solution:** 2.5 mg/mL of USP Iodixanol RS in water**Sample solution:** 2.5 mg/mL of Iodixanol in water**Chromatographic system**(See *Chromatography* { 621 }, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L8**Flow rate:** 1.7 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *System suitability solution*[Note—See *Table 5* for relative retention times.]**Suitability requirements****Resolution:** NLT 5.0 between iodixanol related compound E (first peak) and iodixanol (first peak)**Analysis****Samples:** *Identification solution* and *Sample solution*

[Note—Iodixanol related compound E exhibits two peaks, the second of which may partly overlap with one of iodixanol peaks; use only the area of the first and larger peak of iodixanol related compound E, which corresponds to approximately 60% of the total area of iodixanol related compound E.]

Use the chromatograms obtained from the *Identification solution* and *Sample solution* for *Identification test B*.Calculate the percentage of iodixanol related compound E and iodixanol impurity H in the *Sample solution*:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

 r_U peak response of each impurity in the *Sample solution* r_T sum of all peak responses greater than 0.05% of the principal peaks in the *Sample solution* F relative response factor (see *Table 5*)

Acceptance criteria: See Table 5.

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Iodixanol related compound E (first peak)	0.7	0.58	Sum of both peaks 0.3
Iodixanol related compound E (second peak)	0.8	1.0	
Iodixanol (first peak)	1.0	—	—
Iodixanol impurity H ^a	1.4	1.0	0.6

^a 5-[[[3-[[[3-[[[3-[[3,5-bis-[[[2,3-Dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl](acetylimino)]-2-hydroxypropyl](acetylimino)]-5-[[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl]carbonyl]amino]-2-hydroxypropyl]oxy]-2-hydroxypropyl](acetylimino)]-*N,N*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzendicarboxamide.

▲USP39

SPECIFIC TESTS

Delete the following:

▲● ~~Microbial Enumeration Tests (61)~~ and ~~Tests for Specified Microorganisms (62)~~

~~Total aerobic microbial count:~~ NMT 100 cfu/g ▲USP39

Delete the following:

▲● ~~Optical Rotation, Specific Rotation (781S):~~ -0.5° to $+0.5^{\circ}$

~~Sample solution:~~ 50 mg/mL ▲USP39

● **Water Determination, Method I (921):** NMT 4.0%

ADDITIONAL REQUIREMENTS

Change to read:

● **Packaging and Storage:** Preserve in well-closed, light-resistant containers. Store at 25° ; excursions permitted between 15° and 30° .

▲room temperature. ▲USP39

● **USP Reference Standards (11)**

USP Iodixanol RS

USP Iodixanol Related Compound C RS

5-[Acetyl[3-[[3,5-bis-[(2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]-2-hydroxypropyl]amino]*N,N*-bis-(2,3-dihydroxypropyl)-2,4,6-

triiodo-1,3-benzenedicarboxamide.

$C_{33}H_{42}I_6N_6O_{14}$ 1508.15

USP Iodixanol Related Compound D RS

5-[Acetyl(2-hydroxy-3-methoxypropyl)amino]-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

$C_{20}H_{28}I_3N_3O_9$ 835.16

USP Iodixanol Related Compound E RS

(5-{*N*-[3-(*N*-{3-Carbamoyl-5-[(2,3-dihydroxypropyl)carbamoyl]-2,4,6-triiodophenyl}acetamido)-2-hydroxypropyl]acetamido}-*N1,N3*-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamide.

USP Iohexol Related Compound B RS

5-Amino-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

$C_{14}H_{18}I_3N_3O_6$ 705.02

±

~~▲ 5 (Acetylamino)-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide. ▲*USP39*~~

2

~~▲ 2 [[Acetyl[3,5-bis[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]-*N,N'*-bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4*H*-1,4-benzoxazine-6,8-dicarboxamide. ▲*USP39*~~

3

~~▲ 4 Acetyl-2 [[acetyl[3,5-bis[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]-*N,N'*-bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4*H*-1,4-benzoxazine-6,8-dicarboxamide. ▲*USP39*~~

4

~~▲ 5 [[3-[[3-[[3-[[3-[[3,5-bis[[2,3-Dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl](acetylimino)]-2-hydroxypropyl](acetylimino)]-5-[[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl]carbonyl]amino]-2-hydroxypropyl]oxy]-2-hydroxypropyl](acetylimino)]-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzendicarboxamide. ▲*USP39*~~

BRIEFING

Iohexol, *USP 38* page 3910 and *PF 39(3)* [May–June 2013]. On the basis of comments received, it is proposed to cancel the *PF 39(3)* proposal. It is proposed to publish the revision addressing the comments:

1. Replace *Identification test B* with a retention time match based on the test for *Organic Impurities*.
2. Delete *Identification test C* using thin-layer chromatography, because the test is similar to the proposed new *Identification test B* and therefore is redundant.
3. Delete *Identification test D* because it generates iodine vapors that can irritate the nose and eyes, and therefore is a potential safety hazard.
4. Revise the *Assay* to include a formula for the calculation of the content of iohexol.
5. Rename the *Ionic Compounds* test as the test for *Limit of Ionic Compounds*. Revise the test by deleting the formula and making the *Acceptance criteria* consistent with *European Pharmacopoeia 8.4*.

6. Delete the test for *Free Iodine* because the manufacturing history indicates that no free iodine has been observed.
7. Rename the test for *Free Iodide* as *Limit of Free Iodide*. Revise the test to include a formula for the calculation of iodide.
8. Revise the *System suitability* criteria in the test for *Organic Impurities* to be consistent with *European Pharmacopoeia 8.4*. Also, include a clarifying note to indicate the presence of endo- and exo-isomers of iohexol. The procedure is based on analyses performed with the Spheri-5 RP-18 (Brownlee) brand of L1 column manufactured by PerkinElmer Corporation. In this procedure the retention times for endo- and exo-isomers are about 18 and 19 min, respectively.
9. Replace the test for *Limit of Methanol, Isopropyl Alcohol, and 2-Methoxyethanol* with a modified gas chromatographic procedure for quantifying 2-methoxyethanol. The procedure is based on analyses performed with the DB-Wax brand of G16 column manufactured by JW Scientific (Agilent Technologies) in which the retention time of 2-methoxyethanol is about 8 min.
10. Rename the test for *Limit of 3-Chloro-1,2-propanediol* as *Limit of 3-Chloropropane-1,2-diol*. Revise the test to be consistent with *European Pharmacopoeia 8.4* except the temperature gradient. The change in the gradient is necessary to avoid carry over. The procedure is based on analyses performed with the JW Scientific (Agilent Technologies) DB 1701 brand of G46 column. The retention time of 3-chloro-1,2-propanediol is about 8 min.
11. Rename the test for *Free Aromatic Amine* as *Limit of Free Aromatic Amine*. Revise the test to be consistent with *European Pharmacopoeia 8.4*.
12. It is proposed to delete the test for *Optical Rotation* to be consistent with *European Pharmacopoeia 8.4*.
13. On the basis of stability data, revise the existing *Packaging and Storage* conditions to specify storage at room temperature.

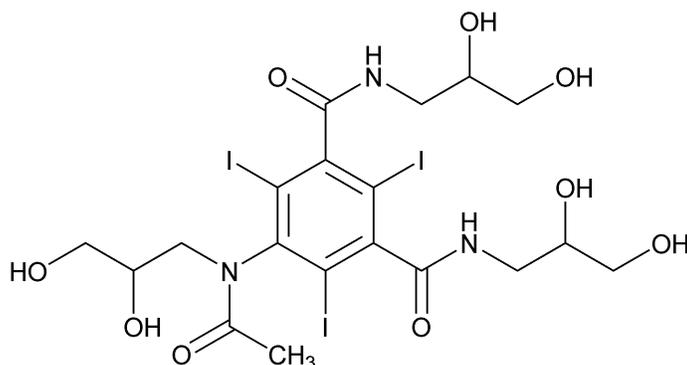
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R. Ravichandran.)

Correspondence Number—C103376

Comment deadline: March 31, 2015

Iohexol



$C_{19}H_{26}I_3N_3O_9$ 821.14

1,3-Benzenedicarboxamide, 5-[acetyl(2,3-dihydroxypropyl)amino]-*N,N*-bis(2,3-

dihydroxypropyl)-2,4,6-triiodo;
N,N'-Bis(2,3-dihydroxypropyl)-5-[*N*-(2,3-dihydroxypropyl)acetamido]-2,4,6-triiodoisophthalamide [66108-95-0].

DEFINITION

Iohexol contains NLT 98.0% and NMT 102.0% of iohexol (C₁₉H₂₆I₃N₃O₉), calculated on the anhydrous basis.

IDENTIFICATION

• A. Infrared Absorption < 197K >

Change to read:

- **B.** ~~The UV absorption spectrum of a 1 in 100,000 solution in water exhibits a maximum and a minimum at the same wavelengths as that of a similar solution of USP Iohexol RS, concomitantly measured.~~

▲ The retention times of the two principal peaks of the *Sample solution* correspond to those of the *System suitability solution*, as obtained in the test for *Organic Impurities*. ▲*USP39*

Delete the following:

▲• **C. Thin-Layer Chromatographic Identification Test** < 201 >

Standard solution: 10 mg/mL in methanol

Sample solution: 10 mg/mL in methanol

Developing solvent system: *n*-butyl alcohol, water, and glacial acetic acid (50:25:11)

Analysis

Samples: ~~Standard solution and Sample solution~~

~~Develop the chromatogram, and locate the spots by short-wavelength UV light.~~

Acceptance criteria: ~~The presence of *exo* and *endo* isomers in the *Sample solution* is shown by the appearance of two spots, each of which corresponds in size and intensity to the corresponding principal spot, at the same R_F value from the *Standard solution*. The spot having the R_F value is the *endo* isomer. ▲*USP39*~~

Delete the following:

▲• **D. Procedure**

Analysis: ~~Heat 500 mg in a crucible.~~

Acceptance criteria: ~~Violet vapors are evolved. ▲*USP39*~~

ASSAY

Change to read:

• Procedure

Analysis: ~~Transfer 500 mg of Iohexol, to a glass stoppered, 125 mL conical flask. Add 25 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a~~

reflux condenser, and reflux the *Sample solution* for 1 h. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly with small portions of water, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid, and titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 27.37 mg of $C_{19}H_{26}I_3N_3O_9$.

▲**Sample:** 500 mg of Iohexol

Sample solution: Transfer the *Sample* to a glass-stoppered, 125-mL conical flask. Add 25 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc. Connect the flask to a reflux condenser, and reflux for 1 h. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and pass the mixture through a filter. Rinse the flask and the filter thoroughly with small portions of water, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* 〈 541 〉.)

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* with 0.1 N silver nitrate VS.

Calculate the percentage of iohexol ($C_{19}H_{26}I_3N_3O_9$) in the portion of Iohexol taken:

$$\text{Result} = [(V \times N \times F)/W] \times 100$$

V

= *Titrant* volume consumed by the *Sample* (mL)

N

= *Titrant* normality (mEq/mL)

F

= equivalent weight of iohexol, 273.7 mg/mEq

W

= *Sample* weight (mg)

▲*USP39*

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Delete the following:

•

•**Heavy Metals, Method I** 〈 231 〉

: NMT 20 ppm •(Official 1-Dec-2015)

Change to read:

•

▲Limit of▲USP39

Ionic Compounds

Rinse all glassware five times with distilled water.

Standard solution: 0.002 mg/mL of sodium chloride in water**Sample solution:** 1 g of Iohexol in 50 mL of water**Analysis****Samples:** *Standard solution* and *Sample solution*

Measure the ~~specific resistance, (R_{sp}), at 20 ° of a solution in water (1 in 50), using a suitable water purity meter. Calculate the ~~specific conductance, K , taken:~~~~

$$\text{Result} = (1/R_{sp}) \cdot 10^6$$

▲▲USP39

Acceptance criteria: The specific conductance of the *Sample solution* is NMT that of the *Standard solution* (equivalent to 0.01% ionic compounds as sodium chloride).

Delete the following:**▲● Free Iodine**

Sample solution: Transfer 2.1 g to a 50 mL centrifuge tube provided with a stopper, add 20 mL of water, and shake vigorously to dissolve. [Note—The solution may be heated gently to assist in dissolving the sample. Cool to room temperature before proceeding.]

Analysis: Add 5.0 mL of toluene and 5 mL of 2 N sulfuric acid, shake, and centrifuge at high speed for 15 min.

Acceptance criteria: The toluene layer shows no red or pink color.▲USP39

Change to read:

•

▲Limit of▲USP39

Free Iodide

Analysis: Transfer 5.0 g to a suitable container, add about 20 mL of water to dissolve, and titrate with 0.001 N silver nitrate VS using a silver electrode in combination with an appropriate reference electrode, determining the endpoint potentiometrically. Each mL of 0.001 N silver nitrate is equivalent to 126.9 µg of I (0.001%).

▲Sample: 5 g of Iohexol**Sample solution:** Dissolve the *Sample* in 20 mL of water.**Titrimetric system**(See *Titrimetry* (541).)**Mode:** Direct titration**Titrant:** 0.001 N silver nitrate VS**Endpoint detection:** Potentiometric**Analysis**Calculate the percentage of free iodide in the portion of the *Sample* taken:

$$\text{Result} = [(V \times N \times F)/W] \times 100$$

V

= *Titrant* volume consumed by the *Sample* (mL)

N

= *Titrant* normality (mEq/mL)

F

= equivalent weight of iodide, 0.1269 mg/mEq

W

= *Sample* weight (mg)

Acceptance criteria: NMT 0.001%  USP39

Change to read:

• **Organic Impurities**

Solution A: Acetonitrile

Solution B: Water

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	1	99
60	13	87

System suitability solution: 1.5 mg/mL of USP Iohexol RS and 0.0075 mg/mL of USP Iohexol Related Compound A RS and 0.0069 mg/mL of USP Iohexol Related Compound C RS  USP39

in water

Sample solution: 1.5 mg/mL of Iohexol

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: *System suitability solution*

~~[Note—The retention time for the *O*-alkylated compounds is between 1.1 and 1.4 relative to the *exo*-isomer of iohexol. The peak area of iohexol related compound C is 0.5% ± 0.1% by comparison to the total area of all the peaks in the chromatogram.]~~

 [

Note—Iohexol may give two nonresolved peaks due to *exo*-*endo* isomerism. In addition, a small peak due to iohexol usually appears at the leading edge of the first principal peak. This small peak has a retention time about 1.2 min less than the first principal peak.

The relative retention times for the iohexol related compound A, iohexol *endo*-isomer, iohexol *exo*-isomer, and *O*-alkylated compounds peaks are 0.85, 0.96, 1.0, and 1.1–1.4,

respectively.

]▲*USP39*

Suitability requirements

Resolution: ~~NLT 20.0 between iohexol related compound A and iohexol related compound C~~

▲NLT 5.0 between iohexol related compound A and the exo-isomer (the second and greater peak) of iohexol▲*USP39*

Analysis

Sample: *Sample solution*

Calculate the percentage of *O*-alkylated compounds and any other individual impurity in the portion of Iohexol taken. Exclude peaks with a retention time between 0.84 (relative to the *endo*-isomer of iohexol [first main peak]) and the *endo*-isomer of iohexol

▲Disregard any peak less than or equal to 0.03% of the principal peaks.▲*USP39*

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity

r_T = sum of all of the peak responses

Acceptance criteria

***O*-alkylated compounds:** NMT 0.6%

Any individual impurity: NMT 0.1%

Total impurities excluding *O*-alkylated compounds: NMT 0.3%

Delete the following:

▲● ~~Limit of methanol, isopropyl alcohol, and methoxyethanol~~

~~**Internal standard solution:** 0.05 mg/mL of butyl alcohol~~

~~**Standard stock solution:** Transfer 0.6 g of methanol to a 1000 mL volumetric flask, and add 100 mL of water. Add 0.6 g of isopropyl alcohol, and 100 mL of water. Add 0.6 g of methoxyethanol, and 100 mL of water. Dilute with water to volume.~~

~~**Standard solution A:** Transfer 10.0 mL of *Standard stock solution* to a 100 mL volumetric flask, and dilute with water to volume.~~

~~**Standard solution B:** Transfer 5.0 mL of *Standard stock solution* to a 100 mL volumetric flask, and dilute with water to volume.~~

~~**Standard solution C:** Transfer 10.0 mL of *Standard stock solution* to a 100 mL volumetric flask, and dilute with water to volume.~~

~~**Standard solution D:** Transfer 10.0 mL of *Standard solution C* and 10.0 mL of *Internal standard solution* to a 50 mL volumetric flask, dilute with water to volume, and mix. Transfer 6.0 mL of the solution so obtained to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 95 ° for 15 min.~~

~~**Sample stock solution:** Transfer about 6.25 g of Iohexol to a 25 mL volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with water to volume.~~

~~**Sample solution A:** Transfer 5.0 mL of *Sample stock solution* and 1.0 mL of water to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 95 ° for 15 min.~~

~~**Sample solution B:** Transfer 5.0 mL of *Sample stock solution* and 1.0 mL of *Standard*~~

solution A to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 95 ° for 15 min.

Sample solution C: Transfer 5.0 mL of *Sample stock solution* and 1.0 mL of *Standard solution B* to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 95 ° for 15 min.

Sample solution D: Transfer 5.0 mL of *Sample stock solution* and 1.0 mL of *Standard solution C* to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 95 ° for 15 min.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 30-m fused-silica column coated with a 3-µm phase G43

Carrier gas: Helium

Temperature

Injector: 140 °

Detector: 250 °

Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	5
40	10	100	±

Flow rate: 14 mL/min

Injection volume: 2 mL of the head space

System suitability

Sample: *Standard solution D*

[Note—The relative retention times for methanol, isopropyl alcohol, secondary butyl alcohol, and methoxyethanol are 0.3, 0.5, 1.0, and 1.3, respectively.]

Suitability requirements

Resolution: NLT 2.5 between methanol and isopropyl alcohol

Relative standard deviation: NMT 5.0% determined from individual peak responses

Analysis

Samples: *Sample solutions A, B, C, and D*

Calculate the peak area ratio for each analyte to the internal standard. Plot the peak area ratios from the *Sample solutions* against the quantity of each individual analyte standard added in mg/g of Iohexol. Extrapolate the line joining the points until it intercepts the concentration axis. The distance between this point and the intersection of the axis is the concentration C_i , in mg/g, of methanol, isopropyl alcohol, or methoxyethanol in the portion of Iohexol taken.

Acceptance criteria: NMT 0.005% each of methanol and isopropyl alcohol and NMT 0.002% of methoxyethanol ▲*USP39*

Add the following:**▲● Limit of 2-Methoxyethanol****Internal standard solution:** 0.01 mg/mL of secondary butyl alcohol in water**Standard stock solution:** 0.005 mg/mL of methanol and 0.01 mg/mL each of isopropyl alcohol, secondary butyl alcohol, and 2-methoxyethanol in *Internal standard solution***Standard solution:** Transfer about 0.25 g of USP Iohexol RS and 1.0 mL of *Standard stock solution* to a headspace vial, and seal the vial with a septum and crimp cap.**Sample solution:** Transfer about 0.25 g of Iohexol and 1.0 mL of *Internal standard solution* to a headspace vial, and seal the vial with a septum and crimp cap.**Chromatographic system**(See *Chromatography* { 621 }, *System Suitability*.)**Mode:** GC with suitable headspace autosampler**Detector:** Flame ionization**Column:** 0.53-mm × 30-m fused-silica coated with a 1-μm phase G16**Temperatures****Autosampler:** 105°**Needle:** 130°–140°**Injection port:** 150°**Detector:** 200°**Column:** See *Table 2*.**Table 2**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	3
40	8	100	1

Carrier gas: Helium**Flow rate:** 11 mL/min**Injection volume:** 1 mL of the headspace**System suitability****Sample:** *Standard solution*

[Note—The typical relative retention times for methanol, isopropyl alcohol, secondary butyl alcohol, and 2-methoxyethanol are 0.5, 0.6, 1.0, and 1.9, respectively.]

Suitability requirements**Resolution:** NLT 1.0 between methanol and isopropyl alcohol**Relative standard deviation:** NMT 10.0% for the ratio of 2-methoxyethanol to the internal standard**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the amount of 2-methoxyethanol in the portion of Iohexol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

$R_{\overline{F}}$ peak response ratio of 2-methoxyethanol to the internal standard from the *Sample solution*

$R_{\overline{S}}$ peak response ratio of 2-methoxyethanol to the internal standard from the *Standard solution*

$C_{\overline{S}}$ concentration of 2-methoxyethanol in the *Standard solution* ($\mu\text{g/mL}$)

$C_{\overline{F}}$ concentration of Iohexol in the *Sample solution* (g/mL)

Acceptance criteria: NMT 20 $\mu\text{g/g}$ of 2-methoxyethanol▲*USP39*

Delete the following:

▲● Limit of 3-Chloro-1,2-propanediol

System suitability stock solution: 25 $\mu\text{g/mL}$ of 3-Chloropropane-1,2-diol in ethylacetate.

System suitability solution: Transfer 1 g of iohexol containing less than 5 μg to a separator. Dissolve in 1 mL of water. Add 2.0 mL of *System suitability stock solution* to the separator. Transfer the ethyl acetate layer to a separate container, and extract the aqueous layer three additional times with 2 mL of ethyl acetate, combining all four extracts. Dry the combined extracts using anhydrous sodium sulfate. Filter, and wash the filter with a small amount of ethyl acetate. Combine the wash with the filtrate, and concentrate to a volume of 2.0 mL, using a warm water bath and a stream of nitrogen. Pass this solution through a membrane filter, and use the clear filtrate.

Standard solution: 20 $\mu\text{g/mL}$ of 3-chloropropane-1,2-diol in ethyl acetate

Sample solution: Transfer 1 g of Iohexol to a separator. Dissolve in 1 mL of water. Extract 4 times with 2 mL of ethyl acetate, and combine the extracts. Dry the combined extracts with anhydrous sodium sulfate. Filter, and wash the filter with a small amount of ethyl acetate. Combine the wash with the filtrate, and concentrate to a volume of 2.0 mL, using a warm water bath and a stream of nitrogen. Pass this solution through a membrane filter, and use the clear filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32 mm \times 30 m fused-silica capillary column bonded with a 1 μm layer of phase G46

Temperature

Injector: 230 $^{\circ}$

Detector: 250 $^{\circ}$

Column: See *Table 3*.

Table 3

Initial Temperature ($^{\circ}$)	Temperature Ramp ($^{\circ}/\text{min}$)	Final Temperature ($^{\circ}$)	Hold Time at Final Temperature (min)
50	—	50	2
50	10	200	—

Carrier gas: Helium under 760 mm Hg pressure

Injection volume: 2 μ L

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—Retention times of two chloropropanediol isomers, are 12 and 12.5 min.]

Suitability requirements

Relative standard deviation: NMT 10.0% for the sum of the two peaks of 3-chloropropane 1,2-diol, *Standard solution*

Recovery

Calculate the percentage of, of 3-chloropropane 1,2-diol *R*

$$\text{Result} = (r_{SS}/r_S) \times (C_S/C_{SS}) \times 100$$

r_{SS} = sum of the two peaks of 3-chloro-1,2-propanediol peak responses from the *System suitability solution*

r_S = sum of the two peaks of 3-chloropropane 1,2-diol peak response from the *Standard solution*

C_S = concentration of 3-chloropropane 1,2-diol in the *Standard solution* (μ g/mL)

C_{SS} = concentration of 3-chloropropane 1,2-diol in the *System suitability solution* (μ g/mL)

60–90% of 3-chloropropane 1,2-diol is recovered

Analysis

Sample: *Sample solution*

Calculate the percentage of 3-chloropropane 1,2-diol in the portion of Iohexol taken:

$$\text{Result} = (r_U/r_S) \times (2 C_S/R) \times 100$$

r_U = total of the peak responses of the two isomers from the *Sample solution*

r_S = total of the peak responses of the two isomers from the *Standard solution*

C_S = concentration of 3-chloropropane 1,2-diol in the *Standard solution* (μ g/mL)

R = percentage recovery determined under *Chromatographic system*

Acceptance criteria: NMT 0.0025% ▲*USP39*

Add the following:

▲● Limit of 3-Chloropropane-1,2-diol

Standard solution: 0.025 mg/mL of 3-chloropropane-1,2-diol in ethyl acetate

Sample solution: Transfer 1 g of Iohexol to a separator. Dissolve in 1 mL of water. Extract 4 times with 2 mL of ethyl acetate, and combine the extracts. Dry the combined extracts with anhydrous sodium sulfate. Filter, and wash the filter with a small amount of ethyl acetate. Combine the wash with the filtrate, and concentrate to a volume of 0.7 mL, using a warm water bath and a stream of nitrogen. Dilute with ethyl acetate to 2 mL.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm \times 30-m fused-silica capillary bonded with a 1- μ m layer of phase G46

Temperatures

Injection port: 230°

Detector: 250°

Column: See Table 3.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
80	—	80	2
80	15	275	—
275	—	275	2

Carrier gas: Helium at 1 mL/min

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

[Note—The retention time of the 3-chloropropane-1,2-diol peak is about 8 min.]

Suitability requirements

Relative standard deviation: NMT 10.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The area of the principal peak from the *Sample solution* is NMT the area of the principal peak from the *Standard solution* (NMT 0.0025%). ▲*USP39*

Change to read:

• **Limit of Free Aromatic Amine**

Solution A: ~~1 mg/mL~~

▲3 mg/mL▲*USP39*

of *N*-(1-naphthyl)ethylenediamine dihydrochloride in a mixture of propylene glycol and water (70:30)

Standard stock solution: 10 µg/mL of USP Iohexol Related Compound B RS in water

Standard solution: Transfer 5 mL of water and 10.0 mL of the *Standard stock solution* to a ~~50-~~
mL

▲25-mL▲*USP39*

volumetric flask.

Sample solution: Transfer 200 mg

▲of Iohexol▲*USP39*

to a ~~50-~~mL

▲25-mL▲*USP39*

volumetric flask, add 15 mL of water, and mix to dissolve.

Blank: Add 15 mL of water to a ~~50-~~mL

▲25-mL▲*USP39*

volumetric flask.

Instrumental conditions

Mode: Vis

Analytical wavelength: 495 nm

Cell: 5 cm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

~~In conducting the following steps, keep the flasks in the ice bath as much as possible until all of the reagents have been added. Treat each flask as follows. Add 3.0 mL of 5 N hydrochloric acid, and swirl to mix. Add 2.0 mL of sodium nitrite solution (1 in 50), mix, and allow to stand for 4 min. Add 2.0 mL of sulfamic acid solution (1 in 25), shake, and allow to stand for 1 min.~~

~~[**Caution**—Considerable pressure is produced.]~~

~~Remove the flasks from the ice bath. To each flask add 2.0 mL of a freshly prepared 1 in 1000 solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride in dilute propylene glycol (7 in 10), and mix. Dilute with water to volume, mix, and allow to stand for 5 min.~~

▲ In conducting the following steps, keep the flasks in iced water and protected as much as possible from light until all of the reagents have been added.

Treat the *Samples* as follows. Place the flask in an ice bath for 5 min. Add 1.5 mL of 6 N hydrochloric acid, and mix by swirling. Add 1.0 mL of sodium nitrite solution (20 mg/mL), and allow to stand in the ice bath for 4 min. Remove the flask from the ice bath, add 1.0 mL of sulfamic acid solution (40 mg/mL), and swirl gently until gas evolution ceases. [**Caution**—Considerable pressure is produced.] Add 1.0 mL of *Solution A*, dilute with water to volume, and allow to stand for 5 min. Measure the absorbance of the *Standard solution* and *Sample solution* against the *Blank solution*.

▲ USP39

Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (NMT 0.05% of free aromatic amine).

SPECIFIC TESTS

• Color of Solution

Sample solution: 647.2 mg/mL

Blank: Water

Instrumental conditions

Mode: UV-Vis

Analytical wavelengths: 400, 420, and 450 nm

Cell: 1 cm

Analysis

Samples: *Sample solution* and *Blank*

Pass the *Sample solution* through a filter of 0.22- μ m pore size.

Determine the absorbances of the *Sample solution* against the *Blank*.

Acceptance criteria: See *Table 4*.

Table 4

Wavelength (nm)	NMT (au)
400	0.180

420	0.030
450	0.015

Delete the following:

- ▲● **Optical Rotation**, ~~Specific Rotation (781S): -0.5° to $+0.5^{\circ}$~~
Sample solution: ~~50 mg/mL~~▲USP39

- **Water Determination**, *Method I* (921): NMT 4.0%

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers. Store at 25° ,
excursions permitted between 15° – 30°
▲room temperature.▲USP39

Change to read:

- **USP Reference Standards** (11)

USP Iohexol RS

USP Iohexol Related Compound A RS

5-(Acetylamino)-*N,N*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

$C_{16}H_{20}I_3N_3O_7$ 747.06

USP Iohexol Related Compound B RS

5-Amino-*N,N*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

$C_{14}H_{18}I_3N_3O_6$ 705.02

~~USP Iohexol Related Compound C RS~~

~~*N,N*-Bis(2,3-dihydroxypropyl)-5-nitro-1,3-benzenedicarboxamide.~~

~~$C_{14}H_{19}N_3O_8$ 357.32~~

▲▲USP39

BRIEFING

Ketorolac Tromethamine Tablets, USP 38 page 4012. As part of the USP monograph modernization initiative, it is proposed to make the following changes:

1. Add *Identification* test *B* based on the UV spectra as obtained in the *Assay*. Revise the *Detector* in the *Assay* accordingly.
2. Revise *System suitability stock solution* and *System suitability solution* in the *Assay* for clarity.
3. Delete the *Column efficiency* requirement in the *Assay* because there are already three requirements that will ensure the suitability of the *Chromatographic system*.
4. Delete the *Procedure for content uniformity* in the test for *Uniformity of Dosage Units* to allow flexibility.
5. Revise the system suitability requirements in the test for *Organic Impurities* to

ensure the appropriate suitability of the *Chromatographic system*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: H. Cai.)

Correspondence Number—C148172

Comment deadline: March 31, 2015

Ketorolac Tromethamine Tablets

DEFINITION

Ketorolac Tromethamine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$).

IDENTIFICATION

Change to read:

-

▲A. ▲*USP39*

The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

- ▲● **B.** The UV absorption spectra of the ketorolac peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*. ▲*USP39*

ASSAY

Change to read:

- **Procedure**

Mobile phase: Methanol, water, and glacial acetic acid (55:44:1)

Diluent: Methanol and water (1:1). [Note—Protect all volumetric solutions from light.]

Standard stock solution: 0.24 mg/mL of USP Ketorolac Tromethamine RS in methanol

Standard solution: 24 µg/mL of USP Ketorolac Tromethamine RS in *Diluent* from *Standard stock solution*

System suitability stock solution: 25 µg/mL each of ~~USP Ketorolac Tromethamine RS,~~
▲▲*USP39*

USP Ketorolac Related Compound A RS, USP Ketorolac Related Compound B RS, USP Ketorolac Related Compound C RS, and USP Ketorolac Related Compound D RS in methanol

System suitability solution: 0.25 µg/mL each of ~~USP Ketorolac Tromethamine RS,~~

▲▲*USP39*

USP Ketorolac Related Compound A RS, USP Ketorolac Related Compound B RS, USP Ketorolac Related Compound C RS, and USP Ketorolac Related Compound D RS in *Standard solution* from *System suitability stock solution*

Sample stock solution: 0.2 mg/mL of ketorolac tromethamine prepared as follows. Transfer 10

Tablets to a suitable volumetric flask. Add a quantity of water equivalent to about 10% of the volume of the flask, and sonicate until the Tablets are disintegrated. Add a quantity of methanol equivalent to 40% of the volume of the flask, and sonicate for 10 min to dissolve the ketorolac tromethamine. Cool to ambient temperature, dilute with methanol to volume, and mix. Centrifuge, or allow to settle.

Sample solution: 0.02 mg/mL of ketorolac tromethamine in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

~~**Detector:** UV 254 nm~~

▲Detectors

Assay: UV 254 nm

Identification test B: Diode array, UV 200–400 nm

▲USP39

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1.2 mL/min

Injection volume: 100 μL

Run time: 3.8 times the retention time of the ketorolac peak

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times are 0.8 for ketorolac related compound B and 1.0 for the ketorolac peaks.]

Suitability requirements

Resolution: NLT 1.5 each between the ketorolac and ketorolac related compound B, and ketorolac and ketorolac related compound C peaks, *System suitability solution*

~~**Column efficiency:** NLT 2700 theoretical plates, *Standard solution*~~

▲▲USP39

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U response of the ketorolac peak from the *Sample solution*

r_S response of the ketorolac peak from the *Standard solution*

C_S concentration of USP Ketorolac Tromethamine RS in the *Standard solution* (mg/mL)

C_U nominal concentration of ketorolac tromethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110%

PERFORMANCE TESTS

- **Dissolution** { 711 }

Medium: Water; 600 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: USP Ketorolac Tromethamine RS in *Medium*

Sample solutions: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

Instrumental conditions

Mode: UV absorption spectroscopy

Analytical wavelength: 322 nm

Tolerances: NLT 75% (Q) of the labeled amount of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$) is dissolved.

Change to read:

• **Uniformity of Dosage Units** 〈 905 〉:

Procedure for content uniformity

Blank: Methanol

Standard solution: 12 µg/mL of USP Ketorolac Tromethamine RS in methanol

Sample solution: Transfer 1 Tablet to a suitable volumetric flask that will provide a final concentration of about 0.1 mg/mL of ketorolac tromethamine. Add a quantity of water equivalent to about 10% of the volume of the flask, and sonicate until the Tablet is disintegrated. Add a quantity of methanol equivalent to 40% of the volume of the flask, and sonicate for about 10 min to dissolve the ketorolac tromethamine. Cool to ambient temperature, dilute with methanol to volume, and mix. Centrifuge or allow to settle. Transfer 6.0 mL of the clear supernatant to a 50 mL volumetric flask, and dilute with methanol to volume.

Spectrometric conditions

Mode: UV absorption spectroscopy

Analytical wavelength: UV 322 nm

Calculate the percentage of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_S absorbance of the *Sample solution*

A_U absorbance of the *Standard solution*

C_S concentration of USP Ketorolac Tromethamine RS in the *Standard solution* (µg/mL)

C_U concentration of ketorolac tromethamine in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

▲Meet the requirements▲*USP39*

IMPURITIES

Change to read:

• **Organic Impurities**

Mobile phase, System suitability solution,

▲Chromatographic system, ▲USP39

and Diluent: Proceed as directed in the Assay.

Standard solution: Use the *System suitability solution*, prepared as directed in the Assay.

Sample solution: Proceed as directed for the *Sample solution* in the Assay.

Chromatographic system and System suitability: Proceed as directed in the Assay

▲System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 each between the ketorolac and ketorolac related compound B, and ketorolac and ketorolac related compound C peaks

Relative standard deviation: NMT 5.0% for ketorolac related compound A, ketorolac related compound B, ketorolac related compound C, and ketorolac related compound D

▲USP39**Analysis**

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each known impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each known impurity in the *Sample solution*

r_S peak response of each known impurity in the *Standard solution*

C_S concentration of each impurity in the *Standard solution* (mg/mL)

C_U nominal concentration of ketorolac tromethamine in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U response of each individual impurity peak in the *Sample solution*

r_T sum of responses for all the peaks in the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ketorolac related compound A	0.5	0.5
Ketorolac related compound B	0.8	0.5
Ketorolac	1.0	—
Ketorolac related compound C	1.2	0.5
Ketorolac related compound D	2.6	0.5
Total unspecified impurity	—	0.5
Total impurities	—	1.0

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers at controlled room temperature, protected from light and excessive humidity.

- **USP Reference Standards** (11)

USP Ketorolac Tromethamine RS

USP Ketorolac Related Compound A RS

5-Benzoyl-*N*-[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]-2,3-dihydro-1*H*-pyrrolizine-1-carboxamide.

$C_{19}H_{22}N_2O_5$ 358.39

USP Ketorolac Related Compound B RS

5-Benzoyl-2,3-dihydro-1*H*-pyrrolizin-1-ol.

$C_{14}H_{13}NO_2$ 227.26

USP Ketorolac Related Compound C RS

5-Benzoyl-2,3-dihydro-1*H*-pyrrolizin-1-one.

$C_{14}H_{11}NO_2$ 225.24

USP Ketorolac Related Compound D RS

5-Benzoyl-2,3-dihydro-1*H*-pyrrolizine.

$C_{14}H_{13}NO$ 211.26

BRIEFING

Memantine Hydrochloride, *USP 38* page 4238. On the basis of comments received, it is proposed to widen the *Relative standard deviation* requirement in the test for *Organic Impurities* from NMT 5.0% to NMT 10.0%.

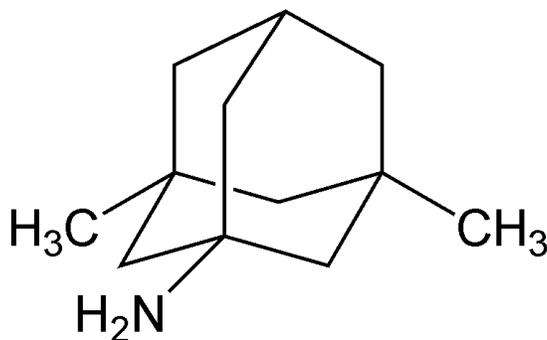
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R. Ravichandran.)

Correspondence Number—C150122

Comment deadline: March 31, 2015

Memantine Hydrochloride



• HCl

$C_{12}H_{21}N \cdot HCl$ 215.76

Tricyclo[3.3.1.1^{3,7}]decan-1-amine, 3,5-dimethyl-, hydrochloride;
1-Amino-3,5-dimethyladamantane hydrochloride [41100-52-1].

DEFINITION

Memantine Hydrochloride contains NLT 98.0% and NMT 102.0% of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈 197K 〉
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General, Chloride** 〈 191 〉: Meets the requirements

ASSAY• **Procedure**

Internal standard solution: 4.0 mg/mL of adamantane in *n*-hexane

Standard solution: 4.0 mg/mL of USP Memantine Hydrochloride RS in *Internal standard solution* prepared as follows. Transfer 100 mg of USP Memantine Hydrochloride RS to a 50-mL centrifuge tube. Add 15 mL of 1 N sodium hydroxide, and mix. Add 25 mL of *Internal standard solution*, and shake for 15 min. Allow the layers to separate, and filter a portion of the top hexane layer through anhydrous sodium sulfate. Use the clear filtrate.

Sample solution: 4.0 mg/mL of Memantine Hydrochloride in *Internal standard solution* prepared as follows. Transfer 100 mg of Memantine Hydrochloride to a 50-mL centrifuge tube. Add 15 mL of 1 N sodium hydroxide, and mix. Add 25 mL of *Internal standard solution*, and shake for 15 min. Allow the layers to separate, and filter a portion of the top hexane layer through anhydrous sodium sulfate. Use the clear filtrate.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 50-m × 0.32-mm; 0.52- μ m packing G27

Temperatures

Injection port: 220 $^{\circ}$

Detector: 300 $^{\circ}$

Column: See *Table 1*.

Table 1

Initial Temperature ($^{\circ}$)	Temperature Ramp ($^{\circ}$ /min)	Final Temperature ($^{\circ}$)	Hold Time at Final Temperature (min)
50	5	145	0
145	10	250	20

Carrier gas: Helium

Flow rate: 4.0 \pm 0.4 mL/min

Injection volume: 1 μ L

Injection type: Split ratio, 1:50

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 each for memantine and adamantane

Relative standard deviation: NMT 2.0% each for memantine and adamantane

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$) in the portion of Memantine Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U peak response ratio of memantine to the internal standard from the *Sample solution*

R_S peak response ratio of memantine to the internal standard from the *Standard solution*

C_S concentration of USP Memantine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U concentration of Memantine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Delete the following:

-

- **Heavy Metals, Method II** (231)

: NMT 10 ppm (Official 1-Dec-2015)

- **Residue on Ignition** (281) : NMT 0.1%

Change to read:

- **Organic Impurities**

Standard stock solution A: 2.5 mg/mL each of USP Memantine Related Compound A RS, USP Memantine Related Compound B RS, USP Memantine Related Compound C RS, USP Memantine Related Compound D RS, and USP Memantine Related Compound E RS in *n*-hexane

Standard stock solution B: 2.5 mg/mL of USP Memantine Hydrochloride RS prepared as follows. To the flask containing a weighed amount of the USP Memantine Hydrochloride RS, add 5.0 N sodium hydroxide to fill 20% of the final volume and *n*-hexane to fill 20% of the final volume. Shake for 10 min, and transfer the contents to a separator. Allow the layers to separate, filter a portion of the top hexane layer, dry the organic layer by swirling with anhydrous sodium sulfate, and allow to stand for a few min to ensure all the remaining water has been removed. Use the clear filtrate.

System suitability solution: 25 µg/mL each of USP Memantine Related Compound A RS, USP Memantine Related Compound B RS, USP Memantine Related Compound C RS, USP Memantine Related Compound D RS, and USP Memantine Related Compound E RS, from *Standard stock solution A* in *Standard stock solution B*. The concentration of USP Memantine Hydrochloride RS is 2.5 mg/mL. [~~Note—Memantine related compounds D and E are used for identification purposes only.~~]

▲▲USP39

Standard solution: 25 µg/mL each of USP Memantine Related Compound A RS, USP Memantine Related Compound B RS, USP Memantine Related Compound C RS, USP Memantine Related Compound D RS, USP Memantine Related Compound E RS, and USP Memantine Hydrochloride RS, from *Standard stock solution A* and *Standard stock solution B*, respectively, in *n*-hexane

Sample solution: 25 mg/mL of Memantine Hydrochloride prepared as follows. Transfer the weighed amount of Memantine Hydrochloride to a suitable volumetric flask. Add 5.0 N sodium

hydroxide to fill 30% of the final volume and *n*-hexane to fill 40% of the final volume. Shake for 10 min, and transfer the contents to a separator. Allow the layers to separate, filter a portion of the top hexane layer, dry the organic layer by swirling with anhydrous sodium sulfate, and allow to stand for a few min to ensure all the remaining water has been removed. Use the clear filtrate.

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 6.0 between memantine and memantine related compound B; NLT 2.0 between memantine related compound B and memantine related compound C, *System suitability solution*

Tailing factor: NMT 2.0 for memantine, *Standard solution*

Relative standard deviation: NMT 5.0%

▲NMT 10.0%▲USP39

for memantine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—Ignore the peaks at the relative retention times 0.11, 0.12, 0.13, 0.18, and 0.26 with respect to the memantine peak, as they correspond to residual solvents.]

Calculate the percentage of each of memantine related compounds A, B, C, D, and E in the portion of Memantine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of memantine related compounds A, B, C, D, or E from the *Sample solution*

r_S peak response of the corresponding USP Memantine Related Compound RS from the *Standard solution*

C_S concentration of the corresponding USP Memantine Related Compound RS in the *Standard solution* (mg/mL)

C_U concentration of Memantine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Memantine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any other impurity from the *Sample solution*

r_S peak response of memantine hydrochloride from the *Standard solution*

C_S concentration of USP Memantine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U concentration of Memantine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Memantine related compound A	0.77	0.15
Memantine	1.0	—
Memantine related compound B	1.03	0.15
Memantine related compound C	1.07	0.15
Memantine related compound D	1.19	0.15
Memantine related compound E	1.44	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	0.50

SPECIFIC TESTS

- **Water Determination, Method I (921)**: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage**: Preserve in well-closed containers. Store at controlled room temperature.

- **USP Reference Standards (11)**

USP Memantine Hydrochloride RS

USP Memantine Related Compound A RS
1,3-Dimethyladamantane.

$C_{12}H_{20}$ 164.29

USP Memantine Related Compound B RS
3,5-Dimethyladamantane-1-ol.

$C_{12}H_{20}O$ 180.29

USP Memantine Related Compound C RS
1-Chloro-3,5-dimethyladamantane.

$C_{12}H_{19}Cl$ 198.73

USP Memantine Related Compound D RS
1-Bromo-3,5-dimethyladamantane.

$C_{12}H_{19}Br$ 243.18

USP Memantine Related Compound E RS
N-3,5-Dimethyladamantan-1-yl formamide.

$C_{13}H_{21}NO$ 207.31

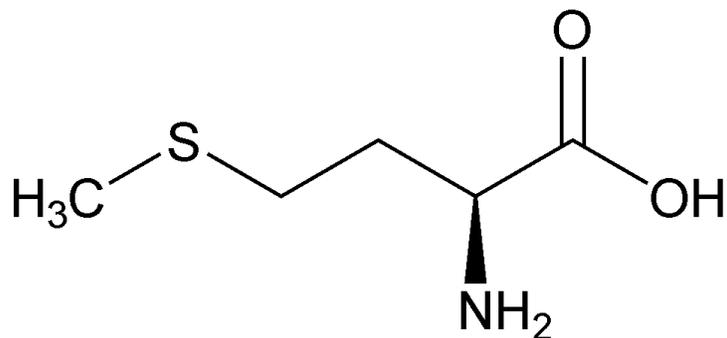
BRIEFING

Methionine, *USP 38* page 4312. As part of the USP modernization efforts, it is proposed to replace the TLC procedure with an HPLC procedure in the test for *Related Compounds* to better characterize the organic impurities. The liquid chromatographic procedure is based on analyses performed with the Hichrom Kromasil C18 brand of L1 column. The typical retention times for methionine and *N*-acetyl-d,l-methionine are 2.0 and 6.8 min, respectively.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(DS: H. Dinh.)

Correspondence Number—C135111

Comment deadline: March 31, 2015**Methionine**C₅H₁₁NO₂S 149.21

l-Methionine [63-68-3].

DEFINITION

Methionine contains NLT 98.5% and NMT 101.5% of l-methionine (C₅H₁₁NO₂S), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈 197K〉

ASSAY

- **Procedure**

Sample: 140 mg of Methionine**Blank:** Mix 3 mL of formic acid and 50 mL of glacial acetic acid.**Titrimetric system**(See *Titrimetry* 〈 541〉.)**Mode:** Direct titration**Titrant:** 0.1 N perchloric acid VS**Endpoint detection:** Potentiometric**Analysis:** Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with the *Titrant*.Calculate the percentage of l-methionine (C₅H₁₁NO₂S) in the portion of the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

 V_S *Titrant* volume consumed by the *Sample* (mL) V_B *Titrant* volume consumed by the *Blank* (mL) N = actual normality of the *Titrant* (mEq/mL) F = equivalency factor, 149.2 mg/mEq W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.4%
- **Chloride and Sulfate, Chloride** 〈 221 〉
Standard solution: 0.50 mL of 0.020 N hydrochloric acid
Sample: 0.73 g of Methionine
Acceptance criteria: NMT 0.05%
- **Chloride and Sulfate, Sulfate** 〈 221 〉
Standard solution: 0.10 mL of 0.020 N sulfuric acid
Sample: 0.33 g of Methionine
Acceptance criteria: NMT 0.03 %
- **Iron** 〈 241 〉: NMT 30 ppm

Change to read:

- **Related Compounds**

~~**System suitability solution:** 0.4 mg/mL each of USP I Methionine RS and USP I Serine RS in 0.3 N hydrochloric acid~~

~~**Standard solution:** 0.05 mg/mL of USP I Methionine RS in 0.3 N hydrochloric acid. [Note — This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]~~

~~**Sample solution:** 10 mg/mL of Methionine in 0.3 N hydrochloric acid~~

~~**Chromatographic system**~~

~~(See *Chromatography* 〈 621 〉, *Thin-Layer Chromatography*.)~~

~~**Mode:** TLC~~

~~**Adsorbent:** 0.25-mm Layer of chromatographic silica-gel mixture~~

~~**Application volume:** 5 µL~~

~~**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (3:1:1)~~

~~**Spray reagent:** 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)~~

~~**System suitability**~~

~~**Suitability requirements:** The chromatogram of the *System suitability solution* exhibits two clearly separated spots.~~

~~**Analysis**~~

~~**Samples:** *System suitability solution*, *Standard solution*, and *Sample solution*~~

~~After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.~~

~~**Acceptance criteria:** Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.~~

~~**Individual impurities:** NMT 0.5%~~

~~**Total impurities:** NMT 2.0%~~

▲Buffer solution: 0.1 M phosphoric acid

Mobile phase: Acetonitrile, *Buffer solution*, and water (10:6:84)

System suitability solution: Transfer 5 mg of USP I-Methionine RS and 5 mg of USP N-

Acetyl-d,l-methionine RS to a 10-mL volumetric flask, dissolve in and dilute with water to volume. Transfer 1.0 mL of the resultant solution to a 10-mL volumetric flask and dilute with *Mobile phase* to volume.

Methionine standard solution: 0.001 mg/mL of USP l-Methionine RS in *Mobile phase*

N-Acetyl-d,l-methionine standard solution: Transfer 5.0 mg of USP *N*-Acetyl-d,l-methionine RS to a 50-mL volumetric flask, dissolve in and dilute with water to volume. Transfer 1.0 mL of the resultant solution to a 50-mL volumetric flask and dilute with *Mobile phase* to volume.

Sample solution: 1.0 mg/mL of Methionine in *Mobile phase*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

Run time: 50–60 min

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for methionine and *N*-acetyl-d,l-methionine are 1.0 and 3.4, respectively.]

Suitability requirements

Resolution: NLT 5.0 between methionine and *N*-acetyl-d,l-methionine

Relative standard deviation: NMT 5.0% for each of methionine and *N*-acetyl-d,l-methionine

Analysis

Samples: *Standard solutions* and *Sample solution*

Calculate the percentage of *N*-acetyl-d,l-methionine in the portion of Methionine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of *N*-acetyl-d,l-methionine from the *Sample solution*

r_S

= peak response of *N*-acetyl-d,l-methionine from the *N-Acetyl-d,l-methionine standard solution*

C_S

= concentration of USP *N*-Acetyl-d,l-methionine RS in the *N-Acetyl-d,l-methionine standard solution* (mg/mL)

C_U

= concentration of Methionine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Methionine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of any unspecified impurity from the *Sample solution*

r_S

= peak response of methionine from the *Methionine standard solution*

C_S

= concentration of USP I-Methionine RS in the *Methionine standard solution* (mg/mL)

C_U

= concentration of Methionine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methionine	1.0	—
<i>N</i> -Acetyl-d,l-methionine	3.4	0.2
Any unspecified impurity	—	0.05
Total unspecified impurities	—	0.20

▲USP39

SPECIFIC TESTS

- **Optical Rotation, Specific Rotation** 〈 781S 〉
Sample solution: 20 mg/mL in 6 N hydrochloric acid
Acceptance criteria: +22.4° to +24.7°
- **pH** 〈 791 〉
Sample solution: 10 mg/mL of solution
Acceptance criteria: 5.6–6.1
- **Loss on Drying** 〈 731 〉
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP I-Serine RS

▲USP N-Acetyl-d,l-methionine RS ▲USP39

USP I-Methionine RS

BRIEFING

Methocarbamol Injection, USP 38 page 4314. Based on the comments received, it is proposed to revise the monograph as follows:

1. Clarify the preparation of *Solution C* in the test for *Limit of Aldehydes* to be consistent with the use of formaldehyde solution.
2. Revise the *Sample solution* in the test for *Limit of Aldehydes* to reflect the use of composite sample to be consistent with the sponsor's approved procedure.
3. Revise the acceptance criterion for the *Limit of Aldehydes* from NMT 0.01% as formaldehyde to NMT 10 µg/mL of the Injection as formaldehyde.
4. Delete the reference to the general chapter in the test for *Limit of Aldehydes* because it is not necessary.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: R. Ravichandran.)

Correspondence Number—C152215

Comment deadline: March 31, 2015

Methocarbamol Injection

DEFINITION

Methocarbamol Injection is a sterile solution of Methocarbamol in an aqueous solution of Polyethylene Glycol 300. It contains NLT 95.0% and NMT 105.0% of the labeled amount of methocarbamol (C₁₁H₁₅NO₅).

IDENTIFICATION

- **A. Infrared Absorption** (197K)
Sample: Mix a volume with 40 mL of water equivalent to 500 mg of methocarbamol from Injection in a small separator. Extract with 10 mL of ethyl acetate, and dry the ethyl acetate layer over anhydrous sodium sulfate. Evaporate the ethyl acetate with the use of a water bath maintained at 40° under a stream of nitrogen to dryness.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of 4.5.
Mobile phase: Methanol and *Buffer* (30:70)
Standard solution: 1 mg/mL of USP Methocarbamol RS in *Mobile phase*

Sample solution: Nominally 1 mg/mL of methocarbamol from a suitable volume of Injection containing NLT 100 mg of methocarbamol in *Mobile phase*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 274 nm

Column: 4.6-mm × 10.0-cm; 3-µm or 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methocarbamol ($C_{11}H_{15}NO_5$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)

C_U nominal concentration of methocarbamol in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

Change to read:

• **Limit of Aldehydes**

Diluent: Alcohol and water (20:80)

Solution A: 10 mg/mL of phenylhydrazine hydrochloride in *Diluent*

Solution B: 10 mg/mL of potassium ferricyanide in water

Solution C: 0.001% (v/v) of formaldehyde solution in water

▲10 µg/mL of formaldehyde in water prepared as follows. Dissolve 1.37 g of formaldehyde solution in 1 L of water. Dilute 10 mL of the resulting solution with water to 500 mL. ▲USP39

Standard solution: Transfer 4 mL of *Solution C* to a 25-mL volumetric flask. Add 2.0 mL of filtered *Solution A*. Allow to stand for 10 min. Add 1 mL of *Solution B*, and allow to stand for 5 min. Add 4 mL of hydrochloric acid, and dilute with alcohol to volume.

Sample solution: Transfer an equivalent to 400 mg of methocarbamol from Injection to a 25-mL volumetric flask.

▲Empty the contents of NLT 10 vials of Injection to a suitable container. Transfer 4.0 mL of the composite sample of Injection to a 25-mL volumetric flask. ▲USP39

Add 2.0 mL of filtered *Solution A*, and allow to stand for 10 min. Add 1 mL of *Solution B*, and allow to stand for 5 min. Add 4 mL of hydrochloric acid, and dilute with alcohol to volume.

Blank: Transfer 4 mL of water to a 25-mL volumetric flask. Add 2.0 mL of filtered *Solution A*, and allow to stand for 10 min. Add 1 mL of *Solution B*, and allow to stand for 5 min. Add 4 mL of hydrochloric acid, and dilute with alcohol to volume.

Instrumental conditions

(See *Spectrophotometry and Light Scattering* ~~(851)~~.)

▲▲USP39

Mode: Vis

Analytical wavelength: 515 nm

Cell: 1 cm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Determine the absorbances of the *Samples*.

Acceptance criteria: The absorbance of the *Sample solution* is NMT the absorbance of the *Standard solution* ~~(NMT 0.01% as formaldehyde)~~

▲(NMT 10 µg of formaldehyde in each mL of Injection).▲USP39

SPECIFIC TESTS

- pH ~~(791)~~: 3.5–6.0
- **Bacterial Endotoxins Test** ~~(85)~~: NMT 0.2 USP Endotoxin Units/mg of methocarbamol
- **Particulate Matter in Injections** ~~(788)~~: Meets the requirements for small-volume injections
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* ~~(1)~~

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose containers. Store at controlled room temperature.
- **USP Reference Standards** ~~(11)~~
 - USP Endotoxin RS
 - USP Methocarbamol RS

BRIEFING

Metoprolol Fumarate, *USP 38* page 4369. As part of the USP monograph modernization effort, it is proposed to revise the monograph as follows:

1. Replace the current titration-based *Assay* with an HPLC procedure. The procedure was validated using the Agilent Zorbax Stablebond C8 brand of L7 column in which metoprolol elutes at about 8 min.
2. Replace the current TLC procedure for *Organic Impurities* with a stability-indicating

HPLC procedure. The procedure uses similar chromatographic parameters as those proposed in the *Assay* except the use of gradient in the *Mobile phase*.

3. Revise the acceptance criteria in the *Definition* and *Assay* from NLT 99.0% and NMT 100.5% to NLT 98.0% and NMT 102.0%, which is typical for chromatographic procedures.
4. Revise *Identification* test A using TLC with the retention time agreement in the proposed *Assay*.
5. Delete *Melting Range or Temperature* as it is a non-value added test.
6. Add an additional requirement to the *Packaging and Storage* section.
7. Add four new USP impurity Reference Standards to the *USP Reference Standards* section to support the test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM2: D. Min.)

Correspondence Number—C109903

Comment deadline: March 31, 2015

Metoprolol Fumarate

(C₁₅H₂₅NO₃)₂·C₄H₄O₄ 650.80

2-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, (±)-, (E)-2-butanedioate (2:1) (salt);
(±)-1-(Isopropylamino)-3-[p-(2-methoxyethyl)-phenoxy]-2-propanol fumarate (2:1) (salt) [119637-66-0].

DEFINITION

Change to read:

Metoprolol Fumarate contains ~~NLT 99.0% and NMT 100.5%~~

▲NLT 98.0% and NMT 102.0%▲^{USP39}

of metoprolol fumarate (C₁₅H₂₅NO₃)₂·C₄H₄O₄, calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)

Change to read:

- **B.**

~~**Standard solution:** 10 mg/mL of USP Metoprolol Fumarate RS in methanol~~

~~**Sample solution:** 10 mg/mL of Metoprolol Fumarate in methanol~~

~~**Chromatographic system**~~

~~(See *Chromatography* (621), *Thin-Layer Chromatography*.)~~

~~**Adsorbent:** 0.25-mm layer of chromatographic silica-gel mixture~~

~~**Application volume:** 20 µL~~

~~**Developing solvent system:** Alcohol, water, and ammonium hydroxide (8:1:1)~~

~~**Spray reagent:** Bromocresol purple TS~~

Analysis**Samples:** ~~Standard solution and Sample solution~~

~~Separately apply the Application volume of Standard solution and Sample solution to a thin-layer chromatographic plate with the Absorbent. Allow the spots to dry, and develop the chromatogram in an unsaturated chamber with the Developing solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, dry at 110° for 30 min, and spray the plate with the Spray reagent. Examine the chromatograms.~~

Acceptance criteria: ~~The R_f value of the yellow spot from the Sample solution corresponds to that from the Standard solution.~~

▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ▲USP39

ASSAY**Change to read:**● **Procedure****Sample solution:** 325 mg of Metoprolol Fumarate in 20 mL of glacial acetic acid**Titrimetric system**(See *Titrimetry* (541).)**Mode:** Direct titration**Titrant:** 0.1 N perchloric acid VS**Endpoint detection:** Visual

Analysis: ~~Titrate with Titrant, using a glass electrode and a calomel electrode containing glacial acetic acid saturated with lithium chloride. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 32.54 mg of metoprolol fumarate (C₁₅H₂₅NO₃)₂·C₄H₄O₄.~~

Acceptance criteria: ~~99.0%–100.5% on the dried basis~~

▲**Solution A:** 1.3 g/L of sodium dodecyl sulfate in 0.1% (w/v) phosphoric acid

Solution B: Acetonitrile**Mobile phase:** *Solution A* and *Solution B* (60:40)**Standard solution:** 1 mg/mL of USP Metoprolol Fumarate RS in *Mobile phase***Sample solution:** 1 mg/mL of Metoprolol Fumarate in *Mobile phase***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 223 nm**Column:** 4.6-mm × 15-cm; 5-µm packing L7**Column temperature:** 30°**Flow rate:** 1 mL/min**Injection volume:** 10 µL**Run time:** 10 min

System suitability**Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2**Relative standard deviation:** NMT 0.73%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of metoprolol fumarate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_4O_4]$ in the portion of Metoprolol Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of metoprolol from the *Sample solution* r_S = peak response of metoprolol from the *Standard solution* C_S = concentration of USP Metoprolol Fumarate RS in the *Standard solution* (mg/mL) C_U = concentration of Metoprolol Fumarate in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the dried basis ▲*USP39***IMPURITIES**

- **Residue on Ignition** $\langle 281 \rangle$: NMT 0.1%

Delete the following:

●

● **Heavy Metals, Method I** $\langle 231 \rangle$

: NMT 10 ppm (Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

Diluent: Methanol and water (10:1)**Standard stock solution:** 1.0 mg/mL of USP Metoprolol Fumarate RS in *Diluent***Standard solution A:** 0.5 mg/mL of USP Metoprolol Fumarate RS from *Standard stock solution* in *Diluent***Standard solution B:** 0.2 mg/mL USP Metoprolol Fumarate RS from *Standard stock solution* in *Diluent***Standard solution C:** 0.1 mg/mL USP Metoprolol Fumarate RS from *Standard stock solution* in *Diluent***Sample solution:** 100 mg/mL of Metoprolol Fumarate in *Diluent*

Solution A: ~~10 mg/mL solution of potassium iodide. [Note—Prepare just before use.]~~

Solution B: ~~Triturate 3 g in 10 mL of cold water, and add 90 mL of boiling water with constant stirring. [Note—Prepare just before use.]~~

Chromatographic system

~~(See *Chromatography* (621), *Thin-Layer Chromatography*.)~~

Mode: ~~TLC~~

Adsorbent: ~~0.25-mm layer of chromatographic silica gel mixture~~

Application volume: ~~5 µL~~

Developing solvent system: ~~Chloroform, methanol, and ammonium hydroxide (80:15:2)~~

Spray reagent: ~~Alcohol, *Solution A*, and *Solution B* (3:10:10)~~

Chamber: ~~Lined chamber with absorbent paper, and add 250 mL of *Developing solvent system* into the chamber. Saturate the chamber for 1.5 h before use.~~

Analysis

Samples: ~~*Standard stock solution*, *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*~~

~~Proceed as directed in the chapter. Allow the chromatogram to develop until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, and dry in a current of warm air until the odor of ammonia is no longer perceptible (45 min). Place a beaker containing 0.5 g of potassium permanganate in a *Chamber*. Add 5 mL of 6N hydrochloric acid to the beaker, and allow to equilibrate for 5 min. Place the plate in the *Chamber* for 5 min. Remove the plate from the *Chamber*, allow to stand in a current of cool air for 1 h, and spray with *Spray reagent*. If spots other than the principal spot are observed in the lane of the *Sample solution*, estimate the concentration of each by comparison with the *Standard stock solution* and *Standard solutions A, B, and C*.~~

Acceptance criteria

Individual impurities: ~~The spots from the *Standard stock solution*, *Standard solution A*, *Standard solution B*, and *Standard solution C* correspond to 1.0%, 0.5%, 0.2%, and 0.1% of impurities.~~

Total impurities: ~~NMT 1.0%~~

▲Solution A: 1.3 g/L of sodium dodecyl sulfate in 0.1% (w/v) phosphoric acid

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
8	60	40
13	10	90
13.1	60	40
16	60	40

Diluent: *Solution A* and *Solution B* (60:40)

System suitability solution: 5 µg/mL each of USP Metoprolol Fumarate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, and USP

Metoprolol Related Compound C RS in *Diluent*

Standard solution: 2.5 µg/mL each of USP Metoprolol Fumarate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, USP Metoprolol Compound C RS, and USP Metoprolol Related Compound D RS in *Diluent*

Sample solution: 1 mg/mL of Metoprolol Fumarate in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 223 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between metoprolol related compound A and metoprolol related compound B; NLT 2.5 between metoprolol related compound B and metoprolol related compound C, *System suitability solution*

Relative standard deviation: NMT 2.0% for metoprolol, metoprolol related compound A, metoprolol related compound B, metoprolol related compound C, and metoprolol related compound D, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the corresponding metoprolol related compound in the portion of Metoprolol Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of the corresponding metoprolol related compound in the *Sample solution*

r_S

= peak response of the corresponding metoprolol related compound in the *Standard solution*

C_S

= concentration of the corresponding USP Metoprolol Related Compound RS in the *Standard solution* (µg/mL)

C_U

= concentration of Metoprolol Fumarate in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified impurity in the portion of Metoprolol Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of each unspecified impurity in the *Sample solution*

r_S

= peak response of metoprolol in the *Standard solution*

C_S

= concentration of USP Metoprolol Fumarate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= concentration of Metoprolol Fumarate in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 2*. Disregard peaks below 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fumaric acid	0.2	—
Metoprolol related compound C ^a	0.6	0.10
Metoprolol related compound B ^b	0.7	0.10
Metoprolol related compound A ^c	0.8	0.10
Metoprolol	1.0	—
Metoprolol related compound D ^d	1.5	0.10
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

a (±)-4-[2-Hydroxy-3-(isopropylamino)propoxy]benzaldehyde.

b (±)-1-Chloro-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.

c (±)-1-(Ethylamino)-3-[4-(2-methoxyethyl)phenoxy]-propan-2-ol.

d (±)-*N,N*-Bis[2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl](1-methylethyl)amine 3,3'-(isopropylazanediyl)bis{1-[4-(2-methoxyethyl)phenoxy]propan-2-ol}.

▲USP39

SPECIFIC TESTS

Delete the following:

▲● **Melting Range or Temperature** ~~(741)~~: 145°–148° ▲*USP39*

● **pH** (791): 5.5–6.5, in a solution (1 in 10)

● **Loss on Drying** (731)

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS**Change to read:**

● **Packaging and Storage:** Preserve in tight, light-resistant containers.

▲Store at controlled room temperature. ▲*USP39*

Change to read:

● **USP Reference Standards** (11)

USP Metoprolol Fumarate RS

▲USP Metoprolol Related Compound A RS

(±)-1-(Ethylamino)-3-[4-(2-methoxyethyl)phenoxy]-propan-2-ol.

C₁₄H₂₃NO₃ 253.34

USP Metoprolol Related Compound B RS

(±)-1-Chloro-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.

C₁₂H₁₇ClO₃ 244.71

USP Metoprolol Related Compound C RS

(±)-4-[2-Hydroxy-3-(isopropylamino)propoxy]benzaldehyde.

C₁₃H₁₉NO₃ 237.29

USP Metoprolol Related Compound D RS

(±)-*N,N*-Bis[2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl](1-methylethyl)amine
3,3'-(Isopropylazanediy)bis{1-[4-(2-methoxyethyl)phenoxy]propan-2-ol}.

C₂₇H₄₁NO₆ 475.62 ▲*USP39*

BRIEFING

Naphazoline Hydrochloride, *USP 38* page 4492. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. The *Ordinary Impurities* (466) procedure in the test for *Organic Impurities* is revised based on the *Related substances* procedure in the current *Naphazoline Hydrochloride* monograph in the *European Pharmacopoeia*. The evaluation of the 4- μ m Agilent Superspher 60 RP Select B brand of L7 column, listed as a suitable HPLC column in the *European Pharmacopoeia*, and the 4- μ m Merck Superspher 60-RP-8 brand of L7 column showed the reversed elution order of naphazoline and 1-naphthylacetic acid. The use of the 5- μ m Agilent Zorbax SB-C8 and 3- μ m Ace ACE C8 brands of L7 columns resulted in the elution order to be consistent with *Table 1*,

- with the typical retention time for the naphazoline peak at about 17–20 min.
- The UV procedure in *Identification* test B is replaced with the retention time agreement in the *Assay*.
 - The criteria of the *Capacity factor* and *Column efficiency* in the *System suitability* section of the *Assay* are deleted, because the remaining requirements are adequate to evaluate the system suitability.
 - The *Relative standard deviation* in the *System suitability* section in the *Assay* is revised to be consistent with the repeatability requirements in *Chromatography* (621).
 - USP Naphazoline Related Compound A RS is added to the *USP Reference Standards* section.

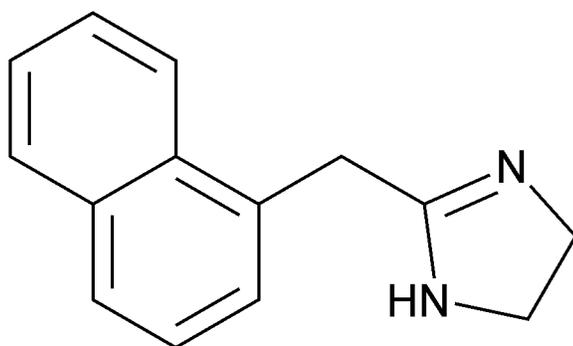
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM3: F. Mao.)

Correspondence Number—C126907

Comment deadline: March 31, 2015

Naphazoline Hydrochloride



• HCl

$C_{14}H_{14}N_2 \cdot HCl$ 246.74

1*H*-Imidazole, 4,5-dihydro-2-(1-naphthalenylmethyl)-, monohydrochloride;
2-(1-Naphthylmethyl)-2-imidazoline monohydrochloride [550-99-2].

DEFINITION

Naphazoline Hydrochloride contains NLT 98.0% and NMT 102.0% of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)

Delete the following:

- **B. Ultraviolet Absorption** (197U)

Analytical wavelength: 280 nm

Sample solution: 20 µg/mL in methanol

Acceptance criteria: Absorptivities, calculated on the dried basis, differ by NMT 3.0%.

▲USP39

Add the following:

- ▲● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP39
- **C. Identification Tests—General, Chloride** 〈 191 〉
 - Sample solution:** 10 mg/mL in water
 - Acceptance criteria:** Meets the requirements

ASSAY**Change to read:**● **Procedure**

Buffer: In a 1000-mL volumetric flask, dissolve 3.0 g of monobasic potassium phosphate in 800 mL of water. Add 3.0 mL of triethylamine, adjust with phosphoric acid to a pH of 3.0, and dilute with water to volume.

Mobile phase: Acetonitrile and *Buffer* (20:80)

Standard solution: 0.05 mg/mL of USP Naphazoline Hydrochloride RS in water

Sample solution: 0.05 mg/mL of Naphazoline Hydrochloride in water

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor, k' : NLT 2.0

Column efficiency: NLT 1500 theoretical plates

▲▲USP39

Tailing factor: NMT 2.0

Relative standard deviation: NMT ~~2.0~~%

▲0.73%▲USP39

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$) in the portion of Naphazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_U peak response from the *Standard solution*

C_S concentration of USP Naphazoline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U concentration of Naphazoline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.2%

Change to read:

- **Organic Impurities**

~~Procedure: Ordinary Impurities (466)-~~

~~Sample solution:~~ Methanol

~~Standard solution:~~ Methanol

~~Eluant:~~ Methanol, glacial acetic acid, and water (8:1:1)

~~Visualization:~~ 2

▲Solution A: Acetonitrile, glacial acetic acid, and water (30: 0.5: 70)

Mobile phase: 1.1 g/L of anhydrous sodium 1-octanesulfonate in *Solution A*

System suitability solution: 0.025 mg/mL of USP Naphazoline Hydrochloride RS and 0.05 mg/mL of 1-naphthylacetic acid in *Mobile phase*

Standard solution: 0.5 µg/mL each of USP Naphazoline Hydrochloride RS and USP Naphazoline Related Compound A RS in *Mobile phase*

Sample solution: 0.5 mg/mL of Naphazoline Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm × 25-cm; 3-µm or 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 3 times of the retention time of the naphazoline peak

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 5.0 between naphazoline and 1-naphthylacetic acid peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of naphazoline related compound A in the portion of Naphazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of naphazoline related compound A from the *Sample solution*

r_S

= peak response of naphazoline related compound A from the *Standard solution*

C_S

= concentration of USP Naphazoline Related Compound A RS in the *Standard solution* (mg/mL)

C_U

= concentration of Naphazoline Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Naphazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of any individual unspecified impurity from the *Sample solution*

r_S

= peak response of naphazoline from the *Standard solution*

C_S

= concentration of USP Naphazoline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= concentration of Naphazoline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria

Individual impurities: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Naphazoline related compound A	0.76	0.1
Naphazoline	1.0	—
1-Naphthylacetic acid	1.4	0.10
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

▲USP39

SPECIFIC TESTS

- pH 〈 791 〉

Sample solution: 10 mg/mL in carbon dioxide-free water

Acceptance criteria: 5.0–6.6. The *Sample solution* is clear and colorless.

- Loss on Drying 〈 731 〉

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Naphazoline Hydrochloride RS

▲USP Naphazoline Related Compound A RS

N-(2-Aminoethyl)-2-(naphthalen-1-yl)acetamide.

C₁₄H₁₆N₂O 228.29▲USP39

BRIEFING

Nitrofurazone, *USP 38* page 4570. As part of USP monograph modernization efforts, the following revisions are proposed:

1. Delete the drying condition in the *Definition* as it is listed in the test for *Loss on Drying* and add the statement “calculated on the dried basis” to be consistent with current FDA approvals.
2. Delete the nonspecific *Identification* test *C* as the other two tests are sufficient.
3. Replace the nonspecific TLC tests for *Ordinary Impurities* and *Limit of 5-Nitro-2-furfuraldazine* with a test for *Organic Impurities* based on the *Related substances* procedure in the *European Pharmacopoeia 8.0* monograph for *Nitrofurazone* with some modifications. The liquid chromatography procedure is performed with the Lichrosphere RP18 brand of L1 column. The typical retention time for the nitrofurazone peak is about 4 min.
4. The *USP Reference Standards* section is revised to include additional Reference Standards used in the test for *Organic Impurities*.

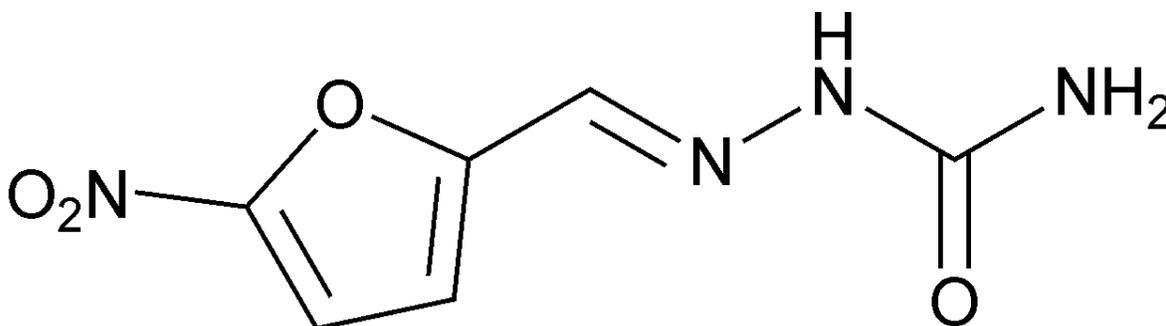
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM1: S. Shivaprasad.)

Correspondence Number—C147431

Comment deadline: March 31, 2015

Nitrofurazone



$C_6H_6N_4O_4$ 198.14

Hydrazinecarboxamide, 2-[(5-nitro-2-furanyl)methylene]-;
5-Nitro-2-furaldehyde semicarbazone [59-87-0].

DEFINITION

Change to read:

Nitrofurazone, dried at 105° for 1 h,

▲▲USP39

contains NLT 98.0% and NMT 102.0% of nitrofurazone ($C_6H_6N_4O_4$),

▲calculated on the dried basis. ▲USP39

[Note—Avoid at all times exposing solutions of nitrofurazone to direct sunlight, excessive heat, strong fluorescent lighting, and alkaline materials.]

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B. Ultraviolet Absorption** (197U)

Sample solution: 8 $\mu\text{g/mL}$, prepared as directed in the Assay

Acceptance criteria: The ratio A_{306}/A_{375} is NMT 0.25.

Delete the following:

▲• **C. Procedure**

Analysis: Dissolve 400 mg of potassium hydroxide in 10 mL of alcohol. Immediately before use, dilute with dimethylformamide to 100 mL. To 10 mL of this solution, add a few crystals of Nitrofurazone.

Acceptance criteria: A purple solution results. ▲USP39

ASSAY

• **Procedure**

Standard stock solution: 0.4 mg/mL of USP Nitrofurazone RS prepared as follows. Transfer 100 mg of USP Nitrofurazone RS, previously dried, to a 250-mL volumetric flask. Dissolve in 50 mL of dimethylformamide, and dilute with water to volume.

Standard solution: 8 $\mu\text{g/mL}$ of USP Nitrofurazone RS in water from *Standard stock solution*

Sample stock solution: 0.4 mg/mL of Nitrofurazone prepared as follows. Transfer 100 mg of Nitrofurazone, previously dried, to a 250-mL volumetric flask. Dissolve in 50 mL of dimethylformamide, and dilute with water to volume.

Sample solution: 8 µg/mL of Nitrofurazone in water from *Sample stock solution*

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 375 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nitrofurazone ($C_6H_6N_4O_4$) in the portion of Nitrofurazone taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_{\bar{U}}$ absorbance of the *Sample solution*

$A_{\bar{S}}$ absorbance of the *Standard solution*

$C_{\bar{S}}$ concentration of USP Nitrofurazone RS in the *Standard solution* (µg/mL)

$C_{\bar{U}}$ concentration of Nitrofurazone in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

Change to read:

- **Organic Impurities**

~~Procedure 1: Ordinary Impurities (466)~~

~~**Standard solution:** Dimethylformamide~~

~~**Sample solution:** Dimethylformamide~~

~~**Application volume:** 10 µL~~

~~**Eluant:** A mixture of chloroform, methanol, and ammonium hydroxide (60:24:3) in a non-equilibrated chamber~~

~~**Visualization:** 1~~

- **Procedure 2: Limit of 5-Nitro-2-furfuraldazine**

~~**Standard stock solution:** Transfer 50.0 mg of USP Nitrofurazone Related Compound A RS to a 100-mL volumetric flask, and dissolve in dimethylformamide to volume.~~

~~**Standard solution:** Transfer 5.0 mL of *Standard stock solution* to a 25-mL volumetric flask, add 10 mL of dimethylformamide, and dilute with acetone to volume.~~

~~**Sample solution:** Dissolve 2.0 g of sample in 60 mL of dimethylformamide in a 100-mL volumetric flask. Dilute with acetone to volume.~~

~~Chromatographic system~~

~~(See *Chromatography* (621), *Thin-Layer Chromatography*.)~~

~~**Mode:** TLC~~

~~**Adsorbent:** 0.5-mm layer of chromatographic silica gel~~

~~**Application volume:** 5 μ L~~

~~**Developing solvent system:** Cyclohexane and ethyl acetate (1:4)~~

~~**Analysis**~~

~~**Samples:** *Sample solution* and *Standard solution*~~

~~Proceed as directed in the chapter. With a suitable densitometer, equipped with a filter having its maximum transmittance at 254 nm, locate and scan the spot produced by the *Standard solution* and any spot from the *Sample solution* having the same R_f as that produced by the *Standard solution*.~~

~~**Acceptance criteria:** The area and intensity of any spot from the *Sample solution* are not greater than the area and intensity of the spot from the *Standard solution* (0.5%).~~

▲Mobile phase: Acetonitrile and water (40:60)

Standard stock solution A: 0.1 mg/mL each of USP Nitrofurazone RS, USP Nitrofurantoin RS, and USP Nitrofurfural Diacetate RS in *Mobile phase*

Standard stock solution B: 0.1 mg/mL of USP Nitrofurazone Related Compound A RS prepared as follows. Transfer a suitable amount of USP Nitrofurazone Related Compound A RS to a suitable volumetric flask and dissolve in 40% of the flask volume of dimethylformamide. Dilute with *Mobile phase* to volume.

Standard solution: 5 μ g/mL each of USP Nitrofurazone RS, USP Nitrofurantoin RS, USP Nitrofurfural Diacetate RS, and USP Nitrofurazone Related Compound A RS in *Mobile phase* prepared as follows. Transfer 5 mL each of *Standard stock solution A* and *Standard stock solution B* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: 1 mg/mL of Nitrofurazone in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 310 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: 10 times the retention time of nitrofurazone

System suitability

Sample: *Standard solution*

[Note—The relative retention times for nitrofurazone and nitrofurantoin are about 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 2.0 between nitrofurazone and nitrofurantoin

Tailing factor: NMT 1.5 for the nitrofurazone, nitrofurazone related compound A, and nitrofurfural diacetate peaks

Relative standard deviation: NMT 0.73% for the nitrofurazone, nitrofurazone related compound A, and nitrofurfural diacetate peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nitrofurazone related compound A and nitrofurfural diacetate in the portion of Nitrofurazone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of nitrofurazone related compound A or nitrofurfural diacetate from the *Sample solution*

r_S

= peak response of nitrofurazone related compound A or nitrofurfural diacetate from the *Standard solution*

C_S

= concentration of USP Nitrofurazone Related Compound A RS or USP Nitrofurfural Diacetate RS in the *Standard solution* (mg/mL)

C_U

= concentration of Nitrofurazone in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Nitrofurazone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of each unspecified impurity from the *Sample solution*

r_S

= peak response of nitrofurazone from the *Standard solution*

C_S

= concentration of USP Nitrofurazone RS in the *Standard solution* (mg/mL)

C_U

= concentration of Nitrofurazone in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nitrofurazone	1.0	—
Nitrofurfural diacetate	3.4	0.5
Nitrofurazone related compound A	6.0	0.5
Any unspecified impurity	—	0.1
Total impurities	—	2.0

▲USP39

SPECIFIC TESTS● **pH** 〈 791 〉

Sample solution: 1 g of Nitrofurazone in 100 mL of water. Shake for 15 min, allow the suspension to settle, and filter.

Acceptance criteria: 5.0–7.5

● **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 1 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

Change to read:● **USP Reference Standards** 〈 11 〉

▲USP Nitrofurantoin RS▲USP39

USP Nitrofurazone RS USP Nitrofurazone Related Compound A RS

5-Nitro-2-furfuraldazine.

C₁₀H₆N₄O₆ 278.18

▲USP Nitrofurfural Diacetate RS

(5-Nitrofuran-2-yl)methylene diacetate.

C₉H₉NO₇ 243.17▲USP39**BRIEFING**

Orphenadrine Citrate, USP 38 page 4656. Based on the comments received, it is proposed to revise the monograph as follows:

1. Replace *Identification* test B with a retention time agreement based on the *Assay*.
2. Include a third *Identification* test for citrate counter ion to strengthen the monograph.
3. Delete the need to adjust the pH of the *Buffer* in the *Assay* as the robustness study does not support the need for stringent pH control.
4. Introduce a *System suitability solution* to the *Assay* to strengthen the monograph. This introduces the use of three Reference Standards that are already being used in other dosage forms containing orphenadrine citrate.
5. Revise the *Suitability requirements* in the *Assay* to include a *Resolution* requirement. Delete the *Column efficiency* requirement as the proposed resolution is a better indicator of the column performance. Move the *Signal-to-noise ratio* requirement to the test for *Organic Impurities, Procedure 1*, where it is relevant.
6. Rename the test for *Organic Impurities* as *Organic Impurities, Procedure 1*. Revise

the *Suitability requirements* to include *Resolution*. Revise the calculation to include the inverse of the correction factor for consistency with current *USP* style. Include limits for the specified impurities based on International Conference on Harmonization (ICH) guidelines. The currently official monograph only has the requirement for total impurities. Also include relative retention time information for citrate to facilitate the identification of counter ion.

7. Introduce *Organic Impurities, Procedure 2*, to allow the monitoring of the impurities specified in *European Pharmacopoeia 8.2*. The gas chromatographic procedure uses the HP-5 brand of G27 column manufactured by Agilent Technologies. The SPB 5 brand of G27 column manufactured by Supelco is a suitable alternative column. Typical retention time for orphenadrine is about 20.6 min, under the chromatographic conditions. Because the impurities are not the same as in *Organic Impurities, Procedure 1*, a flexible monograph approach has been proposed. This introduces the use of *USP Diphenhydramine Citrate RS* for peak identification purposes.
8. Replace the NMR procedure for *Isomer Content*, which uses carbon tetrachloride, with a gas chromatographic procedure based on *European Pharmacopoeia 8.2*. The proposed limit of NMT 0.3% each for *meta*- and *para*- isomers is more stringent than the currently official limit of NMT 3% for the sum of both *meta*- and *para*- isomers. The gas chromatographic procedure uses the HP-5 brand of G27 column manufactured by Agilent Technologies. The SPB 5 brand of G27 column manufactured by Supelco is a suitable alternative column. Typical retention time for orphenadrine is about 13 min, under the chromatographic conditions. This test requires the use of two new Reference Standards.
9. Delete the tests for *Melting Range or Temperature* and *Clarity and Color of Solution* as the monograph includes other tests that are sufficient to ensure the quality of the drug substance.
10. Include a *Labeling* section to indicate compliance with the appropriate *Organic Impurities* procedure.
11. Revise the *USP Reference Standards* section to include the newly required Reference Standards.

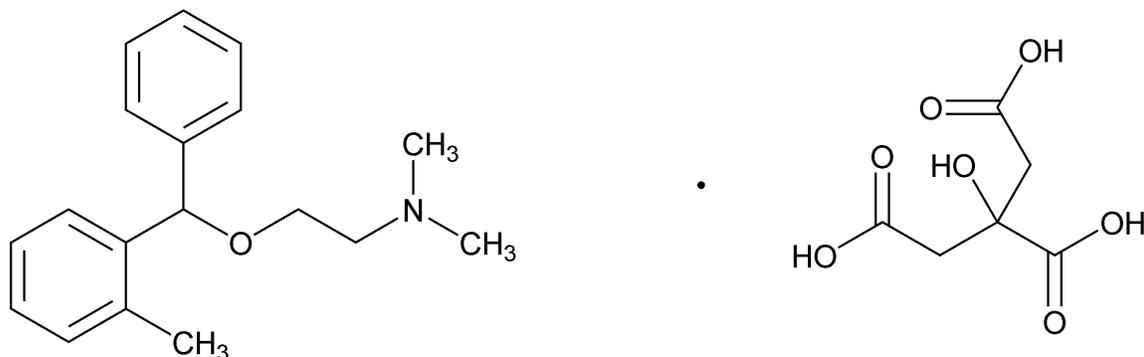
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R. Ravichandran.)

Correspondence Number—C139053

Comment deadline: March 31, 2015

Orphenadrine Citrate



$C_{18}H_{23}NO \cdot C_6H_8O_7$ 461.50

Ethanamine, *N,N*-dimethyl-2-[(2-methylphenyl)phenylmethoxy]-, (\pm)-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1);
 (\pm)-*N,N*-Dimethyl-2-[(*o*-methyl- α -phenylbenzyl)oxy]ethylamine citrate (1:1) [4682-36-4].

DEFINITION

Orphenadrine Citrate contains NLT 98.0% and NMT 101.5% of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** \langle 197M \rangle

Delete the following:

- **B. Ultraviolet Absorption** \langle 197U \rangle

Analytical wavelength: 264 nm

Sample solution: 500 μ g/mL in alcohol

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

▲USP39

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP39

Add the following:

- **C. Identification Tests—General, Citrate** \langle 191 \rangle : Meets the requirement ▲USP39

ASSAY

Change to read:

- **Procedure**

Buffer: 5.8 g/L of monobasic ammonium phosphate in water. Adjust with ammonium hydroxide or phosphoric acid to a pH of 7.9. \pm 0.05.

▲▲USP39

Mobile phase: Methanol, acetonitrile, and *Buffer* (45:15:40)

System sensitivity solution: 0.45 μ g/mL of USP Orphenadrine Citrate RS, from the *Standard solution*, in *Mobile phase*

▲**System suitability solution:** 0.01 mg/mL each of USP Orphenadrine Related Compound B RS, USP Orphenadrine Related Compound C RS, USP Methylbenzhydrol RS, and 1.0 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase* ▲USP39

Standard solution: 0.9

▲1.0 ▲USP39

mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*

Sample solution: 0.9

▲1.0▲*USP39*

mg/mL of Orphenadrine Citrate in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

▲**Run time:** NLT 2.5 times the retention time of orphenadrine▲*USP39*

System suitability

Samples: *System suitability solution* and *Standard solution*

▲[

Note—See *Table 1* for the relative retention times.]▲*USP39*

Suitability requirements

~~**Column efficiency:** NLT 4500 theoretical plates, *Standard solution*~~

▲**Resolution:** NLT 3.0 between orphenadrine related compound B and C; NLT 3.0 between orphenadrine related compound C and methylbenzhydrol▲*USP39*

Tailing factor: NMT 2.0, *Standard solution*

~~**Relative standard deviation:** NMT 2.0%~~

▲NMT 0.73%,▲*USP39*

Standard solution

~~**Signal-to-noise ratio:** NLT 10, *System sensitivity solution*, *Standard solution*~~

▲▲*USP39*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the portion of Orphenadrine Citrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)

C_U concentration of Orphenadrine Citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the dried basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Change to read:

- **Organic Impurities: Procedure 1**

▲[

Note—If methyl orphenadrine is a known manufacturing impurity, *Procedure 1* and the test for *Isomer Content* are recommended. If diphenhydramine and didesmethyl orphenadrine are known manufacturing process impurities, *Procedure 2* is recommended.]▲*USP39*

Buffer, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

▲**Standard solution:** 0.001 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*

Sensitivity solution: 0.5 µg/mL of USP Orphenadrine Citrate RS, from the *Standard solution*, in *Mobile phase*

Sample solution: 1 mg/mL of Orphenadrine Citrate in *Mobile phase*▲*USP39*

System suitability

Samples: ~~*Standard solution* and *System sensitivity solution*~~

▲~~*System suitability solution*, *Standard solution*, and *Sensitivity solution*~~

[~~Note—See *Table 1* for the relative retention times.~~]▲*USP39*

Suitability requirements

~~**Column efficiency:** NLT 4500 theoretical plates, *Standard solution*~~

▲**Resolution:** NLT 3.0 between orphenadrine related compound B and C; NLT 3.0 between orphenadrine related compound C and 2-methylbenzhydrol, *System suitability solution*▲*USP39*

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

[~~Note—Record the chromatogram for at least 2.5 times the retention time of orphenadrine citrate, and measure all the peak areas.~~]

▲▲*USP39*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Orphenadrine Citrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

▲ $\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$ ▲*USP39*

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of Orphenadrine Citrate from the *Standard solution*

C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor
Ethyldimethyl [2-(2-methylbenzhydroxy)ethyl] ammonium chloride	0.25	0.75
2-Methylbenzhydrol	0.51	0.41
Orphenadrine Citrate	1.0	—
<i>N,N</i> -Dimethyl-2-(<i>o</i> -tolyl- <i>o</i> -xylyloxy)ethylamine	1.54	0.52
Any other individual impurity	—	1.0

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citric acid ^a	0.14	—	—
Orphenadrine related compound B	0.25	1.3	0.1
Orphenadrine related compound C	0.39	1.0	0.1
Methylbenzhydrol	0.51	2.4	0.1
Orphenadrine	1.0	—	—
Methyl orphenadrine ^b	1.54	1.9	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities ^c	—	—	0.5

^a Counter ion peak; not to be reported; not to be included in total impurities.

^b 2-(Di-*o*-tolylmethoxy)-*N,N*-dimethylethan-1-amine.

^c Excluding orphenadrine related compound E and orphenadrine related compound F from the *Isomer Content* test.

▲USP39

Add the following:

▲● **Organic Impurities: Procedure 2**

[Note—If diphenhydramine and didesmethyl orphenadrine are known manufacturing process impurities, *Procedure 2* is recommended. The labeling indicates that the article complies with *Organic Impurities, Procedure 2*.]

System suitability solution: 0.3 mg/mL each of USP Orphenadrine Citrate RS, USP Diphenhydramine Citrate RS, USP Methylbenzhydrol RS, USP Orphenadrine Related Compound E RS, and USP Orphenadrine Related Compound F RS in toluene prepared as follows. Dissolve 6 mg each of the USP Reference Standards in 10 mL of water. Add 0.2 mL of ammonium hydroxide and shake with 3 mL of toluene. Separate the organic layer.

Repeat the extraction of the aqueous layer two more times with 3 mL of toluene. To the combined organic layer add anhydrous sodium sulfate, shake, and filter. Evaporate the filtrate at NMT 50° using a rotary evaporator. Dissolve the residue in 20 mL of toluene.

Sample solution: 25 mg/mL of Orphenadrine Citrate in toluene prepared as follows.

Dissolve 500 mg of Orphenadrine Citrate in 50 mL of water. Add 2 mL of ammonium hydroxide and shake with 10 mL of toluene. Separate the organic layer. Repeat the extraction of the aqueous layer two more times with 10 mL of toluene. To the combined organic layers add anhydrous sodium sulfate, shake, and filter. Evaporate the filtrate at NMT 50° using a rotary evaporator. Dissolve the residue in 20 mL of toluene.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 60-m; 1-μm thick coating of phenyl methylpolysiloxane, packing G27

Carrier gas: Helium at 1 mL/min

Temperatures

Injection port: 290°

Detector: 290°

Column: 240°

Injection volume: 2 μL

Injection type: Split ratio, 1:25

Run time: 1.3 times the retention time of orphenadrine

System suitability

Sample: *System suitability solution*

[Note—Relative retention times for the peaks are given in *Table 2*.]

Suitability requirements

Resolution: NLT 1.5 between orphenadrine related compound E and orphenadrine; NLT 2.0 between orphenadrine and orphenadrine related compound F

Analysis

Sample: *Sample solution*

Calculate the sum of the percentage of any individual impurity in the portion of Orphenadrine Citrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of the impurity from the *Sample solution*

r_T sum of all peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (% , w/w)
Methylbenzophenone ^a	0.5	0.3
Methylbenzhydrol	0.6	0.3

Diphenhydramine	0.8	0.3
Didesmethyl orphenadrine ^b	0.9	0.3
Orphenadrine related compound E	0.98	0.3
Orphenadrine	1.0	—
Orphenadrine related compound F	1.1	0.3
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0
^a Phenyl(<i>o</i> -tolyl)methanone.		

^b 2-[Phenyl(*o*-tolyl)methoxy]ethanamine.

▲USP39

Change to read:

• Isomer Content

Solvent: ~~Carbon tetrachloride~~

NMR reference: ~~Tetramethylsilane~~

Sample solution: ~~Place 1 g of Orphenadrine Citrate and 10 mL of water in a separatory funnel, slowly add about 20 drops of sodium hydroxide solution (1 in 2), with swirling, to obtain a solution having a pH of about 10, and extract with three 15-mL portions of ether. Combine the ether extracts in a beaker, discarding the aqueous phase, and evaporate to about one-half the volume by warming on a steam bath under a stream of nitrogen. Transfer to another separatory funnel, wash with three 20-mL portions of water, and dry the ether solution with about 15 g of anhydrous sodium sulfate in a conical flask for 1 h, with intermittent swirling. Decant the dried ether solution through a pledget of glass wool into a small beaker. Rinse the sodium sulfate with two 10-mL portions of ether, and add the rinsings to the beaker. Evaporate most of the ether by warming under a stream of nitrogen, and remove the last traces of ether by drying at a pressure of NMT 2 mm of mercury at 60°. Transfer 400 mg of the orphenadrine so obtained to a small weighing bottle, add 0.5 mL of carbon tetrachloride and 1 drop of tetramethylsilane, and swirl to dissolve.~~

Analysis: ~~Proceed as directed under Nuclear Magnetic Resonance (761), Relative Method of Quantitation, using the calculation formula given therein, in which A_1 is the sum of the average areas of the combined methine peaks associated with the *meta*- and *para*-methylbenzyl isomers, appearing at about 5.23 ppm, and A_2 is the area of the methine peak associated with the *ortho*-methylbenzyl isomer, appearing at about 5.47 ppm, with reference to the tetramethylsilane singlet at 0 ppm, and both n_1 and n_2 are equal to 1.~~

Acceptance criteria: ~~The limit of combined *meta*- and *para*-methylbenzyl isomers is 3.0%.~~

▲If methyl orphenadrine is a known manufacturing impurity, the test for *Isomer Content* is to be performed.

System suitability solution: 0.3 mg/mL each of USP Orphenadrine Citrate RS, USP Orphenadrine Related Compound E RS, and USP Orphenadrine Related Compound F RS in toluene prepared as follows. Dissolve 6 mg each of the USP Reference Standards in 10 mL of water. Add 0.2 mL of ammonium hydroxide and shake with 3 mL of toluene.

Separate the organic layer. Repeat the extraction of the aqueous layer two more times with 3 mL of toluene. To the combined organic layer add anhydrous sodium sulfate, shake, and filter. Evaporate the filtrate at NMT 50° using a rotary evaporator. Dissolve the residue in 20 mL of toluene.

Sample solution and **Chromatographic system:** Proceed as directed in *Organic Impurities, Procedure 2*.

System suitability

Sample: *System suitability solution*

[Note—The relative retention times of orphenadrine related compound E, orphenadrine, and orphenadrine related compound F are about 0.98, 1.0, and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.5 between orphenadrine related compound E and orphenadrine; NLT 2.0 between orphenadrine and orphenadrine related compound F

Analysis

Sample: *Sample solution*

Calculate the percentage of orphenadrine related compound E and orphenadrine related compound F in the portion of Orphenadrine Citrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U

= peak response of orphenadrine related compound E or orphenadrine related compound F from the *Sample solution*

r_T

= sum of all peak responses from the *Sample solution*

Acceptance criteria: NMT 0.3% each of orphenadrine related compound E and orphenadrine related compound F. ▲*USP39*

SPECIFIC TESTS

Delete the following:

▲• ~~Melting Range or Temperature <741>: 134°–138°~~ ▲*USP39*

• ~~Loss on Drying <731>~~

~~**Analysis:** Dry at 105° for 3 h.~~

~~**Acceptance criteria:** NMT 0.5%~~

Delete the following:

▲• ~~Clarity and Color of Solution:~~

~~**Diluent:** alcohol and hydrochloric acid (27:1)~~

~~**Sample:** 1 g in 10 mL of *Diluent*~~

~~**Analysis:** Measure the absorbance of the *Sample* at 436 nm~~

~~**Acceptance criteria:** NMT 0.050 au.~~ ▲*USP39*

ADDITIONAL REQUIREMENTS**Add the following:**

- ▲● **Packaging and Storage:** Preserve in tight, light-resistant containers. ▲USP39

Add the following:

- ▲● **Labeling:** The label states with which *Organic Impurities* procedure the article complies if *Organic Impurities, Procedure 1*, is not used. ▲USP39

Change to read:● **USP Reference Standards** < 11 >

▲USP Diphenhydramine Citrate RS

USP Methylbenzhydrol RS

2-Methylbenzhydrol;

Also known as Phenyl(*o*-tolyl)methanol.

C₁₄H₁₄O 198.26

▲USP39

USP Orphenadrine Citrate RS

▲USP Orphenadrine Related Compound B RS

N-Ethyl-*N,N*-dimethyl-2-[phenyl(*o*-tolyl)methoxy]ethanaminium chloride.

C₂₀H₂₈ClNO 333.90

USP Orphenadrine Related Compound C RS

N-Methyl-2-[phenyl(*o*-tolyl)methoxy]ethanamine hydrochloride.

C₁₇H₂₁NO · HCl 291.82

USP Orphenadrine Related Compound E RS

N,N-Dimethyl-2-[phenyl(*m*-tolyl)methoxy]ethanamine.

C₁₈H₂₃NO 269.38

USP Orphenadrine Related Compound F RS

N,N-Dimethyl-2-[phenyl(*p*-tolyl)methoxy]ethanamine.

C₁₈H₂₃NO 269.38

▲USP39

BRIEFING

Orphenadrine Citrate Injection, USP 38 page 4657. Based on the comments received, it is proposed to revise the monograph as follows:

1. Introduce a *System suitability solution* to the *Assay* to strengthen the monograph. This introduces three Reference Standards that are already being used in other dosage forms containing orphenadrine citrate.
2. Revise the *Suitability requirements* in the *Assay* to include a *Resolution* requirement. Delete the *Column efficiency* requirement because the proposed resolution is a better indicator of the column performance. Move the *Signal-to-noise ratio* requirement to the test for *Organic Impurities*, where it is relevant.
3. Revise the concentration of the *Sensitivity solution* from 0.45 µg/mL to 1 µg/mL, which is 50% of the limit of any individual unspecified degradation product.
4. Revise the concentration of the *Standard solution* from 0.9 mg/mL to 0.002 mg/mL,

which is the limit of any individual unspecified degradation product.

5. Include a second *Resolution* requirement to strengthen the public standard. In addition, widen the *Relative standard deviation* from NMT 2.0% to NMT 5.0% to account for the lower concentration of the *Standard solution*.
6. Revise the calculation in the test for *Organic Impurities* to include the inverse of the correction factor for consistency with current *USP* style.
7. Revise the table of impurities in the test for *Organic Impurities* to include limits for the specified impurities based on International Conference on Harmonization (ICH) guidelines. The currently official monograph only has the requirement for total impurities. Also include relative retention time information for citrate to facilitate the identification of counter ion. Revise the names of the specified degradation products with more appropriate and meaningful names and add appropriate footnotes.
8. Include storage conditions to be consistent with FDA approved label.
9. Update the *USP Reference Standards* section to include the additional Reference Standards.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R. Ravichandran.)

Correspondence Number—C148874

Comment deadline: March 31, 2015

Orphenadrine Citrate Injection

DEFINITION

Orphenadrine Citrate Injection is a sterile solution of Orphenadrine Citrate in Water for Injection, prepared with the aid of Sodium Hydroxide. It contains NLT 93.0% and NMT 107.0% of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$).

IDENTIFICATION

- **A.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*.
- **B. Identification Tests—General, Citrate** $\langle 191 \rangle$: Meets the requirements

ASSAY

Change to read:

- **Procedure**

Buffer: 5.8 g/L of monobasic ammonium phosphate in water. Adjust with ammonium hydroxide or phosphoric acid to a pH of 7.9 ± 0.05 .

Mobile phase: Methanol, acetonitrile, and *Buffer* (45:15:40)

~~**System sensitivity solution:** 0.45 μ g/mL of USP Orphenadrine Citrate RS, from the *Standard solution*, in *Mobile phase*~~

▲System suitability solution: 0.01 mg/mL each of USP Orphenadrine Related Compound B RS, USP Orphenadrine Related Compound C RS, USP Methylbenzhydrol RS, and 0.9 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase* ▲*USP39*

Standard solution: 0.9 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*

Sample solution: Nominally 0.9 mg/mL of orphenadrine citrate from a known volume of the Injection containing NLT 90 mg of orphenadrine citrate in *Mobile phase*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

▲Run time: NLT 2.5 times the retention time of orphenadrine ▲*USP39*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Column efficiency: NLT 4500 theoretical plates, *Standard solution*

Signal-to-noise ratio: NLT 10, *System sensitivity solution*

▲Resolution: NLT 3.0 between orphenadrine related compound B and orphenadrine related compound C; NLT 3.0 between orphenadrine related compound C and methylbenzhydrol

▲*USP39*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the portion of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)

C_U nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

IMPURITIES

Change to read:

- **Organic Impurities**

Buffer, Mobile phase,

▲System suitability solution, ▲*USP39*

Sample solution, and Chromatographic system: Proceed as directed in the Assay.

▲Standard solution: 0.002 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*
Sensitivity solution: 0.001 mg/mL of USP Orphenadrine Citrate RS from the *Standard solution* in *Mobile phase*▲USP39

System suitability

~~**Samples:** *Standard solution* and *System sensitivity solution*~~

~~**Suitability requirements-**~~

~~**Column efficiency:** NLT 4500 theoretical plates, *Standard solution*~~

~~**Tailing factor:** NMT 2, *Standard solution*~~

~~**Relative standard deviation:** NMT 2.0%, *Standard solution*~~

~~**Signal-to-noise ratio:** NLT 10, *System sensitivity solution*. [Note—Record the chromatogram for least 2.5 times the retention time of orphenadrine citrate, and measure all the peak areas.]~~

▲Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 3.0 between orphenadrine related compound B and orphenadrine related compound C; NLT 3.0 between orphenadrine related compound C and methylbenzhydrol

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

▲USP39

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

▲Result = (r_U/r_S) × (C_S/C_U) × (1/F) × 100▲USP39

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of orphenadrine from the *Standard solution*

C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Total impurities: NMT 4.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor
Ethylidimethyl [2-(2-methylbenzhydryloxy)ethyl] ammonium chloride	0.25	0.75
2-Methylbenzhydrol	0.51	0.41
Orphenadrine Citrate	1.0	—

<i>N,N</i>-Dimethyl-2-(<i>o</i>-tolyl-<i>o</i>-xylyloxy)ethylamine	1.54	0.52
Any other individual impurity	—	1.0

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (% , w/w)
Citric acid ^a	0.14	—	—
Orphenadrine related compound B	0.25	1.3	0.2
Orphenadrine related compound C	0.39	1.0	0.2
Methylbenzhydrol	0.51	2.4	0.2
Orphenadrine	1.0	—	—
Methyl orphenadrine ^b	1.54	1.9	0.2
Any individual unspecified degradation product	—	1.0	0.20
Total degradation products	—	—	4.0

^a Counter ion peak; not to be reported; not to be included in total impurities.

^b 2-(Di-*o*-tolylmethoxy)-*N,N*-dimethylethan-1-amine.

▲*USP39***SPECIFIC TESTS**

- **pH** 〈 791 〉: 5.0–6.0
- **Bacterial Endotoxins Test** 〈 85 〉: NMT 5.8 USP Endotoxin Units/mg of orphenadrine citrate
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* 〈 1 〉.

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

▲Store at controlled room temperature.▲*USP39***Change to read:**

- **USP Reference Standards** 〈 11 〉

USP Endotoxin RS

▲USP Methylbenzhydrol RS

2-Methylbenzhydrol;

Also known as Phenyl(*o*-tolyl)methanol.C₁₄H₁₄O 198.26▲*USP39*

USP Orphenadrine Citrate RS **▲USP Orphenadrine Related Compound B RS**

N-Ethyl-*N,N*-dimethyl [2-(2(methylbenzhydryloxy)ethyl)ammonium chloride; also known as *N*-Ethyl-*N,N*-dimethyl-2-[phenyl(*o*-tolyl)methoxy]ethanaminium chloride.

C₂₀H₂₈ClNO 333.90

USP Orphenadrine Related Compound C RS

N-Methyl [2-(2-methylbenzhydryloxy)ethyl]amine hydrochloride; also known as *N*-Methyl-2-[phenyl(*o*-tolyl)methoxy]ethanamine hydrochloride.

C₁₇H₂₁NO · HCl 291.82

▲USP39**BRIEFING**

Orphenadrine Citrate Extended-Release Tablets, *USP 38* page 4658. Based on the comments received, it is proposed to revise the monograph as follows:

1. Include a *Resolution* requirement in the *Assay* to strengthen the monograph. This will not cause any burden to the user as the monograph requires the same resolution requirement as the test for *Organic Impurities*.
2. Delete the need to adjust the pH of the *Buffer* in the *Assay* as the robustness study does not support the need for stringent pH control.
3. Revise the calculation formulae in *Dissolution, Test 1*, to be consistent with current *USP* style.
4. Include calculation formulae in *Dissolution, Test 2*, to be consistent with current *USP* style.
5. Revise the table of impurities by adding appropriate footnotes to provide guidance to the analyst. Also, include relative retention time information for citrate to facilitate the identification of counter ion.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R. Ravichandran.)

Correspondence Number—C148875

Comment deadline: March 31, 2015

Orphenadrine Citrate Extended-Release Tablets**DEFINITION**

Orphenadrine Citrate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of orphenadrine citrate (C₁₈H₂₃NO·C₆H₈O₇).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

Buffer: 5.75

▲5.8▲*USP39*

g/L of monobasic ammonium phosphate. Adjust with phosphoric acid to a pH of 3.2. ~~±0.1.~~

▲▲*USP39*

Mobile phase: Acetonitrile and *Buffer* (40:60)

▲**System suitability solution:** 0.1 mg/mL of USP Orphenadrine Citrate RS and 0.01 mg/mL each of USP Orphenadrine Related Compound B RS and USP Orphenadrine Related Compound C RS, in *Mobile phase*▲*USP39*

Standard solution: 0.1 mg/mL of USP Orphenadrine Citrate RS

Sample stock solution: Nominally 0.5 mg/mL of orphenadrine citrate prepared as follows.

Transfer a quantity of powder equivalent to NLT 100 mg of orphenadrine citrate, from finely powdered Tablets (NLT 20), to a suitable volumetric flask. Add 50% of the flask volume of *Mobile phase*. Sonicate for 5 min and shake for 15 min. Dilute with *Mobile phase* to volume. Pass through a suitable filter.

Sample solution: 0.1 mg/mL of orphenadrine citrate in *Mobile phase* from *Sample stock solution*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 3.9-mm × 30-cm;

▲10-μm▲*USP39*

L1 packing

Flow rate: 2 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

▲**Resolution:** NLT 1.2 between orphenadrine and orphenadrine related compound C; NLT 2.0 between orphenadrine citrate and orphenadrine related compound B▲*USP39*

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)

C_T nominal concentration of orphenadrine citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** 〈 711 〉

Test 1

Medium: Water; 900 mL, deaerated

Apparatus 2: 50 rpm

Times: 1, 2, 6, and 12 h

Buffer: 5.75

▲5.8▲USP39

g/L of monobasic ammonium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (40:60). Adjust with phosphoric acid to a pH of 3.2 ± 0.1.

Standard stock solution: 1 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*.

▲Sonication may be used to promote dissolution.▲USP39

Standard solution 0.1 mg/mL of USP Orphenadrine Citrate RS in *Medium* from a suitable volume of *Standard stock solution* and *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter with 0.45-µm pore size and discard the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 3.9-mm × 30-cm;

▲10-µm▲USP39

packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis:

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved by using the following formulas:

The percentage dissolved after 1 h is determined by the formula:

$$A = (r_U \times C_S \times 900 \times 100) / (r_S \times L)$$

The percentage dissolved after 2 h is determined by:

$$\text{Result} = B + A \times (V_1/900)$$

where

$$B = (r_U \times C_S \times (V - V_1/900) \times 100) / (r_S \times L)$$

The percentage dissolved after 6 h is determined by:

$$\text{Result} = C + [A \times (V_1/900)] + [B \times [V_2/(900 - V_1)]]$$

where

$$C = [r_U \times C_S \times [900 - (V_1 + V_2)] \times 100] / (r_S \times L)$$

The percentage dissolved after 12 h is determined by:

$$\text{Result} = D + [A \times (V_1/900)] + [B \times [V_2/(900 - V_1)]] + [C \times [V_3/(900 - (V_1 + V_2))]]$$

where

$$D = [r_U \times C_S [900 - (V_1 + V_2 + V_3)] \times 100] / (r_S \times L)$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = Tablet label claim (mg)

V = volume (mL)

V_1 = volume of sample withdrawn at the first hour (mL)

V_2 = volume of the sample withdrawn at the second hour (mL)

V_3 = volume of the sample withdrawn at the sixth hour (mL)

▲ Calculate the concentration (C_i) of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved in the portion of the sample withdrawn at each time point (i) (mg/mL):

$$C_i = (r_U/r_S) \times C_S$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{C_3 \times [V - (2 \times V_S)] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{C_4 \times [V - (3 \times V_S)] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i

= concentration of orphenadrine citrate in the portion of sample withdrawn at time point (i) (mg/mL)

V

= volume of *Medium*, 900 mL

L

= label claim (mg/Tablet)

V_S

= volume of the *Sample solution* withdrawn at each time point (mL)

▲USP39

Tolerances: See Table 1.

Table 1

Time point (i)	Time (h)	Amount Dissolved
1	1	10%–40%
2	2	30%–50%
3	6	50%–80%
4	12	NLT 80%

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Times: 1, 4, and 12 h

Standard solution: 0.02 mg/mL of USP Orphenadrine Citrate RS in *Medium*

Sample solution: Withdraw 10 mL of the solution under test from each vessel at each specified time point. Replace 10 mL of *Medium* in each vessel. Pass a portion of the solution under test through a suitable filter having a porosity of 0.45 μm . Transfer 1.0 mL of the filtrate to a 50-mL volumetric flask, and dilute with *Medium* to volume.

Blank: *Medium*

Instrumental conditions

Mode: UV 210 nm

Path length: 1 cm

▲**Analysis**

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of orphenadrine citrate ($\text{C}_{18}\text{H}_{23}\text{NO}\cdot\text{C}_6\text{H}_8\text{O}_7$) in the sample

withdrawn from the vessel at each time point (i):

$$\text{Result} = (A_U/A_S) \times C_S \times D_S \times (1/L) \times V \times 100$$

A_U

= absorbance of the *Sample solution*

A_S

= absorbance of the *Standard solution*

C_S

= concentration of the *Standard solution* (mg/mL)

D_S

= dilution factor of the *Sample solution*, 50

L

= label claim (mg/Tablet)

V

= volume of *Medium*, 900 mL

Calculate the percentage of the labeled amounts (Q_i) of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i

= concentration of orphenadrine citrate in the portion of sample withdrawn at time point (i) (mg/mL)

V

= volume of *Medium*, 900 mL

L

= label claim (mg/Tablet)

V_S

= volume of the *Sample solution* withdrawn at each time point (mL)

▲USP39

Tolerances: See Table 2.

Table 2

Time point (i)	Time (h)	Amount Dissolved
1	1	10%–40%
2	4	40%–70%
3	12	NLT 80%

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

Change to read:

- **Organic Impurities**

Buffer, Mobile phase,

▲System suitability solution, ▲USP39

Sample solution, Chromatographic system, and

▲System suitability: ▲USP39

Proceed as directed in the Assay.

Analysis

Sample: *Sample solution*

Calculate the percentage of each of the (known or unknown) individual orphenadrine citrate related compounds

▲degradation product ▲USP39

in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak response of each impurity

▲degradation product ▲USP39

from the *Sample solution*

r_S = peak response of orphenadrine from the *Sample solution*

F = relative response factor for each impurity

▲degradation product ▲USP39

(see Table 3)

Acceptance criteria: See Table 3.

Individual impurities: NMT 0.10%

Total impurities: NMT 1.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citric acid	0.4	—	—
Orphenadrine related compound C ^a	0.9	1.5	0.5
Orphenadrine citrate	1	—	—
Orphenadrine related compound B ^b	1.3	1.3	0.5
2-Methylbenzhydrol	2.1	2.1	0.5

2-Methylbenzophenone	4	1.0	0.5
a N-Methyl [2-(2-methylbenzhydryloxy)ethyl]amine hydrochloride.			
b N-Ethyl-N,N-dimethyl[2-2(methylbenzhydryloxy)ethyl]ammonium chloride.			

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citric acid ^a	0.4	—	—
Orphenadrine related compound C	0.9	1.5	0.5
Orphenadrine citrate	1	—	—
Orphenadrine related compound B	1.3	1.3	0.5
2-Methylbenzhydrol ^b	2.1	2.1	0.5
2-Methylbenzophenone ^c	4	1.0	0.5
Any individual unspecified degradation product	—	1.0	0.10
Total degradation products	—	—	1.5

a The peak is due to counter ion and is not to be reported or included in total degradation products.

b Also known as phenyl(*o*-tolyl)methanol.

c Also known as phenyl(*o*-tolyl)methanone.

▲USP39

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in well-closed, tight,

▲▲USP39

light-resistant containers, and store at controlled room temperature.

- **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

- **USP Reference Standards** (11)

USP Orphenadrine Citrate RS

USP Orphenadrine Related Compound B RS

N-Ethyl-*N,N*-dimethyl [2-2(methylbenzhydryloxy)ethyl]ammonium chloride;

▲also known as *N*-Ethyl-*N,N*-dimethyl-2-[phenyl(*o*-tolyl)methoxy]ethanaminium chloride.

▲USP39

C₂₀H₂₈ClNO 333.90

USP Orphenadrine Related Compound C RS

N-Methyl [2-(2-methylbenzhydryloxy)ethyl]amine hydrochloride;

▲also known as *N*-Methyl-2-[phenyl(*o*-tolyl)methoxy]ethanamine hydrochloride. ▲*USP39*

C₁₇H₂₂ClNO 291.82

BRIEFING

Palonosetron Hydrochloride. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods of analyses, is proposed. The liquid chromatographic procedure in the *Assay* and in the test for *Limit of Unspecified Impurities* is based on analyses performed with the Zorbax SB-C8 brand of L7 column. The typical retention time for palonosetron is about 11 min. The liquid chromatographic procedure in the test for *Limit of Specified Impurities* is based on analyses performed with the Astec Chirobiotic-V brand of L88 column. The typical retention time for palonosetron is about 23–27 min.

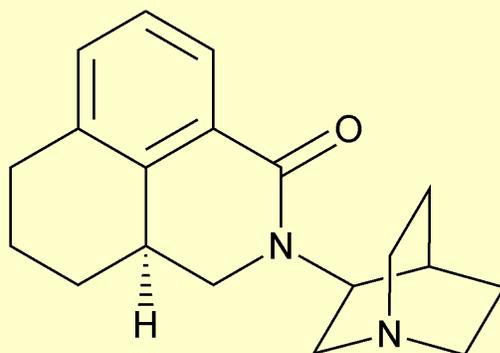
(SM3: A. Wise, E. Gonikberg.)

Correspondence Number—C125958

Comment deadline: March 31, 2015

Add the following:

▲Palonosetron Hydrochloride



C₁₉H₂₄N₂O·HCl 332.87

1*H*-Benzo[*de*]isoquinoline-1-one, 2,3,3*a*,4,5,6-hexahydro-2-[(3*S*)-1-azabicyclo[2.2.2]octan-3-yl],(3*S*)-, hydrochloride;
(3*aS*)-2-[(3*S*)-Quinuclidin-3-yl]-2,3,3*a*,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride [135729-62-3].

DEFINITION

Palonosetron Hydrochloride contains NLT 98.0% and NMT 102.0% of palonosetron hydrochloride (C₁₉H₂₄N₂O·HCl), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** { 197K }
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- **C. Identification Tests—General, Chloride $\langle 191 \rangle$:** Meets the requirements

ASSAY

- **Procedure**

Mobile phase: Acetonitrile, water, and trifluoroacetic acid (280: 720: 0.67)

Standard stock solution: 0.7 mg/mL of USP Palonosetron Hydrochloride RS in methanol.

Sonicate as needed to dissolve. This solution is stable for 6 weeks if stored in the refrigerator and protected from light.

Standard solution: 0.014 mg/mL of USP Palonosetron Hydrochloride RS from the *Standard stock solution* in *Mobile phase*

Sample stock solution: 0.7 mg/mL of Palonosetron Hydrochloride in methanol. Sonicate as needed to dissolve.

Sample solution: 0.014 mg/mL of Palonosetron Hydrochloride from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography $\langle 621 \rangle$, System Suitability.*)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Flow rate: 1 mL/min

Injection volume: 80 μ L

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of palonosetron hydrochloride ($C_{19}H_{24}N_2O \cdot HCl$) in the portion of Palonosetron Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Palonosetron Hydrochloride RS in the *Standard solution* (mg/mL)

C_U concentration of Palonosetron Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition $\langle 281 \rangle$:** NMT 0.1%

- **Limit of Specified Impurities**

Buffer: 1.5 g/L of ammonium acetate, adjusted with glacial acetic acid to a pH of 6.0

Mobile phase: Tetrahydrofuran and *Buffer* (1:9)

System suitability stock solution: 0.07 mg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound C RS, USP Palonosetron Related Compound D RS, USP Palonosetron Related Compound E RS, and USP Palonosetron Enantiomer RS in methanol. Sonicate as needed to dissolve.

System suitability solution: 3.5 µg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound C RS, USP Palonosetron Related Compound D RS, USP Palonosetron Related Compound E RS, and USP Palonosetron Enantiomer RS from the *System suitability stock solution* and 0.7 mg/mL of USP Palonosetron Hydrochloride RS in *Buffer*

Impurity standard stock solution: 0.35 mg/mL of USP Palonosetron Related Compound D RS in methanol. Sonicate as needed to dissolve.

Impurity standard solution: 0.0035 mg/mL of USP Palonosetron Related Compound D RS from the *Impurity standard stock solution* in *Buffer*

Sensitivity solution: 0.35 µg/mL of USP Palonosetron Related Compound D RS from the *Impurity standard stock solution* in *Buffer*

Sample solution: 0.7 mg/mL of Palonosetron Hydrochloride in *Buffer*. Sonicate as needed to dissolve.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 238 nm

Column: 4.6-mm × 25-cm; 5-µm packing L88. [Note—A suitable column is Astec Chirobiotic-V from www.sigma-aldrich.com.]

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 100 µL

Run time: At least 2.5 times the retention time of palonosetron

System suitability

Samples: *System suitability solution*, *Impurity standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.4 between palonosetron related compound D and palonosetron enantiomer; NLT 1.2 between palonosetron enantiomer and palonosetron related compound E, *System suitability solution*

Tailing factor: NMT 2.5 for palonosetron related compound E, *System suitability solution*

Relative standard deviation: NMT 5.0% for palonosetron related compound D, *Impurity standard solution*

Signal-to-noise ratio: NLT 10 for palonosetron related compound D, *Sensitivity solution*

Analysis

Samples: *Impurity standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Palonosetron Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{\bar{f}}$ peak response of each impurity from the *Sample solution*

$r_{\bar{s}}$ peak response of palonosetron related compound D from the *Impurity standard solution*

$C_{\bar{s}}$ concentration of USP Palonosetron Related Compound D RS in the *Impurity standard solution* (mg/mL)

$C_{\bar{f}}$ concentration of Palonosetron Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Palonosetron related compound A	0.45	1.0	0.1
Palonosetron related compound B	0.54	1.0	0.1
Palonosetron	1.0	—	—
Palonosetron related compound C	1.2	1.0	0.1
Palonosetron related compound D	1.3	1.0	0.5
Palonosetron enantiomer	1.4	1.0	0.1
Palonosetron related compound E	1.5	2.5	0.5

● **Limit of Unspecified Impurities**

Mobile phase and Chromatographic system: Proceed as directed in the *Assay*.

Peak identification stock solution: 0.07 mg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound D RS, and USP Palonosetron Related Compound E RS in methanol. Sonicate as needed to dissolve.

Peak identification solution: 0.07 µg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound D RS, and USP Palonosetron Related Compound E RS from the *Peak identification stock solution* and 3.5 µg/mL of USP Palonosetron Hydrochloride RS from the *Standard stock solution* in *Mobile phase*

Sensitivity solution: 0.0175 µg/mL of USP Palonosetron Hydrochloride RS in *Mobile phase*

Sample stock solution: 0.175 mg/mL of Palonosetron Hydrochloride in methanol. Sonicate as needed to dissolve.

Sample solution: 0.035 mg/mL of Palonosetron Hydrochloride from the *Sample stock solution* in *Mobile phase*

System suitability

Sample: *Sensitivity solution*

Suitability requirements

Signal-to-noise ratio: NLT 10

Analysis

Samples: *Peak identification solution* and *Sample solution*

Chromatograph the *Peak identification solution* and identify specified impurities based on the relative retention times listed in *Table 2*.

Calculate the percentage of each unspecified impurity in the portion of Palonosetron Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_{\bar{U}}$ peak response of each unspecified impurity from the *Sample solution*

$r_{\bar{T}}$ sum of all peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Palonosetron related compound E ^a	0.91	—
Palonosetron related compound D ^a	0.94	—
Palonosetron	1.0	—
Palonosetron related compound B ^a	1.1	—
Palonosetron related compound A ^a	1.2	—
Any individual unspecified impurity	—	0.1
Total impurities ^b	—	1.0

^a These impurities are controlled in the test for *Limit of Specified Impurities* and are not to be reported here.

^b Total impurities includes impurities controlled in the test for *Limit of Specified Impurities* and in the test for *Limit of Unspecified Impurities*.

SPECIFIC TESTS

- **pH** 〈 791 〉
Sample solution: 10 mg/mL in water
Acceptance criteria: 5.0–6.0
- **Loss on Drying** 〈 731 〉
Analysis: Dry under vacuum at 80° for 3 h.
Acceptance criteria: NMT 1.0%
- **Microbial Enumeration Tests** 〈 61 〉 and **Tests for Specified Microorganisms** 〈 62 〉: The total aerobic microbial count does not exceed 10² cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g.
- **Bacterial Endotoxins Test** 〈 85 〉: Where the label states that Palonosetron Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 30 USP Endotoxin Units/mg of palonosetron hydrochloride.
- **Sterility Tests** 〈 71 〉: Meets the requirements where the label states that Palonosetron Hydrochloride is sterile

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature, protected from light.
- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states

that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

● **USP Reference Standards** { 11 }

USP Endotoxin RS

USP Palonosetron Enantiomer RS

(3*aR*)-2-[(3*R*)-Quinuclidin-3-yl]-2,3,3*a*,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride.

C₁₉H₂₄N₂O·HCl 332.87

USP Palonosetron Hydrochloride RS

USP Palonosetron Related Compound A RS

Palonosetron *N*-oxide;

(3*aS*)-1-Oxo-2-[(3*S*)-quinuclidin-3-yl]-2,3,3*a*,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinoline 1-oxide.

C₁₉H₂₄N₂O₂ 312.41

USP Palonosetron Related Compound B RS

Palonosetron-3-ene *N*-oxide;

(3*S*)-3-(1-Oxo-5,6-dihydro-1*H*-benzo[*de*]isoquinolin-2(4*H*)-yl)quinuclidine 1-oxide.

C₁₉H₂₂N₂O₂ 310.39

USP Palonosetron Related Compound C RS

Palonosetron diastereomer;

(3*aS*)-2-[(3*R*)-Quinuclidin-3-yl]-2,3,3*a*,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride.

C₁₉H₂₄N₂O·HCl 332.87

USP Palonosetron Related Compound D RS

Palonosetron 3*a*-epimer;

(3*aR*)-2-[(3*S*)-Quinuclidin-3-yl]-2,3,3*a*,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride.

C₁₉H₂₄N₂O·HCl 332.87

USP Palonosetron Related Compound E RS

Palonosetron-3-ene;

2-[(3*S*)-Quinuclidin-3-yl]-2,4,5,6-tetrahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride.

C₁₉H₂₂N₂O·HCl 330.85

▲**USP39**

BRIEFING

Palonosetron Injection. Because there is no existing *USP* monograph for this dosage form, a new monograph, based on validated methods of analyses, is proposed. The liquid chromatographic procedure in the *Assay* and in the test for *Limit of Unspecified Impurities* is based on analyses performed with the Zorbax SB-C8 brand of L7 column. The typical retention time for palonosetron is about 11 min. The liquid chromatographic procedure in the test for *Limit of Specified Impurities* is based on analyses performed with the Astec Chirobiotic-V brand of L88 column. The typical retention time for palonosetron is about 23–27 min.

(SM3: A. Wise, E. Gonikberg.)

Correspondence Number—C125959

Comment deadline: March 31, 2015

Add the following:

▲Palonosetron Injection

DEFINITION

Palonosetron Injection contains an amount of palonosetron hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of palonosetron ($C_{19}H_{24}N_2O$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY

• **Procedure**

Mobile phase: Acetonitrile, water, and trifluoroacetic acid (280: 720: 0.67)

Standard stock solution: 0.07 mg/mL of USP Palonosetron Hydrochloride RS in methanol. Sonicate as needed to dissolve. This solution is stable for 6 weeks if stored in the refrigerator and protected from light.

Standard solution: 0.014 mg/mL of USP Palonosetron Hydrochloride RS from the *Standard stock solution* in *Mobile phase*

Sample solution: Nominally 0.012 mg/mL of palonosetron from Injection in *Mobile phase*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm. When this procedure is used for *Identification* test *B*, use a diode array detector set at 200–400 nm.

Column: 4.6-mm × 25-cm; 5- μ m packing L7

Flow rate: 1 mL/min

Injection volume: 80 μ L

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of palonosetron ($C_{19}H_{24}N_2O$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Palonosetron Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of palonosetron in the *Sample solution* (mg/mL)

$M_{r\bar{r}}$ = molecular weight of palonosetron, 296.41

$M_{r\bar{z}}$ = molecular weight of palonosetron hydrochloride, 332.87

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Uniformity of Dosage Units** { 905 } : Meets the requirements

IMPURITIES

- **Limit of Specified Impurities**

Buffer: 1.5 g/L of ammonium acetate in water, adjusted with acetic acid to a pH of 6.0

Mobile phase: Tetrahydrofuran and *Buffer* (1:9)

System suitability stock solution: 0.07 mg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound C RS, USP Palonosetron Related Compound D RS, USP Palonosetron Related Compound E RS, and USP Palonosetron Enantiomer RS in methanol. Sonicate as needed to dissolve.

System suitability solution: 3.5 µg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound C RS, USP Palonosetron Related Compound D RS, USP Palonosetron Related Compound E RS, and USP Palonosetron Enantiomer RS from the *System suitability stock solution* and 50 µg/mL of USP Palonosetron Hydrochloride RS in *Buffer*

Impurity standard stock solution: 0.06 mg/mL of USP Palonosetron Related Compound D RS in methanol. Sonicate as needed to dissolve.

Impurity standard solution: 0.003 mg/mL of USP Palonosetron Related Compound D RS from the *Impurity standard stock solution* in *Buffer*

Sensitivity solution: 0.3 µg/mL of USP Palonosetron Related Compound D RS from the *Impurity standard stock solution* in *Buffer*

Sample solution: Use the Injection. (This solution has a concentration of 0.05 mg/mL of palonosetron.)

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: 238 nm

Column: 4.6-mm × 25-cm; 5-µm packing L88. [Note—A suitable column is Astec Chirobiotic-V from www.sigma-aldrich.com.]

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 200 µL

Run time: At least 2.5 times the retention time of palonosetron

System suitability

Samples: *System suitability solution*, *Impurity standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.4 between palonosetron related compound D and palonosetron enantiomer; NLT 1.2 between palonosetron enantiomer and palonosetron related compound E, *System suitability solution*

Tailing factor: NMT 2.5 for palonosetron related compound E, *System suitability solution*

Relative standard deviation: NMT 5.0% for palonosetron related compound D, *Impurity standard solution*

Signal-to-noise ratio: NLT 10 for palonosetron related compound D, *Sensitivity solution*

Analysis

Samples: *Impurity standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of palonosetron related compound D from the *Impurity standard solution*

C_S = concentration of USP Palonosetron Related Compound D RS in the *Impurity standard solution* (mg/mL)

C_U = nominal concentration of palonosetron in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Palonosetron related compound A ^a	0.45	—	—
Palonosetron related compound B ^a	0.54	—	—
Palonosetron	1.0	—	—
Palonosetron related compound C ^b	1.2	—	—
Palonosetron related compound D	1.3	1.0	0.5
Palonosetron enantiomer ^b	1.4	1.0	—
Palonosetron related compound E	1.5	2.5	0.5
^a These degradation products are controlled using the limit for unspecified impurities (see <i>Table 2</i>).			

^b These are included in the table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and should not be included in the total impurities.

- Limit of Unspecified Impurities**

Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Peak identification stock solution: 0.07 mg/mL each of USP Palonosetron Related

Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound D RS, and USP Palonosetron Related Compound E RS in methanol. Sonicate as needed to dissolve.

Peak identification solution: 0.07 µg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound D RS, and USP Palonosetron Related Compound E RS, from the *Peak identification stock solution* in *Mobile phase*

Sensitivity solution: 0.04 µg/mL of USP Palonosetron Hydrochloride RS from the *Standard solution* in *Mobile phase*

System suitability

Sample: *Sensitivity solution*

Suitability requirements

Signal-to-noise ratio: NLT 10 for palonosetron

Analysis

Samples: *Peak identification solution* and *Sample solution*

Chromatograph the *Peak identification solution* and identify specified impurities based on the relative retention times listed in *Table 2*.

Calculate the percentage of each unspecified impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of each unspecified impurity from the *Sample solution*

r_T sum of all peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Palonosetron related compound E ^a	0.91	—
Palonosetron related compound D ^a	0.94	—
Palonosetron	1.0	—
Palonosetron related compound B ^b	1.1	—
Palonosetron related compound A ^b	1.2	—
Any individual unspecified impurity	—	0.5
Total impurities ^c	—	2.0
^a These impurities are controlled in the test for <i>Limit of Specified Impurities</i> and are not to be reported here.		

^b These degradation products are controlled using the limit for unspecified impurities.

^c Total impurities includes impurities controlled in the test for *Limit of Specified Impurities* and in the test for *Limit of Unspecified Impurities*.

SPECIFIC TESTS

- **pH** 〈 791 〉: 4.5–5.5
- **Particulate Matter in Injections** 〈 788 〉: Meets the requirements
- **Bacterial Endotoxins Test** 〈 85 〉: NMT 30 USP Endotoxin Units/mg of palonosetron hydrochloride
- **Sterility Tests** 〈 71 〉: Meets the requirements
- **Other Requirements**: Meets the requirements in *Injections and Implanted Drug Products* 〈 1 〉

ADDITIONAL REQUIREMENTS

- **Packaging and Storage**: Preserve in tight containers. Store at controlled room temperature, protected from light.
- **USP Reference Standards** 〈 11 〉
 - USP Endotoxin RS
 - USP Palonosetron Enantiomer RS
(3aR)-2-[(3R)-Quinuclidin-3-yl]-2,3,3a,4,5,6-hexahydro-1H-benzo[de]isoquinolin-1-one hydrochloride.
C₁₉H₂₄N₂O·HCl 332.87
 - USP Palonosetron Hydrochloride RS
 - USP Palonosetron Related Compound A RS
Palonosetron N-oxide;
(3aS)-1-Oxo-2-[(3S)-quinuclidin-3-yl]-2,3,3a,4,5,6-hexahydro-1H-benzo[de]isoquinoline 1-oxide.
C₁₉H₂₄N₂O₂ 312.41
 - USP Palonosetron Related Compound B RS
Palonosetron-3-ene N-oxide;
(3S)-3-(1-Oxo-5,6-dihydro-1H-benzo[de]isoquinolin-2(4H)-yl)quinuclidine 1-oxide.
C₁₉H₂₂N₂O₂ 310.39
 - USP Palonosetron Related Compound C RS
Palonosetron diastereomer;
(3aS)-2-[(3R)-Quinuclidin-3-yl]-2,3,3a,4,5,6-hexahydro-1H-benzo[de]isoquinolin-1-one hydrochloride.
C₁₉H₂₄N₂O·HCl 332.87
 - USP Palonosetron Related Compound D RS
Palonosetron 3a-epimer;
(3aR)-2-[(3S)-Quinuclidin-3-yl]-2,3,3a,4,5,6-hexahydro-1H-benzo[de]isoquinolin-1-one hydrochloride.
C₁₉H₂₄N₂O·HCl 332.87
 - USP Palonosetron Related Compound E RS
Palonosetron-3-ene;
2-[(3S)-Quinuclidin-3-yl]-2,4,5,6-tetrahydro-1H-benzo[de]isoquinolin-1-one hydrochloride.
C₁₉H₂₂N₂O·HCl 330.85

▲USP39

BRIEFING

Perindopril Erbumine. Because there is no existing *USP* monograph for this drug substance,

a new monograph, based on validated methods of analysis, is being proposed.

1. The liquid chromatographic procedure in the *Assay* is based on analyses performed with the Waters Spherisorb brand of L7 column. The typical retention time for perindopril is about 12.5 min under the conditions specified in the *Assay*.
2. The liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with the Merck LiChroCART Superspher 60 Å RP8 brand of L7 column. The typical retention time for perindopril is about 27.2 min.
3. The liquid chromatographic procedure in the test for *Limit of Perindopril Related Compound A and Imidazole* is based on analyses performed with the Eclipse XDB-C8 brand of L7 column. The typical retention times for imidazole and perindopril related compound A are about 3.3 and 4.4 min, respectively.
4. The liquid chromatographic procedure in the test for *Limit of Perindopril Related Compound I* is based on analyses performed with the Waters Xterra RP 18 brand of L1 column. The typical retention times for perindopril and perindopril related compound I are about 12 and 19 min, respectively.

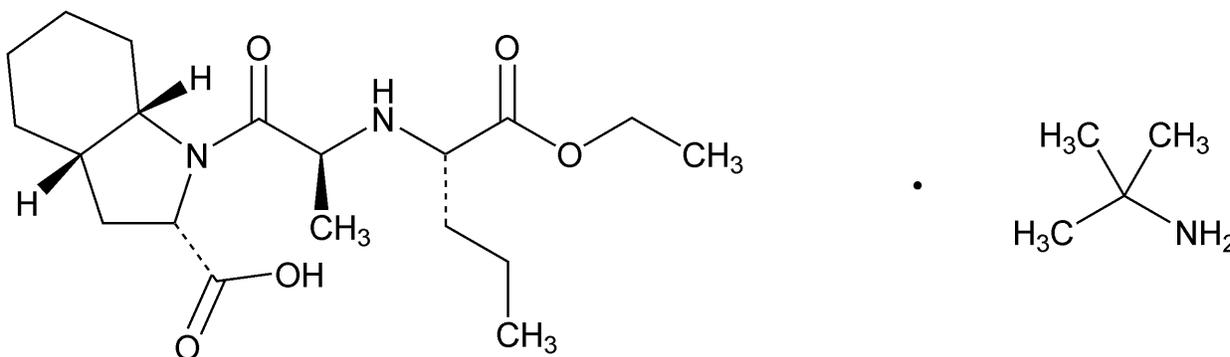
(SM2: S. Ramakrishna.)

Correspondence Number—C120791

Comment deadline: March 31, 2015

Add the following:

▲Perindopril Erbumine



$C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$ 441.6

1*H*-Indole-2-carboxylic acid, 1-[2-[[1-(ethoxycarbonyl)butyl]amino]-1-oxopropyl]octahydro-, [2*S*-[1[*R**(*R**)],2 α ,3 α β ,7 α β]-, compound with 2-methyl-2-propanamine (1:1); (2*S*,3 α *S*,7 α *S*)-1-[(*S*)-*N*-[(*S*)-1-Carboxybutyl]alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, compound with *tert*-butylamine (1:1); (2*S*,3 α *S*,7 α *S*)-1-[(*S*)-2-[(*R*)-1-Ethoxy-1-oxopentan-2-ylamino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid [107133-36-8].

DEFINITION

Perindopril Erbumine contains NLT 99.0% and NMT 101.0% of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈 197K 〉
- **B.** The retention time of the major peak of the *Sample solution* corresponds to the major peak of *System suitability solution 1*, as obtained in the test for *Limit of Perindopril Related Compound I*.

ASSAY

• Procedure

Buffer: Dissolve 0.92 g of sodium 1-heptanesulfonate in 1 L of water and add 1 mL of triethylamine. Adjust with a solution of perchloric acid and water (1:1) to a pH of 2.0.

Mobile phase: Acetonitrile and *Buffer* (35:65)

Standard solution: 0.1 mg/mL of USP Perindopril Erbumine RS in *Buffer*. Initially add *Buffer* to about 60% of the flask volume, sonicate to dissolve, and dilute with *Buffer* to volume.

Sample solution: 0.1 mg/mL of Perindopril Erbumine in *Buffer*. Initially add *Buffer* to about 60% of the flask volume, sonicate to dissolve, and dilute with *Buffer* to volume.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5- μ m packing L7

Column temperature: 60 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$) in the portion of Perindopril Erbumine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak area of perindopril from the *Sample solution*

r_S peak area of perindopril from the *Standard solution*

C_S concentration of USP Perindopril Erbumine RS in the *Standard solution* (mg/mL)

C_U concentration of Perindopril Erbumine in the *Sample solution* (mg/mL)

Acceptance criteria: 99.0%–101.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

• Organic Impurities

Solution A: Proceed as directed for the *Buffer* as described in the *Assay*.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
27	68	32
45	50	50
60	20	80
70	20	80
71	80	20
80	80	20

Diluent: *Solution B* and *Solution A* (20:80)

System suitability stock solution A: 0.03 mg/mL of USP Imidazole RS in *Diluent*

System suitability stock solution B: 0.03 mg/mL each of USP Perindopril Erbumine RS, USP Perindopril Related Compound B RS, USP Perindopril Related Compound C RS, USP Perindopril Related Compound D RS, and USP Perindopril Related Compound F RS in *Diluent*

System suitability solution: Transfer 5 mL each of *System suitability stock solution A* and *System suitability stock solution B* to a 50-mL volumetric flask and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- μ m pore size, discard the first 3 mL of filtrate, and use the clear filtrate.

Standard stock solution: 0.03 mg/mL of USP Perindopril Erbumine RS in *Diluent* prepared as follows. Dissolve a suitable quantity of USP Perindopril Erbumine RS in 80% of the total volume of *Diluent*, sonicate for about 5 min, and dilute with *Diluent* to volume.

Standard solution: 0.003 mg/mL of USP Perindopril Erbumine RS in *Diluent* from the *Standard stock solution*. Pass through a suitable filter of 0.45- μ m pore size and discard the first 3 mL of filtrate.

Sample solution: 3 mg/mL of Perindopril Erbumine in *Diluent* prepared as follows. Dissolve a suitable quantity of Perindopril Erbumine in 80% of the total volume of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- μ m pore size and discard the first 3 mL of filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.0-mm \times 25-cm; 4- μ m packing L7

Column temperature: 60 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *System suitability solution*

Suitability requirements

Tailing factor: NMT 1.5 for the perindopril peak

Relative standard deviation: NMT 5.0% for the perindopril peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Perindopril Erbumine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of perindopril erbumine from the *Standard solution*

C_S = concentration of USP Perindopril Erbumine RS in the *Standard solution* (mg/mL)

C_U = concentration of Perindopril Erbumine in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Imidazole ^a	0.08	—	—
Perindopril related compound B ^b	0.42	1.20	0.3
Perindopril related compound C ^c	0.74	0.96	0.1
Perindopril related compound D ^d	0.85	0.98	0.1
Perindopril erbumine	1.0	—	—
Perindopril related compound E ^e	1.38	0.85	0.2
Any individual unspecified impurity	—	—	0.10
Total impurities ^f	—	—	1

^a Imidazole is quantitated using the test for *Limit of Perindopril Related Compound A and Imidazole* and is included in the table for identification purposes only.

^b ((2*S*,3*aS*,7*aS*)-1-{(*S*)-2-[(*S*)-1-carboxybutylamino]propanoyl}octahydro-1*H*-indole-2-carboxylic acid).

^c (2*S*)-2-[(3*S*,5*aS*,9*aS*,10*aS*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid.

^d (2*S*)-2-[(3*S*,5*aS*,9*aS*,10*aR*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid.

^e Ethyl (2*S*)-2-[(3*S*,5*aS*,9*aS*,10*aS*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoate.

^f Total impurities include all specified and unspecified impurities and imidazole from the test for *Limit of Perindopril Related Compound A and Imidazole*. Perindopril related compound A is not included.

• **Limit of Perindopril Related Compound A and Imidazole**

Solution A: Proceed as directed in *Organic Impurities*.

Solution B: Acetonitrile

Mobile phase: See Table 3.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	83	17
5	83	17
6	20	80
20	20	80
21	83	17
33	83	17

Diluent: *Solution B* and *Solution A* (17:83)

Standard stock solution: 0.25 mg/mL of USP Perindopril Related Compound A RS and 0.1 mg/mL of USP Imidazole RS in *Diluent*. Sonicate if necessary.

Standard solution: 10 mg/mL of USP Perindopril Erbumine RS, 0.025 mg/mL of USP Perindopril Related Compound A RS, and 0.01 mg/mL of USP Imidazole RS in *Diluent* prepared as follows. Transfer a weighed amount of USP Perindopril Erbumine RS and a suitable amount of *Standard stock solution* to a suitable volumetric flask, and dissolve with sonication in *Diluent* equivalent to 60% of the final volume. Dilute with *Diluent* to volume, pass through a suitable filter of 0.45- μ m pore size, and discard the first 3 mL of filtrate.

Sample solution: 10 mg/mL of Perindopril Erbumine in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detectors

Perindopril related compound A: UV 210 nm

Imidazole: UV 225 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Temperatures

Column: 60 $^{\circ}$

Sample cooler: 5 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 50 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times for imidazole and perindopril related compound A are 1.0 and 1.4, respectively.]

Suitability requirements

Tailing factor: NMT 1.8 for perindopril related compound A

Relative standard deviation: NMT 5.0% for perindopril related compound A

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of perindopril related compound A or imidazole in the portion of Perindopril Erbumine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of perindopril related compound A or imidazole from the *Sample solution*

r_S peak response of perindopril related compound A or imidazole from the *Standard solution*

C_S concentration of USP Perindopril Related Compound A RS or USP Imidazole RS in the *Standard solution* (mg/mL)

C_U concentration of Perindopril Erbumine in the *Sample solution* (mg/mL)

Acceptance criteria

Perindopril related compound A: NMT 0.25%

Imidazole: NMT 0.1%

• Limit of Perindopril Related Compound I

Solution A: Dissolve 5 g of potassium phosphate monobasic in 1900 mL of water. Adjust with triethylamine to a pH of 6.50 and add 100 mL of acetonitrile.

Solution B: Acetonitrile

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	83	17
22	83	17
23	20	80
33	20	80
34	83	17
46	83	17

Diluent: *Solution B* and *Solution A* (17:83)

System suitability solution 1: 3 mg/mL of USP Perindopril Erbumine RS in *Diluent*. [Note —This solution is used in *Identification test B*.]

System suitability solution 2: 3 µg/mL of USP Perindopril Erbumine RS in *Diluent* from *System suitability solution 1*

Sample solution: 3 mg/mL of Perindopril Erbumine in *Diluent* prepared as follows. Dissolve a suitable quantity of Perindopril Erbumine in 80% of the total volume of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution 2*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 5.0%

Analysis

Sample: *Sample solution*

Calculate the percentage of perindopril related compound I in the portion of Perindopril Erbumine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of perindopril related compound I from the *Sample solution*

r_T total of all peak responses from the *Sample solution*

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

- **Water Determination, Method Ia** (921): NMT 1.0%
- **Optical Rotation, Specific Rotation** (781S)
Sample solution: 10 mg/mL of Perindopril Erbumine in ethanol
Acceptance criteria: -66° to -69° , at 20°

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers at controlled room temperature.
- **USP Reference Standards** (11)
 - USP Imidazole RS
 - USP Perindopril Erbumine RS
 - USP Perindopril Related Compound A RS
 [(2S,3aS,7aS)Octahydro-1H-indole-2-carboxylic acid] hydrochloride.
 $C_{17}H_{28}N_2O_5 \cdot HCl$ 205.68
 - USP Perindopril Related Compound B RS
 [(2S,3aS,7aS)-1-{(S)-2-[(S)-1-Carboxybutylamino]propanoyl}octahydro-1H-indole-2-carboxylic acid].
 $C_{17}H_{28}N_2O_5$ 340.41
 - USP Perindopril Related Compound C RS
 (S)-2-{(3S,5aS,9aS,10aS)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1H)-yl}pentanoic acid.
 $C_{17}H_{26}N_2O_4$ 322.40
 - USP Perindopril Related Compound D RS
 (S)-2-{(3S,5aS,9aS,10aR)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1H)-yl}pentanoic acid.
 $C_{17}H_{26}N_2O_4$ 322.40
 - USP Perindopril Related Compound F RS
 (S)-Ethyl 2-{(3S,5aS,9aS,10aS)-3-methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1H)-yl}pentanoate.
 $C_{19}H_{30}N_2O_4$ 350.45

▲USP39

BRIEFING

Perindopril Erbumine Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is being proposed.

1. The liquid chromatographic procedure in the *Assay* is based on analyses performed with the Merck Superspher 60 Å RP8 brand of L7 column. The typical retention time for perindopril is about 6.2 min.
2. The liquid chromatographic procedure in the *Dissolution* test is based on analyses performed with the Agilent Zorbax XDB-C8 brand of L7 column. The typical retention time for perindopril is about 4.2 min.
3. The liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with the Merck Superspher 60 Å RP8 brand of L7 column. The typical retention time for perindopril is about 27.2 min.
4. The liquid chromatographic procedure in the test for *Limit of Perindopril Related Compound I* is based on analyses performed with the Waters Xterra RP 18 brand of L1 column. The typical retention times for perindopril and perindopril related compound I are about 12 and 19 min, respectively.

(SM2: S. Ramakrishna.)

Correspondence Number—C96679; C120793

Comment deadline: March 31, 2015

Add the following:

▲Perindopril Erbumine Tablets

DEFINITION

Perindopril Erbumine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$).

IDENTIFICATION

- **A.** The UV absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

● **Procedure**

Solution A: Dissolve 0.92 g of sodium 1-heptanesulfonate in 1 L of water and add 1 mL of triethylamine. Adjust with a solution of perchloric acid and water (1:1) to a pH of 2.0.

Mobile phase: Acetonitrile and *Solution A* (38:62)

Diluent: Acetonitrile and *Solution A* (40:60)

Standard solution: 0.08 mg/mL of USP Perindopril Erbumine RS in *Diluent* prepared as follows. Dissolve a suitable quantity of USP Perindopril Erbumine RS in 80% of the total volume of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- μ m pore size and discard the first 3 mL of filtrate.

Sample solution: Nominally equivalent to 0.08 mg/mL of perindopril erbumine in *Diluent*

prepared as follows. Weigh and transfer the number of Tablets into a suitable volumetric flask, as indicated in *Table 1*.

Table 1

Tablet Strength	Number of Tablets (NLT)	Volumetric Flask, (mL)
2	20	500
4	10	500
8	10	1000

Add *Diluent* to about 70% of the flask volume, shake mechanically for about 60 min at 180 rpm, and sonicate for 20 min. Dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- μ m pore size and discard the first 3 mL of filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm \times 25-cm; 4- μ m packing L7

Temperatures

Column: 60 $^{\circ}$

Sample cooler: 5 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: NLT 2.5 times the retention time of perindopril

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Perindopril Erbumine RS in the *Standard solution* (mg/mL)

C_U nominal concentration of perindopril erbumine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- Dissolution** { 711 }

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Solution A: Proceed as directed in the *Assay*.

Mobile phase: Acetonitrile and *Solution A* (350:650)

Standard stock solution: 0.55 mg/mL of USP Perindopril Erbumine RS in acetonitrile

Standard solution: Prepare solutions of USP Perindopril Erbumine RS in *Medium* from the *Standard stock solution*, with final concentrations from *Table 2*.

Table 2

Tablet Strength (mg)	Concentration (mg/mL)
2	0.0022
4	0.0044
8	0.0088

Sample solution: Pass a portion of the solution under test through a suitable filter and discard the first 1 mL of filtrate.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 50°

Flow rate: 1.2 mL/min

Injection volume: 100 µL

Run time: NLT 1.6 times the retention time of perindopril

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of perindopril erbumine (C₁₉H₃₂N₂O₅·C₄H₁₁N) dissolved.

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (Q) of the labeled amount of perindopril erbumine (C₁₉H₃₂N₂O₅·C₄H₁₁N) is dissolved.

- **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

IMPURITIES● **Organic Impurities****Solution A:** Proceed as directed in the *Assay*.**Solution B:** Acetonitrile**Mobile phase:** See *Table 3*.**Table 3**

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
27	68	32
45	50	50
60	20	80
70	20	80
71	80	20
80	80	20

Diluent: *Solution B* and *Solution A* (20:80)**System suitability stock solution A:** 0.03 mg/mL of USP Imidazole RS in *Diluent***System suitability stock solution B:** 0.12 mg/mL each of USP Perindopril RelatedCompound C RS and USP Perindopril Related Compound D RS in *Diluent*. Initially add 80% of the total volume of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume.**System suitability solution:** Accurately weigh about 1.5 mg each of USP Perindopril Related Compound B RS and USP Perindopril Related Compound F RS into a 50-mL volumetric flask. Add 30 mL of *Diluent* and sonicate for 5 min. Transfer 5.0 mL each of *System suitability stock solution A*, *System suitability stock solution B*, and *Standard stock solution*. Dilute with *Diluent* to volume.**Standard stock solution:** 0.05 mg/mL of USP Perindopril Erbumine RS in *Diluent*. Dissolve a suitable quantity of USP Perindopril Erbumine RS in 80% of the total volume of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume.**Standard solution:** 0.005 mg/mL of USP Perindopril Erbumine RS in *Diluent* from the *Standard stock solution*. Pass through a suitable filter of 0.45- μ m pore size and discard the first 3 mL of filtrate.**Sample solution:** Nominally equivalent to 2 mg/mL of perindopril erbumine in *Diluent* prepared as follows. Transfer a quantity equivalent to about 20 mg of perindopril erbumine from powdered Tablets (NLT 20) into a test tube. Pipet 10.0 mL of *Diluent* into the test tube, sonicate for about 10 min, and vortex for about 1 min. Pass through a suitable filter of 0.45- μ m pore size and discard the first 3 mL of filtrate.**Chromatographic system**(See *Chromatography* { 621 }, *System Suitability*.) Proceed as directed in the *Assay*, except for the *Run time*. [Note—The run time is determined by the gradient from *Table 3*.]**System suitability****Sample:** *System suitability solution***Suitability requirements****Tailing factor:** NMT 1.5 for the perindopril peak**Relative standard deviation:** NMT 5.0%**Analysis**

Samples: *Standard solution and Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of perindopril erbumine from the *Standard solution*

C_S = concentration of USP Perindopril Erbumine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of perindopril erbumine in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (see *Table 4*)

Acceptance criteria: See *Table 4*. Disregard peaks less than 0.1%.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Imidazole ^a	0.08	—	—
Perindopril related compound B ^b	0.42	1.21	2.0
Perindopril related compound C ^c	0.74	0.97	0.5
Perindopril related compound D ^d	0.85	0.98	0.5
Perindopril erbumine	1.0	—	—
Perindopril related compound F ^e	1.38	0.86	2.0
Any unspecified impurity	—	—	0.2
Total impurities ^f	—	—	1.5

^a Imidazole is given for identification only and is not quantitated using this procedure.

^b (2*S*,3*aS*,7*aS*)-1-[(2*S*)-2-[[*(1S)*]-1-carboxybutyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid.

^c (2*S*)-2-[(3*S*,5*aS*,9*aS*,10*aS*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid.

^d (2*S*)-2-[(3*S*,5*aS*,9*aS*,10*aR*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid.

^e Ethyl (2*S*)-2-[(3*S*,5*aS*,9*aS*,10*aS*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoate.

^f Total impurities includes perindopril related compound I from the test for *Limit of Perindopril Related Compound I* and excludes perindopril related compound F and perindopril related compound B.

- **Limit of Perindopril Related Compound I**

Solution A: Dissolve 5 g of potassium phosphate monobasic in 1900 mL of water. Adjust

with triethylamine to a pH of 6.5 and add 100 mL of acetonitrile.

Solution B: Acetonitrile

Mobile phase: See Table 5.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	83	17
22	83	17
23	20	80
33	20	80
34	83	17
46	83	17

Diluent: *Solution A* and *Solution B* (17:83)

System suitability solution: 3 µg/mL of USP Perindopril Erbumine RS in *Diluent*. Sonicate for 5 min and pass through a suitable filter of 0.45-µm pore size.

Standard solution: 0.0125 mg/mL of USP Perindopril Erbumine RS in *Diluent* prepared as follows. Dissolve a suitable quantity of USP Perindopril Erbumine RS in 80% of the total volume of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of filtrate.

Sample solution: Nominally equivalent to 2 mg/mL of perindopril erbumine in *Diluent* prepared as follows. Transfer a quantity equivalent to about 16 mg of perindopril erbumine from powdered Tablets (NLT 20) into a test tube. Pipet 8.0 mL of *Diluent* into the test tube, sonicate for about 10 min, and vortex for about 1 min. Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Column: 60°

Sample cooler: 5°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for perindopril

Relative standard deviation: NMT 5.0% for perindopril

Analysis

Sample: *Sample solution*

Calculate the percentage of perindopril related compound I in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

\bar{r} peak response of perindopril related compound I from the *Sample solution*

r total of all peak responses from the *Sample solution*

Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in air-tight containers. Protect from heat and moisture.
- **USP Reference Standards** { 11 }

USP Imidazole RS

USP Perindopril Erbumine RS

USP Perindopril Related Compound B RS

[(2*S*,3*aS*,7*aS*)-1-{(*S*)-2-[(*S*)-1-Carboxybutylamino]propanoyl}octahydro-1*H*-indole-2-carboxylic acid].

C₁₇H₂₈N₂O₅ 340.41

USP Perindopril Related Compound C RS

(*S*)-2-{(3*S*,5*aS*,9*aS*,10*aS*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl}pentanoic acid.

C₁₇H₂₆N₂O₄ 322.40

USP Perindopril Related Compound D RS

(*S*)-2-{(3*S*,5*aS*,9*aS*,10*aR*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl}pentanoic acid.

C₁₇H₂₆N₂O₄ 322.40

USP Perindopril Related Compound F RS

(*S*)-Ethyl 2-{(3*S*,5*aS*,9*aS*,10*aS*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl}pentanoate.

C₁₉H₃₀N₂O₄ 350.45

▲USP39

BRIEFING

Pyrimethamine Compounded Oral Suspension, *USP 38* page 5096. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* { 7 } published in *USP 38-NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Pyrimethamine▲**Compounded**▲*USP39*

Oral Suspension

DEFINITION***Change to read:***

Pyrimethamine

▲**Compounded**▲*USP39*

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of pyrimethamine (C₁₂H₁₃ClN₄).

Prepare Pyrimethamine

▲**Compounded**▲*USP39*

Oral Suspension 2 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile*

Preparations 〈 795 〉).

Pyrimethamine powder	200 mg
Vehicle: a 1:1 mixture of Methylcellulose 1% Solution, and Syrup, <i>NF</i> , a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. Place *Pyrimethamine powder* in a suitable mortar. Add 2 mL of the *Vehicle*, and triturate to make a smooth paste. Add an additional 10 mL of the *Vehicle* to the paste, and levigate to form a suspension. Add increasing volumes of *Vehicle* to make a liquid that is pourable. Transfer the contents of the mortar to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY● **Procedure**

Mobile phase: Acetonitrile and 50 mM anhydrous sodium acetate (40:60). Pass through a PVDF membrane filter of 0.45-µm pore size, and degas.

System suitability solution: 0.20 mg/mL of USP Pyrimethamine RS (previously dried) and 0.10 mg/mL of USP Phenacetin RS (previously dried) in methanol

Standard solution: 0.20 mg/mL of USP Pyrimethamine RS (previously dried) in methanol

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.20 mg/mL of pyrimethamine from Oral Suspension in methanol. Sonicate, and mix on a vortex mixer.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Autosampler temperature: 10°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for phenacetin and pyrimethamine are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6.0 between the pyrimethamine and phenacetin peaks, *System suitability solution*

Column efficiency: NLT 8000 theoretical plates, *System suitability solution*

Tailing factor: NMT 1.5, *System suitability solution*

Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pyrimethamine ($C_{12}H_{13}ClN_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pyrimethamine from the *Sample solution*

r_S = peak response of pyrimethamine from the *Standard solution*

C_S = concentration of pyrimethamine in the *Standard solution* (mg/mL)

C_U = nominal concentration of pyrimethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- pH (791): 6.6–7.6

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at ~~controlled cold temperature~~

▲2°–8° ▲USP39

or at controlled room temperature.

Change to read:

- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded, when stored at ~~controlled cold temperature~~

▲2°–8° ▲USP39

or at controlled room temperature

- **Labeling:** Label it to indicate that it is to be well shaken before use, protect from light, and to state the *Beyond-Use Date*.

- **USP Reference Standards** (11)

USP Phenacetin RS

USP Pyrimethamine RS

Quinidine Sulfate Compounded Oral Suspension, *USP 38* page 5118. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* 〈7〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Quinidine Sulfate

▲▲Compounded▲*USP39*

Oral Suspension

DEFINITION

Change to read:

Quinidine Sulfate

▲Compounded▲*USP39*

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of quinidine sulfate [(C₂₀H₂₄N₂O₂)₂·H₂SO₄·2H₂O]. Prepare Quinidine Sulfate

▲Compounded▲*USP39*

Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈795〉).

Quinidine Sulfate	1 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), <i>NF</i> , and Vehicle for Oral Suspension, <i>NF</i> , a sufficient quantity to make	100 mL

If using *Quinidine Sulfate* tablets, place in a suitable mortar, and comminute into a fine powder, or add *Quinidine Sulfate* powder to the mortar. Add 15 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar to the calibrated bottle. Add sufficient *Vehicle* to volume, and mix well.

ASSAY

● **Procedure**

Solution A: Add 35.0 mL of methanesulfonic acid to 20.0 mL of glacial acetic acid, and dilute with water to 500 mL.

Solution B: Dissolve 10.0 mL of diethylamine in water to obtain 100 mL of solution.

Mobile phase: Acetonitrile, *Solution A*, *Solution B*, and water (10:1:1:40)

Standard solution: 100 µg/mL of USP Quinidine Sulfate RS in *Mobile phase*

Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at -70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix on a vortex mixer for 30 s. Pipet 1.0 mL of the *Sample solution* into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[Note—The retention time for quinidine sulfate is about 8.5 min.]

Suitability requirements

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of quinidine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$ in the volume of Oral Suspension taken:

$$\text{Result} = [(r_{B,U} + r_{D,U}) / (r_{B,S} + r_{D,S})] \times (C_S / V) \times 100$$

$r_{B,\bar{U}}$ peak response of quinidine from the *Sample solution*

$r_{D,\bar{U}}$ peak response of dihydroquinidine from the *Sample solution*

$r_{B,\bar{S}}$ peak response of quinidine from the *Standard solution*

$r_{D,\bar{S}}$ peak response of dihydroquinidine from the *Standard solution*

C_S = concentration of USP Quinidine Sulfate RS in the *Standard solution* (mg/mL)

V = volume of Oral Suspension taken (mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** 〈 791 〉: 3.4–4.4

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at room temperature, or in a cold place.

Change to read:

- **Beyond-Use Date:** 60 days after the day on which it was compounded

▲when stored at room temperature, or in a cold place▲*USP39*

- **Labeling:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP Reference Standards** { 11 }
 USP Quinidine Sulfate RS

BRIEFING

Rifabutin Compounded Oral Suspension, *USP 38* page 5169. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* { 7 } published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Rifabutin

▲**Compounded**▲*USP39*

Oral Suspension

DEFINITION

Change to read:

Rifabutin

▲**Compounded**▲*USP39*

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of rifabutin (C₄₆H₆₂N₄O₁₁).

Prepare Rifabutin

▲**Compounded**▲*USP39*

Oral Suspension 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* { 795 }).

Rifabutin capsules ^a equivalent to	3 g
Vehicle: a 1:1 mixture of Ora-Sweet ^b and Ora-Plus ^b , a sufficient quantity to make	150 mL
^a Mycobutin 150-mg capsules, Pfizer Inc., New York, NY. ^b Paddock Laboratories, Minneapolis, MN.	

Calculate the required quantity of each ingredient for the total amount to be prepared. Empty

the required number of capsules in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a rifabutin liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

• Procedure

Solution A: 100 mM monobasic potassium phosphate buffer, adjusted with 2 N sodium hydroxide to a pH of 6.5

Mobile phase: Acetonitrile and *Solution A* (50:50). Filter and degas.

Standard stock solution: 2.0 mg/mL of USP Rifabutin RS in *Mobile phase*

Standard solution: Transfer 10 mL of *Standard stock solution* to a 100-mL volumetric flask. Immediately rinse the volumetric apparatus with 10 mL of acetonitrile and 10 mL of *Mobile phase*. Dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 0.2 mg/mL of rifabutin. Pass through a filter of 0.45- μ m pore size.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of Oral Suspension into a 100-mL volumetric flask. Immediately rinse the pipette with 10 mL of acetonitrile and 10 mL of *Mobile phase*. Dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 0.2 mg/mL of rifabutin. Pass through a filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Flow rate: 1.0 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

[Note—The retention time for the rifabutin is about 14.0 min.]

Suitability requirements

Column efficiency: NLT 4500 theoretical plates

Tailing factor: NMT 3.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of rifabutin ($C_{46}H_{62}N_4O_{11}$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of rifabutin in the *Standard solution* (mg/mL)

C_U nominal concentration of rifabutin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** 〈 791 〉: 4.5–5.5

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at ~~controlled cold temperature~~

▲2°–8° ▲USP39

or at controlled room temperature.

Change to read:

- **Beyond-Use Date:** NMT 84 days after the date on which it was compounded, when stored at ~~controlled cold temperature~~

▲2°–8° ▲USP39

or at controlled room temperature

- **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

- **USP Reference Standards** 〈 11 〉

USP Rifabutin RS

BRIEFING

Rifampin Compounded Oral Suspension, *USP 38* page 5172. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compdial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* 〈 7 〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Rifampin

▲▲Compounded▲USP39

Oral Suspension

DEFINITION**Change to read:**

Rifampin

▲Compounded▲USP39

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of rifampin ($C_{43}H_{58}N_4O_{12}$).

Prepare Rifampin

▲Compounded▲USP39

Oral Suspension

▲10 mg/mL▲USP39

as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈 795 〉).

Rifampin	1.20 g
Citric Acid or Sodium Citrate	To adjust pH
Syrup, a sufficient quantity to make	120 mL

Empty the required number of capsules into a suitable mortar, or use *Rifampin* powder. If necessary, gently crush the capsule contents with a pestle to produce a fine powder. Add 2 mL of *Syrup* to the mortar, and triturate until a smooth paste is formed. Add 10 mL of *Syrup*, and triturate to form a suspension. Continue to add *Syrup* until 80 mL has been added. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add *Syrup* in portions to rinse the mortar, and add the rinses to the bottle. If necessary, add *Citric Acid* or *Sodium Citrate* to adjust to a pH of 5.0. Add a suitable flavor if desired. Add sufficient *Syrup* to bring the preparation to final volume. Shake vigorously.

ASSAY● **Procedure**

Solution A: 1.0 M monobasic potassium phosphate containing 6.3 mL/L of phosphoric acid

Solution B: Acetonitrile, 1.0 M dibasic potassium phosphate, 1.0 M monobasic potassium phosphate, 1.0 M citric acid, and water (25: 7.7: 2.3: 1: 64)

Mobile phase: Acetonitrile, *Solution A*, 1.0 M citric acid, 0.5 M sodium perchlorate, and water (36:10:2:2:50). Pass through a suitable filter of 0.7- μ m or finer pore size, and degas.

Diluent: Acetonitrile and water (50:50)

System suitability solution: 0.1 mg/mL of USP Rifampin RS and 0.1 mg/mL of USP Rifampin Quinone RS in acetonitrile. Transfer 1.0 mL of the solution into a 10-mL volumetric flask, and dilute with *Solution B* to volume.

Standard solution: 0.5 mg/mL of USP Rifampin RS in *Diluent*. If necessary, sonicate for 30 s to dissolve. Transfer 5.0 mL of the solution to a 50-mL, low-actinic volumetric flask, and dilute with *Diluent* to volume. Use the solution within 1 h.

Sample solution: Transfer 5.0 mL of Oral Suspension, freshly mixed and free of air bubbles, to a 100-mL, low-actinic volumetric flask, and dissolve in and dilute with *Diluent* to volume. Transfer 5.0 mL of the resulting solution to a 50-mL, low-actinic volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC**Detector:** UV 254 nm**Column:** 4.6-mm × 10-cm; 5-μm packing L7**Injection volume:** 20 μL**System suitability****Samples:** *System suitability solution* and *Standard solution*

[Note—The relative retention times for rifampin quinone and rifampin are about 0.6 and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 4.0 between rifampin quinone and rifampin, *System suitability solution***Relative standard deviation:** NMT 1.0% for replicate injections, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of rifampin (C₄₃H₅₈N₄O₁₂) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak area from the *Sample solution* r_S peak area from the *Standard solution* C_S concentration of USP Rifampin RS in the *Standard solution* (mg/mL) C_U nominal concentration of rifampin in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**SPECIFIC TESTS**

- **pH** { 791 } : 4.5–5.5

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in a tight, light-resistant glass or plastic prescription bottle with a child-resistant closure. Store at controlled room temperature.

Change to read:

- **Beyond-Use Date:** NMT 30 days after the date on which it was compounded

▲when stored at controlled room temperature▲USP39

- **Labeling:** Label it to state that it is to be well shaken. Label it to state that it contains 50 mg of rifampin per 5 mL of Oral Suspension.

- **USP Reference Standards** { 11 }

USP Rifampin RS USP Rifampin Quinone RS **BRIEFING**

Sildenafil Compounded Oral Suspension, USP 38 page 5293. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded

preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* 〈 7 〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Sildenafil Citrate

▲▲Compounded▲*USP39*

Oral Suspension

DEFINITION

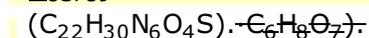
Change to read:

Sildenafil Citrate

▲Compounded▲*USP39*

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of sildenafil citrate

▲▲*USP39*



▲▲*USP39*

Prepare Sildenafil Citrate

▲Compounded▲*USP39*

Oral Suspension

▲containing▲*USP39*

2.5 mg/mL

▲of sildenafil▲*USP39*

as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈 795 〉).

Sildenafil Citrate tablets ^a equivalent to	250 mg
Vehicle: a 1:1 mixture of Ora-Sweet ^b and Ora-Plus ^b , a sufficient quantity to make	100 mL
<p>^a Viagra 25-mg tablets, Pfizer Inc., New York, NY.</p> <p>^b Paddock Laboratories, Minneapolis, MN.</p>	

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar, and comminute to a fine powder with a pestle. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing

volumes of the *Vehicle* to make a sildenafil citrate

▲▲USP39

liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

Change to read:

● Procedure

Mobile phase: Acetonitrile and 0.2 M ammonium acetate (50:50). Pass through a nylon 66 filter of 0.45- μ m pore size, and degas.

Standard stock solution: 2.5 mg/mL of

▲sildenafil prepared from USP▲USP39

Sildenafil Citrate

▲RS▲USP39

in *Mobile phase* [~~Note—The *Standard solutions* should be prepared from the appropriate reference material.~~]

▲▲USP39

Standard solution: 0.25 mg/mL of sildenafil citrate

▲▲USP39

prepared from *Standard stock solution* and *Mobile phase*

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.25 mg/mL of sildenafil citrate

▲▲USP39

from Oral Suspension and *Mobile phase*, and centrifuge.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 3.0-mm \times 15-cm; 5- μ m packing L1

Column temperature: 25 $^{\circ}$

Flow rate: 0.5 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

[~~Note—The retention time for sildenafil citrate~~

▲▲USP39

is about 7.1 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sildenafil citrate

▲▲USP39



▲▲USP39

in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of sildenafil citrate

▲▲USP39

in the *Standard solution* (mg/mL)

C_U = nominal concentration of sildenafil citrate

▲▲USP39

in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- pH (791): 3.9–4.9

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at ~~controlled cold~~ temperature

▲2°–8° ▲USP39

or at controlled room temperature.

Change to read:

- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded, when stored at ~~controlled cold~~ temperature

▲2°–8° ▲USP39

or at controlled room temperature

- **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

Add the following:

- ▲• **USP Reference Standards (11)**

USP Sildenafil Citrate RS

▲USP39

BRIEFING

Simvastatin Tablets, USP 38 page 5300. As part of USP monograph modernization efforts, it is proposed to make following changes:

1. Add *Identification* test *B* to strengthen the monograph.
2. Remove the concentration of acid or base for the adjustment of pH in the *Assay*.

3. Preparation of the *Sample solution* in the *Assay* is modified to accommodate different Tablet strengths.
4. Delete *Capacity factor* and *Column efficiency* from the system suitability requirements in the *Assay* as the remaining requirements adequately measure the system suitability. Use of diode array detector for the *Assay* is included in order to support the proposed addition of *Identification* test *B*.
5. Add a new stability indicating liquid chromatographic procedure for the test for *Organic Impurities*. The liquid chromatographic procedure is based on analysis performed with the Agilent Zorbax Eclipse XDB of L1 packing. The typical retention time for simvastatin is about 31 min.
6. Add USP Tenvastatin Calcium RS and USP Lovastatin RS under the section for *USP Reference Standards* to support the proposed revision for the test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: R.S. Prasad.)

Correspondence Number—C136694

Comment deadline: March 31, 2015

Simvastatin Tablets

DEFINITION

Simvastatin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of simvastatin ($C_{25}H_{38}O_5$).

IDENTIFICATION

Change to read:

-

▲A. ▲USP39

The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

- ▲● B. The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP39

ASSAY

Change to read:

- **Procedure**

Buffer solution: Dissolve 3.9 g of monobasic sodium phosphate in 900 mL water. Adjust, if necessary, with either 50%

▲▲USP39

sodium hydroxide or 85%

▲▲USP39

phosphoric acid to a pH of 4.5. Dilute with water to 1000 mL and mix.

Mobile phase: Acetonitrile and *Buffer solution* (65:35)

Solution A: Add 3.0 mL of glacial acetic acid to 900 mL of water. Adjust with 5 N sodium hydroxide to a pH of 4.0 and dilute with water to 1 L.

Diluent: Acetonitrile and *Solution A* (8:2)

Standard solution: 0.1 mg/mL of USP Simvastatin RS in *Diluent*

Sample solution: Prepare nominally 0.1 mg/mL of simvastatin from Tablets in *Diluent* as follows. Transfer NLT 10 Tablets to a suitable volumetric flask. Add a small volume of water (NMT 4% of the total volume), and swirl to disintegrate the Tablets. Dilute with *Diluent* to volume, sonicate for 15 min, and cool to room temperature. Centrifuge a portion of the mixture, and use the clear supernatant.

▲Crush NLT 20 Tablets into a fine powder and transfer the powder equivalent to 100 mg of simvastatin to a suitable volumetric flask. Add *Diluent* to fill 70% of the volume of the flask, and sonicate with intermittent swirling for 10 min. Equilibrate to room temperature and dilute with *Diluent* to volume. Centrifuge a portion of the mixture, and pass the clear supernatant through a filter of 0.45 µm pore size. Discard first few mL of filtrate. ▲USP39

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 238 nm.

▲For *Identification* test B, use diode array detector in the range of 190 nm–300 nm. ▲USP39

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 45°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor: NLT 3.0

Column efficiency: NLT 4500 theoretical plates

▲▲USP39

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of simvastatin (C₂₅H₃₈O₅) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of simvastatin from the *Sample solution*

r_S = peak area of simvastatin from the *Standard solution*

C_S concentration of USP Simvastatin RS in the *Standard solution* (mg/mL)

C_T nominal concentration of simvastatin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution 〈 711 〉

Medium: Prepare a pH 7.0 buffer solution containing 0.5% sodium dodecyl sulfate in 0.01 M sodium phosphate as follows. Dissolve 30 g of sodium dodecyl sulfate and 8.28 g of monobasic sodium phosphate in 6000 mL of water and adjust with 50% (w/v) sodium hydroxide solution to a pH of 7.0; 900 mL.

Apparatus 2: 50 rpm

Time: 30 min

Prewashed manganese dioxide: Transfer 10 g of manganese dioxide to a suitable container, and treat as follows. Add 50 mL of *Medium*, and shake vigorously for 5 min. Centrifuge, decant the supernatant layer, and discard. Repeat twice, first with *Medium* and then with water. Dry the solid at 100° for 1 h before use.

Standard solution: USP Simvastatin RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Transfer a portion of the solution to a centrifuge tube containing about 10 mg of *Prewashed manganese dioxide* per mL of transferred solution under test, and mix. Allow the mixture to stand for 30 min with occasional shaking, centrifuge, and use a portion of the clear supernatant.

Instrumental conditions

Mode: UV

Analytical wavelength: 247 nm and 257 nm

Blank: Proceed as directed for the *Sample solution*, except use the *Medium*.

Analysis: Calculate the percentage of the labeled amount of simvastatin ($C_{25}H_{38}O_5$) dissolved from the difference between the UV absorbances at the wavelengths of maximum and minimum absorbances at about 247 and 257 nm, respectively, of the *Sample solution*, in comparison with the *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of simvastatin ($C_{25}H_{38}O_5$) is dissolved.

• Uniformity of Dosage Units 〈 905 〉: Meet the requirements

IMPURITIES

Add the following:

▲• Organic Impurities

Solution A: Prepare a mixture of acetonitrile and dilute phosphoric acid (1 mL in 1 L of water) (45:55).

Solution B: Prepare a mixture of acetonitrile and dilute phosphoric acid (1 mL in 1 L of water) (90:10).

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
------------	----------------	----------------

0	100	0
38	100	0
40	65	35
50	65	35
70	0	100
90	0	100
92	100	0
100	100	0

Solution C: 1.4 g/L of monobasic potassium phosphate in water. Adjust with diluted ammonia solution to a pH of 7.0.

Diluent: Acetonitrile and *Solution C* (60:40)

System suitability stock solution: 0.2 mg/mL each of USP Simvastatin RS and USP Lovastatin RS in *Diluent*. Sonicate if necessary.

System suitability solution: 0.02 mg/mL each of USP Simvastatin RS and USP Lovastatin RS in *Diluent* from *System suitability stock solution*

Standard stock solution: 0.15 mg/mL of USP Simvastatin RS and 1.3 mg/mL of USP Tenivastatin Calcium RS in *Diluent*. Sonicate if necessary.

Standard solution: 7.5 µg/mL of USP Simvastatin RS and 0.026 mg/mL of USP Tenivastatin Calcium RS in *Diluent* from *Standard stock solution*

Sample solution: Prepare nominally 1.5 mg/mL of simvastatin in *Diluent* as follows.

Transfer a suitable amount of powdered Tablets (NLT 20) to a volumetric flask. Add *Diluent* to fill 60% of the volume of the flask, and sonicate with intermittent swirling for 5 min. Dilute with *Diluent* to volume. Pass the solution through a filter of 0.45 µm pore size. Discard the first few mL of filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 238 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Temperatures

Column: 30°

Autosampler: 10°

Flow rate: 2.0 mL/min

Injection volume: 20 µL

Run time: NLT 3 times the retention time of simvastatin

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 8.0 between simvastatin and lovastatin, *System suitability solution*

Tailing factor: NMT 1.5 for simvastatin, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tenivastatin in the portion of Tablets taken:

$$\text{Result} = (r_{11}/r_{12}) \times (C_{12}/C_{11}) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of tenivastatin from the *Sample solution*

r_S = peak response of tenivastatin from the *Standard solution*

C_S = concentration of USP Tenivastatin Calcium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of simvastatin in the *Sample solution* (mg/mL)

M_{rT} = molecular weight of tenivastatin, 436.58

M_{rZ} = molecular weight of tenivastatin calcium, 929.24

Calculate the percentage of any unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_I = peak response of any unspecified impurity from the *Sample solution*

r_S = peak response of simvastatin from the *Standard solution*

C_S = concentration of USP Simvastatin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of simvastatin in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tenivastatin ^a	0.43	0.80
Lovastatin ^{b,c}	0.63	—
Epilovastatin ^{b,d}	0.66	—
Methylene simvastatin ^{b,e}	0.80	—
Simvastatin	1.0	—
Tenivastatin methyl ester ^{b,f}	1.14	—
Acetyl simvastatin ^{b,g}	1.54	—
Anhydro simvastatin ^{b,h}	1.59	—
Simvastatin related compound D ^{b,i}	2.30	—
Individual unspecified impurity	—	0.2
Total impurities ^j	—	2.0

^a (3R,5R)-7-(((1S,2S,6R,8S,8aR)-8-[(2,2)-Dimethylbutanoyl]oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl)-3,5-dihydroxyheptanoic acid.

^b Process impurity included in the table for identification purposes only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

^c (S)-(1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-Hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2-methylbutanoate.

^d (R)-(1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-Hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl}-3,7-

dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2-methylbutanoate.

^e (1*S*,3*R*,7*S*,8*S*,8*aR*)-8-{2-[(2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbut-3enoate.

^f Methyl (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8*aR*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoate.

^g (1*S*,3*R*,8*S*,8*aR*)-8-{2-[(2*R*,4*R*)-4-Acetoxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate.

^h (1*S*,3*R*,7*S*,8*S*,8*aR*)-3,7-Dimethyl-8-{2-[(*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]ethyl}-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate.

ⁱ (3*R*,5*R*)-(2*R*,4*R*)-2-(2-[(1*S*,2*S*,6*R*,8*S*,8*aR*)-8-[(2,2-Dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]ethyl)-6-oxotetrahydro-2*H*-pyran-4-yl 7-[(1*S*,2*S*,6*R*,8*S*,8*aR*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoate.

^j Excluding Lovastatin.

▲*USP39*

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

Change to read:

- **USP Reference Standards** { 11 }

▲USP Lovastatin RS

(*R*)-(1*S*,3*R*,7*S*,8*S*,8*aR*)-8-{2-[(2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2-methylbutanoate.

C₂₄H₃₆O₅ 404.54▲*USP39*

USP Simvastatin RS

▲USP Tenivastatin Calcium RS

Calcium (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8*aR*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoate (1:2) monohydrate.

C₅₀H₇₈CaO₁₂·H₂O 929.24▲*USP39*

BRIEFING

Sodium Bromide Compounded Injection, Veterinary, *USP 37* page 4706. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also

Compounded Preparations in general chapter *Labeling* 〈 7 〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Sodium Bromide

▲Compounded ▲*USP39*

Injection, Veterinary

DEFINITION

Change to read:

Sodium Bromide

▲Compounded ▲*USP39*

Injection, Veterinary contains an amount of Sodium Bromide equivalent to NLT 21.0 mg and

NMT 25.6 mg of bromide (Br^-) per mL.

Prepare Sodium Bromide

▲Compounded ▲*USP39*

Injection, Veterinary as follows (see *Pharmaceutical Compounding—Sterile Preparations* 〈 797 〉).

Sodium Bromide	3.0 g
Sterile Water for Injection, <i>USP</i> , a sufficient quantity to make	100 mL

Dissolve the *Sodium Bromide* in *Sterile Water for Injection* with mixing. Sterilize by a suitable means such as sterile filtration or autoclaving.

ASSAY

• **Procedure**

TCA solution: 20% (w/v) Trichloroacetic acid in water

Gold chloride solution: 5 mg/mL of gold chloride in water

Standard stock solution: Dissolve USP Sodium Bromide RS in water to obtain a solution with a nominal concentration of 20 mg/mL of bromide.

Standard solutions: Prepare four solutions of known concentrations of about 2.0, 1.0, 0.5, and 0.25 mg/mL of bromide from *Standard stock solution* and water.

Sample solution: Dilute Injection, Veterinary quantitatively with water (1:19).

Blank: Water

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* 〈 851 〉.)

Mode: Vis

Analytical wavelength: 440 nm

System suitability

Samples: *Standard solutions* and *Blank*

Suitability requirements

Correlation coefficient: NLT 0.99, linear regression of the *Standard solutions*

Analysis

Samples: *Sample solution* and *Blank*

To 750- μ L aliquots of each *Sample* add 500 μ L of *TCA solution* and 250 μ L of *Gold chloride solution*. Mix on a vortex mixer, and immediately read the absorbance of each *Sample*.

Calculate the concentration of bromide (Br^-), in mg/mL, in the portion of Injection, Veterinary taken:

$$\text{Result} = C \times D$$

C = concentration of the *Sample solution* (mg/mL) calculated from the standard curve

D = dilution factor of the *Sample solution* (20)

Acceptance criteria: 21.0–25.6 mg/mL of bromide (Br^-)

SPECIFIC TESTS

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 0.03 USP Endotoxin Units/mg of sodium bromide
- **Sterility Tests** 〈 71 〉: Meets the requirements
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in single-dose containers, preferably of Type I glass, and store in a refrigerator.

Change to read:

- **Beyond-Use Date:**

▲In the absence of passing a sterility test and endotoxin test, the storage conditions for *Pharmaceutical Compounding—Sterile Preparations* 〈 797 〉, *High-Risk Level CSPs* apply. After successful completion of sterility and endotoxin testing, ▲*USP39*

NMT 180 days after the date on which it was compounded

▲when stored in a refrigerator. ▲*USP39*

- **Labeling:** Label to state the *Beyond-Use Date* and to state the nominal content of sodium bromide in the Injection, Veterinary. Label to state that it is to be kept out of the reach of children. Label to state that it is for infusion only at a rate not to exceed 150 mg of sodium bromide per kg of body weight per hour. Label to state that it is for veterinary use only.
- **USP Reference Standards** 〈 11 〉
USP Endotoxin RS

USP Sodium Bromide RS

BRIEFING

Sodium Bromide Compounded Oral Solution, Veterinary, *USP 38* page 5311. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* 〈 7 〉 published in *USP 38-NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:**Sodium Bromide**

▲Compounded▲*USP39*

Oral Solution, Veterinary

DEFINITION**Change to read:**

Sodium Bromide

▲Compounded▲*USP39*

Oral Solution, Veterinary contains an amount of sodium bromide equivalent to NLT 151 mg and

NMT 185 mg of bromide (Br^-) per mL.

Prepare Sodium Bromide

▲Compounded▲*USP39*

Oral Solution, Veterinary as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈 795 〉).

Sodium Bromide	21.6 g
Purified Water	60 mL
Corn Syrup, FCC, a sufficient quantity to make	100 mL

Dissolve the *Sodium Bromide* in *Purified Water*. Add *Corn Syrup* to bring the preparation to final volume with mixing.

ASSAY

- **Procedure**

TCA solution: 20% (w/v) trichloroacetic acid in water

Gold chloride solution: 5 mg/mL of gold chloride in water

Standard stock solution: Dissolve USP Sodium Bromide RS in water to obtain a solution with a nominal concentration of 20 mg/mL of bromide.

Standard solutions: Prepare four solutions of known concentrations of about 2.0, 1.0, 0.5, and 0.25 mg/mL of bromide from *Standard stock solution* in water.

Sample solution: Dilute Oral Solution, Veterinary quantitatively with water (1:99).

Blank: Water

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* 〈 851 〉.)

Mode: Vis

Analytical wavelength: 440 nm

System suitability

Samples: *Standard solutions* and *Blank*

Suitability requirements

Correlation coefficient: NLT 0.99, linear regression of the *Standard solutions*

Analysis

Samples: *Sample solution* and *Blank*

To 750- μ L aliquots of each *Sample* add 500 μ L of *TCA solution* and 250 μ L of *Gold chloride solution*. Mix on a vortex mixer, and immediately read the absorbance of each *Sample*.

Calculate the concentration of bromide (Br^-), in mg/mL, in the portion of Oral Solution, Veterinary taken:

$$\text{Result} = C \times D$$

C = concentration of the *Sample solution* (mg/mL) calculated from the standard curve

D = dilution factor of the *Sample solution* (100)

Acceptance criteria: 151–185 mg/mL of bromide (Br^-)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in a tight container, and store in a refrigerator.

Change to read:

- **Beyond-Use Date:** NMT 180 days after the date on which it was compounded

▲when stored in a refrigerator▲*USP39*

- **Labeling:** Label to state the *Beyond-Use Date* and to state the nominal content of sodium bromide in the Oral Solution, Veterinary. Label to state that it is to be kept out of the reach of children and to state that it is for veterinary use only.

- **USP Reference Standards** 〈 11 〉

USP Sodium Bromide RS

BRIEFING

Sodium Hypochlorite Compounded Topical Solution, *USP 38* page 5323. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify

that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* 〈7〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Sodium Hypochlorite

▲▲Compounded▲▲*USP39*

Topical Solution

DEFINITION

Change to read:

Sodium Hypochlorite

▲Compounded▲*USP39*

Topical Solution contains NLT 0.20 g and NMT 0.32 g of sodium hypochlorite (NaClO) in 1000 mL of Topical Solution.

Prepare Sodium Hypochlorite

▲Compounded▲*USP39*

Topical Solution as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈795〉).

Sodium Hypochlorite Solution	5.0 mL
Monobasic Sodium Phosphate, monohydrate	1.02 g
Dibasic Sodium Phosphate, anhydrous	17.61 g
Purified Water, a sufficient quantity to make	1000 mL

Dissolve the *Dibasic Sodium Phosphate, anhydrous* and the *Monobasic Sodium Phosphate, monohydrate* in about 500 mL of *Purified Water*. Add the *Sodium Hypochlorite Solution* and sufficient *Purified Water* to bring the preparation to final volume, and mix.

[Note—The source of the *Sodium Hypochlorite Solution* may be commercial unscented laundry bleach, nominally 5.25% (w/v) provided that the commercial laundry bleach was acquired recently.]

ASSAY

● **Procedure**

Sample: 50.0 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: To the *Sample* in a glass-stoppered flask add 0.5 g of potassium iodide and 10 mL of 6 N acetic acid. Titrate the liberated iodine with *Titrant*, adding 2 mL of starch TS as the endpoint is approached. Perform a blank determination and make any necessary correction. Each mL of *Titrant* is equivalent to 3.722 mg of sodium hypochlorite (NaClO).

Acceptance criteria: 0.20–0.32 g in 1000 mL

SPECIFIC TESTS

- pH $\langle 791 \rangle$: 7.8–8.2

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant, 1-L plastic containers, and store at controlled room temperature.

Change to read:

- **Beyond-Use Date:** NMT 7 days after the date on which it was compounded

▲when stored at controlled room temperature▲*USP39*

- **Labeling:** Label it to indicate that its strength is 0.025%, and to state the *Beyond-Use Date*. [Note—For external use only; it may be applied to wounds and burns.]

BRIEFING

Sodium Phenylbutyrate Compounded Oral Suspension, *USP 38* page 5330. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* $\langle 7 \rangle$ published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Sodium Phenylbutyrate

▲**Compounded**▲*USP39*

Oral Suspension

DEFINITION

Change to read:

Sodium Phenylbutyrate

▲Compounded▲USP39

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of sodium phenylbutyrate (C₁₀H₁₁O₂Na).

Prepare Sodium Phenylbutyrate

▲Compounded▲USP39

Oral Suspension 200 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile*

Preparations { 795 }).

Sodium Phenylbutyrate powder ^a	20 g
Vehicle: a 1:1 mixture of Ora-Sweet ^b (regular or sugar-free) and Ora-Plus ^b , a sufficient quantity to make	100 mL
<p>^a Ucyclyd Pharma, Inc., Scottsdale, AZ.</p> <p>^b Paddock Laboratories, Minneapolis, MN.</p>	

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the *Sodium Phenylbutyrate powder* in a suitable mortar, and comminute to a fine powder with a pestle. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a sodium phenylbutyrate liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY● **Procedure**

Mobile phase: Acetonitrile and 5 mM phosphoric acid (40:60). Filter and degas.

Standard solution: 0.1 mg/mL of sodium phenylbutyrate in *Mobile phase*. [Note—The *Standard solution* should be prepared from the appropriate reference material.]

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.1 mg/mL of sodium phenylbutyrate from Oral Suspension and *Mobile phase*.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 218 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 60°

Flow rate: 1.0 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

[Note—The retention time for sodium phenylbutyrate is about 3.0 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sodium phenylbutyrate ($C_{10}H_{11}O_2Na$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of sodium phenylbutyrate in the *Standard solution* (mg/mL)

C_U nominal concentration of sodium phenylbutyrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** $\langle 791 \rangle$: 7.0–8.0

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature.
- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature
- **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

BRIEFING

Sotalol Hydrochloride Compounded Oral Suspension, *USP 38* page 5344. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* $\langle 7 \rangle$ published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Sotalol Hydrochloride

▲Compounded▲USP39

Oral Suspension

DEFINITION

Change to read:

Sotalol Hydrochloride

▲Compounded▲USP39

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of sotalol hydrochloride ($C_{12}H_{20}N_2O_3S \cdot HCl$).

Prepare Sotalol Hydrochloride

▲Compounded▲USP39

Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈 795 〉).

Sotalol Hydrochloride tablets ^a equivalent to	600 mg
Vehicle: a 1:1 mixture of Ora-Sweet ^b and Ora-Plus ^b , a sufficient quantity to make	120 mL
<p>^a Betapace 120-mg tablets, Berlex Laboratories, Wayne, NJ.</p> <p>^b Paddock Laboratories, Minneapolis, MN.</p>	

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar, and comminute to a fine powder with a pestle. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a sotalol hydrochloride liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY● **Procedure**

Mobile phase: Acetonitrile and 5 mM octanesulfonic acid (25:75), adjusted to a pH of 3.2.

Pass through a nylon 66 filter of 0.45- μ m pore size, and degas.

Standard solution: 20 μ g/mL of USP Sotalol Hydrochloride RS in *Mobile phase*

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 20 μ g/mL of sotalol hydrochloride from Oral Suspension and *Mobile phase*. Centrifuge.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 3.0-mm \times 15-cm; 5- μ m packing L1

Flow rate: 0.4 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

[Note—The retention time for sotalol hydrochloride is about 5.1 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sotalol hydrochloride ($C_{12}H_{20}N_2O_3S \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of sotalol hydrochloride in the *Standard solution* ($\mu\text{g/mL}$)

C_U nominal concentration of sotalol hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** $\langle 791 \rangle$: 3.8–4.8

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at ~~controlled cold temperature~~

$\blacktriangle 2^\circ - 8^\circ$ \blacktriangle *USP39*

or at controlled room temperature.

Change to read:

- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded when stored at ~~controlled cold temperature~~

$\blacktriangle 2^\circ - 8^\circ$ \blacktriangle *USP39*

or at controlled room temperature

- **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

- **USP Reference Standards** $\langle 11 \rangle$

USP Sotalol Hydrochloride RS

BRIEFING

Spirolactone and Hydrochlorothiazide Compounded Oral Suspension, *USP 38* page 5351. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* $\langle 7 \rangle$ published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Spirolactone and Hydrochlorothiazide

▲**Compounded**▲*USP39*

Oral Suspension

DEFINITION

Change to read:

Spirolactone and Hydrochlorothiazide

▲**Compounded**▲*USP39*

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of spironolactone (C₂₄H₃₂O₄S) and hydrochlorothiazide (C₇H₈ClN₃O₄S₂).

Prepare Spirolactone 5 mg/mL and Hydrochlorothiazide 5 mg/mL Oral Suspension

▲an oral suspension containing 5 mg/mL of spironolactone and 5 mg/mL of hydrochlorothiazide

▲*USP39*

as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈 795 〉).

Spirolactone and Hydrochlorothiazide tablets ^a equivalent to	500 mg and 500 mg
Vehicle: a 1:1 mixture of Ora-Sweet ^b (regular or sugar-free) and Ora-Plus ^b , a sufficient quantity to make	100 mL
<p>^a Spirolactone and hydrochlorothiazide 25-mg/25-mg tablets, Mylan Pharmaceutical Inc., Morgantown, WV.</p> <p>^b Paddock Laboratories, Minneapolis, MN.</p>	

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a spironolactone and hydrochlorothiazide liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

● **Procedure**

Mobile phase: Methanol and water (70:30). Filter and degas.

Standard solution: 0.1 mg/mL of USP Spirolactone RS and 0.1 mg/mL of USP Hydrochlorothiazide RS in methanol

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.1 mg/mL of spironolactone and 0.1 mg/mL of hydrochlorothiazide from Oral Suspension and methanol. Centrifuge.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

[Note—The retention times for hydrochlorothiazide and spironolactone are about 3.5 and 7.4 min, respectively.]

Suitability requirements

Relative standard deviation: NMT 2.0% for spironolactone and NMT 2.0% for hydrochlorothiazide for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of spironolactone ($C_{24}H_{32}O_4S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of spironolactone in the *Standard solution* (mg/mL)

C_U nominal concentration of spironolactone in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Oral Suspension taken by the same formula, changing the terms to refer to hydrochlorothiazide.

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- pH (791): 3.8–4.8

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at ~~controlled cold temperature~~

▲2°–8° ▲USP39

or at controlled room temperature.

Change to read:

- **Beyond-Use Date:** NMT 60 days after the date on which it was compounded, when stored at ~~controlled cold temperature~~

▲2°–8° ▲USP39

or controlled room temperature

- **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP Reference Standards** { 11 }
 USP Hydrochlorothiazide RS
 USP Spironolactone RS

BRIEFING

Sumatriptan Compounded Oral Suspension, *USP 38* page 5418. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compdial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* { 7 } published in *USP 38-NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Sumatriptan Succinate

▲▲Compounded▲*USP39*

Oral Suspension

DEFINITION

Change to read:

Sumatriptan Succinate

▲Compounded▲*USP39*

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of sumatriptan (C₁₄H₂₁N₃O₂S).

Prepare Sumatriptan Succinate

▲Compounded▲*USP39*

Oral Suspension containing ~~7 mg/mL of sumatriptan succinate equivalent to~~

▲▲*USP39*

5 mg/mL of sumatriptan as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* { 795 }).

Sumatriptan (as the ▲Sumatriptan▲ ^{USP39} Succinate)	500 mg (700 mg)
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), <i>NF</i> and Vehicle for Oral Suspension, <i>NF</i> , a sufficient quantity to make	100 mL

Place the required number of tablets in a suitable mortar, and comminute to a fine powder, or add *Sumatriptan Succinate* powder to the mortar. Add 25 mL of *Vehicle* in portions, mixing thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY

Change to read:

● Procedure

Mobile phase: Acetonitrile and 0.01 M dibutylamine in 0.025 M aqueous monobasic sodium phosphate dihydrate (25:75), adjusted with 1 N sodium hydroxide to a pH of 8.0. Filter and degas.

~~**Internal standard solution:** 3.0 mg/mL of *N*-hydroxymethylsumatriptan succinate in *Mobile phase*~~

▲▲^{USP39}

Standard stock solution: 4.0 mg/mL of USP Sumatriptan Succinate RS in *Mobile phase*

Standard solution: 0.12 mg/mL of sumatriptan prepared with *Standard stock solution* and *Mobile phase*. ~~Each solution contains 30 µg/mL of *N*-hydroxymethylsumatriptan succinate from *Internal standard solution*.~~

▲▲^{USP39}

Sample solution: 0.15 mg/mL of sumatriptan succinate

▲▲^{USP39}

prepared from Oral Suspension and 0.1 M hydrochloric acid. Pass through a 0.22-µm syringe filter into a 0.3-mL polypropylene sample vial.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 282 nm

Column: 4.6-mm × 10-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[Note—The retention times

▲▲^{USP39}

for sumatriptan and ~~*N*-hydroxymethylsumatriptan~~ are

▲is▲USP39

11 and 14

▲▲USP39

min. , respectively

▲▲USP39

]

Suitability requirements**Relative standard deviation:** NMT 1.5% for replicate injections**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of sumatriptan ($C_{14}H_{21}N_3O_2S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak response from the *Sample solution* r_S peak response from the *Standard solution* C_S concentration of sumatriptan in the *Standard solution* (mg/mL) C_U nominal concentration of sumatriptan in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**SPECIFIC TESTS**

- **pH** (791): 3.6–4.6

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant containers. Store in a cold place.

Change to read:

- **Beyond-Use Date:** NMT 14 days after the date on which it was compounded

▲when stored in a cold place.▲USP39

Change to read:

- **Labeling:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*. Label to indicate that it contains ~~7 mg/mL of sumatriptan succinate equivalent~~ to

▲▲USP39

5 mg/mL of sumatriptan.

- **USP Reference Standards** (11)

USP Sumatriptan Succinate RS **BRIEFING**

Tacrolimus Compounded Oral Suspension, *USP 38* page 5433. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp->

nf/development-process/compdial-nomenclature). See also *Compounded Preparations* in general chapter *Labeling* 〈 7 〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Tacrolimus

▲Compounded▲*USP39*

Oral Suspension

DEFINITION

Change to read:

Tacrolimus

▲Compounded▲*USP39*

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$).

Prepare Tacrolimus

▲Compounded▲*USP39*

Oral Suspension 0.5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈 795 〉).

Tacrolimus capsules ^a equivalent to	50 mg
Vehicle: a 1:1 mixture of Ora-Plus ^b and Syrup, <i>NF</i> , a sufficient quantity to make	100 mL
^a Prograf 5-mg capsules, Astellas Pharma US, Inc., Deerfield, IL. ^b Paddock Laboratories, Minneapolis, MN.	

Calculate the quantity of each ingredient required for the total amount to be prepared. Empty the required number of capsules in a suitable mortar. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a tacrolimus liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well. Tacrolimus powder is not interchangeable with *Tacrolimus capsules* and should not be used.

ASSAY

● **Procedure**

Mobile phase: Acetonitrile and deionized distilled water (65:35). Filter and degas.

Standard stock solution: 0.5 mg/mL of USP Tacrolimus RS in acetonitrile

Standard solution: Pipet 1.0 mL of *Standard stock solution* into a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution having a nominal concentration of 50 µg/mL of tacrolimus. [Note—The *Standard solution* is relatively unstable, and the *Assay* should proceed immediately.]

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of Oral Suspension to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 50 µg/mL of tacrolimus. [Note—The *Sample solution* is relatively unstable, and the *Assay* should proceed immediately.]

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 70°

Flow rate: 1.7 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[Note—The retention time for tacrolimus is about 6.4 min.]

Suitability requirements

Column efficiency: NLT 2500 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tacrolimus (C₄₄H₆₉NO₁₂) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of tacrolimus in the *Standard solution* (µg/mL)

C_U nominal concentration of tacrolimus in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** 〈 791 〉: 4.1–5.1

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature.
- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature
- **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the

Beyond-Use Date.

- **USP Reference Standards** 〈 11 〉

USP Tacrolimus RS

BRIEFING

Temozolomide Compounded Oral Suspension, *USP 38* page 5481. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* 〈 7 〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Temozolomide

▲**Compounded**▲*USP39*

Oral Suspension

DEFINITION

Change to read:

Temozolomide

▲**Compounded**▲*USP39*

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of temozolomide ($C_6H_6N_6O_2$).

Prepare Temozolomide

▲**Compounded**▲*USP39*

Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈 795 〉).

Temozolomide capsules, ^a equivalent to	1 g
Povidone K-30, <i>USP</i>	500 mg
Anhydrous Citric Acid, <i>USP</i>	25 mg
Purified Water, <i>USP</i>	1.5 mL
Vehicle: a 1:1 mixture of Ora-Plus ^b and Ora-Sweet ^b (regular or sugar-free), a sufficient quantity to make	100 mL

- | |
|--|
| a Temodar 100-mg capsules, Schering Corporation, Whitehouse, NJ. |
| b Paddock Laboratories, Minneapolis, MN. |

Calculate the required quantity of each ingredient for the total amount to be prepared. Empty the required number of capsules in a suitable mortar. Add the *Povidone K-30* powder to the mortar, and triturate to a fine powder. [Note—Povidone is critical for physical stability.] Dissolve the *Anhydrous Citric Acid* in *Purified Water*. Add the mixture of *Anhydrous Citric Acid* and *Purified Water* to the mortar to wet the powder. Mix thoroughly to form a uniform paste. Add the *Vehicle* in small portions, and triturate to make a smooth mixture. Add increasing volumes of the *Vehicle* to make a temozolomide liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well. It is necessary to wear appropriate personal protective equipment and to perform this procedure in a Biologic Safety Cabinet. [Caution—Temozolomide is cytotoxic. Great care should be taken to prevent inhaling particles of temozolomide and exposure to the skin.]

ASSAY

• Procedure

Solution A: 10 mM ammonium phosphate adjusted to a pH of 3.25

Mobile phase: Methanol and *Solution A* (12:88). Filter and degas.

Standard stock solution: 0.5 mg/mL of USP Temozolomide RS in *Mobile phase*. Transfer the mixture to a centrifuge tube, and centrifuge at $1,500 \times g$ for 10 min. Pass the supernatant through a filter of 0.45- μm pore size, into a glass vial.

Standard solution: 0.1 mg/mL of temozolomide prepared from *Standard stock solution* and *Mobile phase*

Sample solution: Shake thoroughly each bottle of Oral Suspension. Immediately transfer 2.5 mL of Oral Suspension to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume. Vortex the sample for 30 s, centrifuge for 10 min at $1,500 \times g$, and pass through a membrane filter of 0.22- μm pore size. Pass at least 0.5 mL of sample through the filter before collecting the sample in a vial. Transfer 1.0 mL of the resultant solution to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 0.1 mg/mL of temozolomide.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L1

Flow rate: 1.0 mL/min

Injection volume: 15 μL

System suitability

Sample: *Standard solution*

[Note—The retention time for temozolomide is about 7.9 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of temozolomide ($C_6H_6N_6O_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of temozolomide in the *Standard solution* (mg/mL)

C_U nominal concentration of temozolomide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- pH (791): 3.5–4.5

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at ~~controlled cold~~ temperature

▲2°–8° . ▲USP39

Change to read:

- **Beyond-Use Date:** NMT 60 days after the date on which it was compounded, when stored at ~~controlled cold~~ temperature

▲2°–8° ▲USP39

- **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

- **USP Reference Standards (11)**

USP Temozolomide RS

BRIEFING

Terbinafine Compounded Oral Suspension, USP 38 page 5490. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling (7)* published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Terbinafine

▲▲Compounded▲▲USP39

Oral Suspension

DEFINITION

Change to read:

Terbinafine

▲Compounded▲USP39

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled content of terbinafine hydrochloride

▲▲USP39

(C₂₁H₂₅N)·HCl

▲▲USP39

Prepare Terbinafine

▲Compounded▲USP39

Oral Suspension (~~28.1 mg/mL as hydrochloride~~) equivalent to 25 mg of Terbinafine per mL

▲containing 25 mg/mL of terbinafine▲USP39

as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈 795 〉).

Terbinafine (as ▲Terbinafine▲USP39 Hydrochloride)	2500 mg (2810 mg)
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, <i>NF</i> , and Vehicle for Oral Suspension, <i>NF</i> , a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using tablets, place the required number of tablets in a suitable mortar, and comminute the tablets to a fine powder or add *Terbinafine Hydrochloride* powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a terbinafine suspension that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

Change to read:

● **Procedure**

Mobile phase: Acetonitrile and water (2:3), with 0.15% triethylamine and 0.15% phosphoric acid. Make adjustments if necessary.

Standard stock solution: 1.0 mg/mL of USP Terbinafine Hydrochloride RS in methanol

Standard solution: Transfer 0.5 mL of *Standard stock solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume to obtain a solution containing 5 µg/mL of

terbinafine, hydrochloride

▲▲USP39

and pass through a suitable filter of 0.22- μ m pore size.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Accurately pipet 1.0 mL to a 25-mL volumetric flask. Dilute with methanol to volume to obtain a nominal concentration of 1 mg/mL of terbinafine, hydrochloride

▲▲USP39

Mix the sample again. Accurately pipet 1.0

▲0.5▲USP39

mL of the diluted terbinafine hydrochloride

▲▲USP39

solution to a 10-mL

▲100-mL▲USP39

volumetric flask, and dilute with *Mobile phase* to volume to obtain a nominal concentration of 5 μ g/mL of terbinafine, hydrochloride

▲▲USP39

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 224 nm

Column: 4.6-mm \times 15-cm; 3.5- μ m packing L1

Flow rate: 0.4 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

[Note—The retention time of the terbinafine peak is 5.1 min.]

Suitability requirements

Relative standard deviation: NMT 5.8%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of terbinafine ($C_{21}H_{25}N$)-HCl

▲▲USP39

in the volume of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of terbinafine hydrochloride

▲▲USP39

in the *Standard solution* (μ g/mL)

C_U = nominal concentration of terbinafine hydrochloride

▲▲USP39

in the *Sample solution* (μ g/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** 〈 791 〉 : 5.3–5.7

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature or ~~controlled cold temperature~~

▲2°–8° .▲USP39

Change to read:

- **Beyond-Use Date:** NMT 30 days after the date on which it was compounded when stored at controlled room temperature or at ~~controlled cold temperature~~

▲2°–8° .▲USP39

- **Labeling:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP Reference Standards** 〈 11 〉
USP Terbutaline Hydrochloride RS

BRIEFING

Terbutaline Sulfate Compounded Oral Suspension, *USP 38* page 5492. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* 〈 7 〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:**Terbutaline**

▲Sulfate Compounded ▲USP39

Oral Suspension

DEFINITION**Change to read:**

Terbutaline

▲Sulfate Compounded▲*USP39*

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled content of terbutaline sulfate [(C₁₂H₁₉NO₃)₂·H₂SO₄]. Prepare Terbutaline

▲Sulfate Compounded▲*USP39*

Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈 795 〉).

Terbutaline Sulfate	100 mg
Syrup, <i>NF</i> , ^a a sufficient quantity to make	100 mL
^a Syrup, <i>NF</i> , containing 0.2% sodium benzoate.	

Calculate the required quantity of each ingredient for the total amount to be prepared. If using tablets, place the required number of tablets in a suitable mortar, and comminute the tablets to a fine powder or add *Terbutaline Sulfate* powder. Add the *Syrup, NF*, to make a terbutaline

▲sulfate▲*USP39*

suspension that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Syrup, NF*, to bring to final volume, and mix well.

ASSAY**Change to read:**● **Procedure**

Mobile phase: A solution of methanol and 20 mM monobasic potassium phosphate (2:23), adjusted with phosphoric acid to a pH of 3.6. Filter and degas.

Standard stock solution: 5 mg/mL of USP Terbutaline Sulfate RS in methanol

Standard solution: Transfer 0.2 mL of *Standard stock solution* to a 100-mL volumetric flask, dilute with water to volume to obtain a solution containing 10 µg/mL of terbutaline sulfate, and pass through a suitable filter of 0.22-µm pore size.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Accurately pipet 1.0 mL to a 10-mL volumetric flask. Dilute with *Mobile phase* to volume to obtain a nominal concentration of 100 µg/mL of terbutaline sulfate. Extract terbutaline sulfate from the suspension with methanol. Accurately pipet 1 mL of Oral Suspension and 3 mL of *Mobile phase* in the barrel of a 5-mL plastic syringe. Shake, and pass through a suitable filter of 0.22-µm pore size into a 10-mL volumetric flask. Repeat the process with an additional 2 mL of methanol. Bring to a final volume of 10 mL with *Mobile phase* to obtain a nominal concentration of 10 µg/mL of terbutaline sulfate.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 3.9-mm × 30-cm; 10-µm microphenyl packing L11

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[Note—The retention time for the terbutaline

▲sulfate▲*USP39*

peak is 5 min.]

Suitability requirements

Relative standard deviation: NMT 2.2% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of terbutaline sulfate $[(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4]$ in the volume of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of terbutaline sulfate in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of terbutaline sulfate in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at ~~controlled cold~~ temperature

▲2°–8° .▲*USP39*

Change to read:

- **Beyond-Use Date:** NMT 30 days after the date on which it was compounded when stored at ~~controlled cold~~ temperature

▲2°–8° ▲*USP39*

- **Labeling:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.

- **USP Reference Standards** { 11 }

USP Terbutaline Sulfate RS

BRIEFING

Tetracycline Hydrochloride Compounded Oral Suspension, *USP 38* page 5517. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* { 7 } published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at

<http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Tetracycline Hydrochloride

▲▲Compounded▲▲USP39

Oral Suspension

DEFINITION

Change to read:

Tetracycline Hydrochloride

▲▲Compounded▲▲USP39

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of tetracycline hydrochloride. ~~in 100 mL of Oral Suspension~~

▲▲USP39

Prepare Tetracycline Hydrochloride

▲▲Compounded▲▲USP39

Oral Suspension

▲25 mg/mL▲USP39

as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Tetracycline Hydrochloride	2.50 g
Cetylpyridinium Chloride	10 mg
Xanthan Gum	0.15 g
Dibasic Sodium Phosphate	60 mg
Monobasic Sodium Phosphate	0.65 g
Sodium Hydroxide	0.30 g
Purified Water	35 mL
Suspension Structured Vehicle or Sugar-Free Suspension Structured Vehicle, a sufficient quantity to make	100 mL

Dissolve *Dibasic Sodium Phosphate* and *Monobasic Sodium Phosphate* in 25 mL of *Purified Water*. Separately dissolve a quantity of *Cetylpyridinium Chloride* in *Purified Water* and dilute, if

necessary, with *Purified Water* to obtain 5 mL of a solution containing 10 mg of *Cetylpyridinium Chloride*. Mix this solution with 5 mL of the aqueous phosphate solution and add the resulting solution, in divided portions, with mixing, to the *Tetracycline Hydrochloride* in a glass mortar to completely wet the powder, and make a smooth paste.

Transfer the remaining 20 mL of the aqueous phosphate solution to a beaker. Using moderate heat, stir to form a vortex, and slowly sprinkle the *Xanthan Gum* into the vortex to produce a uniform dispersion. Add this dispersion to the paste in the glass mortar, and mix until smooth; then add 20 mL of the *Suspension Structured Vehicle* or *Sugar-Free Suspension Structured Vehicle* to the mixture.

Dissolve the *Sodium Hydroxide* in 5 mL of *Purified Water*, and while mixing, slowly add this solution to the prepared mixture. Complete the suspension by adding a sufficient quantity of the *Suspension Structured Vehicle* or *Sugar-Free Suspension Structured Vehicle* to make a final volume of 100 mL, and pass this final dispersion through a hand homogenizer prior to transferring it to the dispensing container.

ASSAY

• Procedure

Diluent: Dimethylformamide and ammonium oxalate (27:68)

Mobile phase: Dimethylformamide, 0.1 M ammonium oxalate, and 0.2 M dibasic ammonium phosphate (27:68:5). Adjust, if necessary, with 3 N ammonium hydroxide or 3 N phosphoric acid to a pH of 7.6–7.7.

System suitability solution: 100 µg/mL of tetracycline hydrochloride and 25 µg/mL of USP 4-Epianhydrotetracycline Hydrochloride RS

Standard solution: 0.5 mg/mL of USP Tetracycline Hydrochloride RS in *Diluent*

Sample solution: Equivalent to 125 mg of tetracycline hydrochloride, from Oral Suspension, to a 250-mL volumetric flask, add 200 mL of *Diluent*, and shake. Add *Diluent* to volume and filter.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Columns

Pre-injection guard: 4.6-mm × 3-cm; 10-µm packing L7

Analytical: 4.6-mm × 25-cm; 5–10-µm packing L7

Flow rate: 2 mL/min

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for 4-epianhydrotetracycline and tetracycline are about 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.2 between the 4-epianhydrotetracycline and tetracycline peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCL$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Tetracycline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of tetracycline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0–110.0%

SPECIFIC TESTS

- **pH** 〈 791 〉: 3.5–6.0

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature, and protect from freezing.

Change to read:

- **Beyond-Use Date:** NMT 30 days after the date on which it was compounded

▲when stored at controlled room temperature▲*USP39*

Change to read:

- **Labeling:**

▲Label it to state the *Beyond-Use Date*.▲*USP39*

Label it to state that it should not be frozen and that it is to be well shaken before using.

- **USP Reference Standards** 〈 11 〉

USP 4-Epianhydrotetracycline Hydrochloride RS

USP Tetracycline Hydrochloride RS

BRIEFING

Theophylline Compounded Oral Suspension, *USP 38* page 5528. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compdial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* 〈 7 〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:**Theophylline**▲▲**Compounded**▲*USP39*

Oral Suspension

DEFINITION**Change to read:**

Theophylline

▲**Compounded**▲*USP39*

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of theophylline (C₇H₈N₄O₂).

Prepare Theophylline

▲**Compounded**▲*USP39*

Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile*

Preparations 〈 795 〉).

Theophylline	500 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), <i>NF</i> , and Vehicle for Oral Suspension, <i>NF</i> , a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. Calculate the amount of *Theophylline* on the anhydrous basis. If using tablets, place the required number in a suitable mortar, and comminute to a fine powder, or use *Theophylline* powder. Add about 20 mL of the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated container. Add enough of the liquid *Vehicle* to bring to final volume, and mix well.

ASSAY● **Procedure**

Solution A: Dissolve 2.500 g of sodium citrate and 1.633 g of citric acid (anhydrous) in 850 mL of ASTM Type I water, add 150 mL of methanol, and pass through 0.45-µm membrane filters.

Solution B: Acetonitrile

Mobile phase: See *Table 1*. Make adjustments, if necessary.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0.0
20.0	100	0.0
20.01	5.0	95.0
25.0	5.0	95.0
25.01	100	0.0

Diluent: *Solution A*

System suitability solution: 0.10 mg/mL of USP Theophylline RS and 0.20 mg/mL of USP

Caffeine RS in *Solution A*

Standard solution: 0.10 mg/mL of USP Theophylline RS in *Solution A*

Sample solution: 0.10 mg/mL of theophylline prepared from Oral Suspension and *Solution A*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 3.0 between the theophylline and caffeine peaks

Column efficiency: NLT 1000 theoretical plates for the theophylline peak

Tailing factor: NMT 1.5 for the theophylline peak

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of theophylline in the *Standard solution* (mg/mL)

C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** 〈 791 〉: 4.0–5.0

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature. Do not refrigerate.
- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature
- **Labeling:** Label to state that it is to be well shaken before use, protected from light, not to be refrigerated, and to state the *Beyond-Use Date*.
- **USP Reference Standards** 〈 11 〉
 - USP Caffeine RS
 - USP Theophylline RS

BRIEFING

Tiagabine Hydrochloride Compounded Oral Suspension, *USP 38* page 5565. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* 〈7〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Tiagabine Hydrochloride

▲▲Compounded▲*USP39*

Oral Suspension

DEFINITION

Change to read:

Tiagabine Hydrochloride

▲Compounded▲*USP39*

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled content of tiagabine hydrochloride (C₂₀H₂₅NO₂S₂·HCl). Prepare Tiagabine Hydrochloride

▲Compounded▲*USP39*

Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile*

Preparations 〈795〉).

Tiagabine Hydrochloride	100 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, <i>NF</i> , and Vehicle for Oral Suspension, <i>NF</i> , a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using tablets, place the required number of tablets in a suitable mortar, and comminute the tablets to a fine powder or add *Tiagabine Hydrochloride* powder. Add the *Vehicle* in small portions and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a tiagabine

▲hydrochloride▲*USP39*

suspension that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

Change to read:● **Procedure**

Mobile phase: Acetonitrile and 5 mM octanesulfonic acid (50:50). Pass through a suitable filter of 0.45- μm pore size and degas.

Standard stock solution: 1.0 mg/mL of USP Tiagabine Hydrochloride RS in methanol

Standard solution: Transfer 0.2 mL of *Standard stock solution* to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution containing 20 $\mu\text{g}/\text{mL}$ of tiagabine hydrochloride. Centrifuge, and pass through a suitable filter of 0.22- μm pore size.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Centrifuge, and pass through a suitable filter of 0.22- μm pore size. Accurately pipet 0.2 mL of the Oral Suspension to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a nominal concentration of 20 $\mu\text{g}/\text{mL}$ of tiagabine hydrochloride.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Flow rate: 0.4 mL/min

Column: 3.0-mm \times 15-cm; 5- μm packing L10

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

[Note—The retention time of the tiagabine

▲hydrochloride▲USP39

peak is 3.2 min.]

Suitability requirements

Relative standard deviation: NMT 1.7%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tiagabine hydrochloride ($\text{C}_{20}\text{H}_{25}\text{NO}_2\text{S}_2\cdot\text{HCl}$) in the volume of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of tiagabine hydrochloride in the *Standard solution* ($\mu\text{g}/\text{mL}$)

C_U nominal concentration of tiagabine hydrochloride in the *Sample solution* ($\mu\text{g}/\text{mL}$)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

● **pH** 〈 791 〉: 4.0–4.5

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature or ~~controlled cold temperature~~

▲at 2°–8° .▲USP39

Change to read:

- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded, when stored at ~~controlled cold temperature~~

▲2°–8° ;▲USP39

NMT 60 days after the date it was compounded, when stored at controlled room temperature

- **Labeling:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.

- **USP Reference Standards** 〈 11 〉

USP Tiagabine Hydrochloride RS

BRIEFING

Tramadol Hydrochloride Compounded Oral Suspension, *USP 38* page 5631. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* 〈 7 〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Tramadol Hydrochloride

▲**Compounded**▲USP39

Oral Suspension

DEFINITION

Change to read:

Tramadol Hydrochloride

▲**Compounded**▲USP39

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of tramadol hydrochloride (C₁₆H₂₅NO₂·HCl).

Prepare Tramadol Hydrochloride

▲Compounded▲USP39

Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* { 795 }).

Tramadol Hydrochloride tablets ^a equivalent to	500 mg
Vehicle: a 1:1 mixture of Ora-Sweet ^b (sugar-free) and Ora-Plus ^b , a sufficient quantity to make	100 mL
<p>^a Ultram 50-mg tablets, Ortho-McNeil Pharmaceutical, Inc., Raritan, NJ.</p> <p>^b Paddock Laboratories, Minneapolis, MN.</p>	

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a tramadol hydrochloride liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY● **Procedure**

Solution A: 20 mM of phosphoric acid and 4 g/L of sodium 1-hexane sulfonate

Mobile phase: Acetonitrile and *Solution A* (50:50). Filter and degas.

Diluent: Acetonitrile and water (50:50)

Standard solution: 0.25 mg/mL of USP Tramadol Hydrochloride RS in *Diluent*

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.25 mg/mL of tramadol hydrochloride from Oral Suspension and *Diluent*, and centrifuge.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

[Note—The retention time for tramadol hydrochloride is about 6 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tramadol hydrochloride (C₁₆H₂₅NO₂·HCl) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_{\bar{f}}$ peak response from the *Sample solution*

$r_{\bar{s}}$ peak response from the *Standard solution*

$C_{\bar{s}}$ concentration of tramadol hydrochloride in the *Standard solution* (mg/mL)

$C_{\bar{f}}$ nominal concentration of tramadol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** 〈 791 〉: 3.8–4.8

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at ~~controlled cold temperature~~

▲2°–8° ▲USP39

or at controlled room temperature.

Change to read:

- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded when stored at ~~controlled cold temperature~~

▲2°–8° ▲USP39

or controlled room temperature

- **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

- **USP Reference Standards** 〈 11 〉

USP Tramadol Hydrochloride RS

BRIEFING

Tramadol Hydrochloride and Acetaminophen Compounded Oral Suspension, USP 38 page 5636. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* 〈 7 〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:**Tramadol Hydrochloride and Acetaminophen****▲Compounded▲^{USP39}**

Oral Suspension

DEFINITION**Change to read:**

Tramadol Hydrochloride and Acetaminophen

▲Compounded▲^{USP39}

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) and acetaminophen ($C_8H_9NO_2$).

Prepare Tramadol Hydrochloride

▲and Acetaminophen Compounded▲^{USP39}

~~Oral Suspension 7.5 mg/mL and Acetaminophen Oral Suspension 65 mg/mL~~

▲containing 7.5 mg/mL of tramadol hydrochloride and 65 mg/mL of acetaminophen▲^{USP39}

as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈 795 〉).

Tramadol Hydrochloride and Acetaminophen tablets ^a	750 mg and 6500 mg
Vehicle: a 1:1 mixture of Ora-Sweet ^b (sugar-free) and Ora-Plus ^b , a sufficient quantity to make	100 mL
<p>^a Ultracet 37.5-mg/325-mg tablets, Ortho-McNeil Pharmaceutical, Inc., Raritan, NJ.</p> <p>^b Paddock Laboratories, Minneapolis, MN.</p>	

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a tramadol hydrochloride and acetaminophen liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

- **Tramadol Hydrochloride**

Solution A: 20 mM of phosphoric acid and 4 g/L of sodium 1-hexane sulfonate

Mobile phase: Acetonitrile and *Solution A* (50:50). Filter and degas.

Diluent: Acetonitrile and water (50:50)

Standard solution: 0.15 mg/mL of USP Tramadol Hydrochloride RS in *Diluent*

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.15 mg/mL of tramadol hydrochloride from Oral Suspension and *Diluent*, and centrifuge.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

[Note—The retention time for tramadol hydrochloride is about 6 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tramadol hydrochloride (C₁₆H₂₅NO₂·HCl) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of tramadol hydrochloride in the *Standard solution* (mg/mL)

C_U nominal concentration of tramadol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

● **Acetaminophen**

Mobile phase: Acetonitrile and water (70:30). Filter and degas.

Standard solution: 65 µg/mL of USP Acetaminophen RS in *Mobile phase*

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 65 µg/mL of acetaminophen from Oral Suspension and *Mobile phase*, and centrifuge.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

[Note—The retention time for acetaminophen is about 2 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen (C₈H₉NO₂) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_{\bar{F}}$ peak response from the *Sample solution*

$r_{\bar{S}}$ peak response from the *Standard solution*

$C_{\bar{S}}$ concentration of acetaminophen in the *Standard solution* ($\mu\text{g/mL}$)

$C_{\bar{T}}$ nominal concentration of acetaminophen in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** $\langle 791 \rangle$: 3.8–4.8

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at ~~controlled cold temperature~~

$\blacktriangle 2^{\circ}$ – 8° \blacktriangle *USP39*

or at controlled room temperature.

Change to read:

- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded, when stored at ~~controlled cold temperature~~

$\blacktriangle 2^{\circ}$ – 8° \blacktriangle *USP39*

or controlled room temperature

- **Labeling:** Label to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP Reference Standards** $\langle 11 \rangle$
 USP Acetaminophen RS
 USP Tramadol Hydrochloride RS

BRIEFING

Tripolidine Hydrochloride, *USP 38* page 5699. As part of the USP monograph modernization efforts, the following revisions are proposed:

1. Revise the acceptance criterion in the *Definition* from "NLT 98.0% and NMT 101.0%" to "NLT 98.0% and NMT 102.0%", which is typical for chromatographic procedures.
2. Replace *Identification* test *B* that uses ultraviolet absorptivities with an HPLC procedure based on the retention time agreement for tripolidine from the proposed *Assay* procedure.
3. Replace the existing *Assay* titration procedure with a validated stability-indicating HPLC procedure. The proposed liquid chromatographic procedure is based on an analysis performed with the Inertsil ODS-3V brand of column containing L1 packing. The typical retention time for tripolidine is about 5.8 min.
4. Add a test for *Organic Impurities* based on the proposed HPLC procedure in the *Assay*.
5. Update the chemical information for USP Tripolidine Hydrochloride Z-Isomer RS in the

USP Reference Standards section.

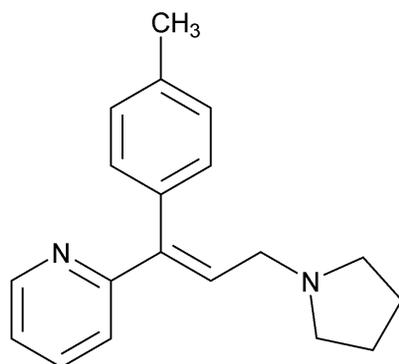
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: C. Anthony.)

Correspondence Number—C133017

Comment deadline: March 31, 2015

Tripolidine Hydrochloride



• HCl • H₂O

C₁₉H₂₂N₂·HCl·H₂O 332.87

C₁₉H₂₂N₂·HCl 314.86

Pyridine, 2-[1-(4-methylphenyl)-3-(1-pyrrolidinyl)-1-propenyl]-, monohydrochloride, monohydrate, (*E*)-;

(*E*)-2-[3-(1-Pyrrolidinyl)-1-*p*-tolylpropenyl]pyridine monohydrochloride monohydrate [6138-79-0].

Anhydrous [550-70-9].

DEFINITION

Change to read:

Tripolidine Hydrochloride contains NLT 98.0% and NMT ~~101.0%~~

~~102.0%~~▲*USP39*

of tripolidine hydrochloride (C₁₉H₂₂N₂·HCl), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** <197K>

Delete the following:

- **B. Ultraviolet Absorption** <197U>

Analytical wavelength: 290 nm

Sample solution: 10 µg/mL in 0.1 N hydrochloric acid

Acceptance criteria: Absorptivities, calculated on the anhydrous basis, do not differ by more than 3.0%.▲*USP39*

Add the following:

▲● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP39*

● **C. Identification Tests—General, Chloride** 〈 191 〉

ASSAY**Change to read:**● **Procedure**

~~**Sample solution:** Dissolve 400 mg of Triprolidine Hydrochloride in 80 mL of glacial acetic acid, warming, if necessary, to effect solution. Add 15 mL of mercuric acetate TS.~~

~~**Titrimetric system**~~

~~**Mode:** Potentiometric titration~~

~~**Titrant:** 0.1 N perchloric acid VS~~

~~**Endpoint detection:** Potentiometric~~

~~**Analysis:** Titrate the *Sample solution* with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 15.74 mg of triprolidine hydrochloride ($C_{19}H_{22}N_2 \cdot HCl$).~~

~~**Acceptance criteria:** 98.0%–101.0% on the anhydrous basis~~

▲**Mobile phase:** Acetonitrile, formic acid, and water (200:1:800) prepared as follows. Mix 200 mL of acetonitrile with 800 mL of water, add 1 mL of formic acid, and sonicate.

Diluent: Acetonitrile and water (20:80)

System suitability solution: 0.01 mg/mL each of USP Triprolidine Hydrochloride RS and USP Triprolidine Hydrochloride Z-Isomer RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Triprolidine Hydrochloride RS in *Diluent* prepared as follows. Transfer a suitable amount of USP Triprolidine Hydrochloride RS to a suitable volumetric flask and dissolve, by sonication, in about 60% of the flask volume of *Diluent*. Dilute with *Diluent* to volume.

Sample solution: 0.1 mg/mL of Triprolidine Hydrochloride in *Diluent* prepared as follows. Transfer a suitable amount of Triprolidine Hydrochloride to a suitable volumetric flask and dissolve, by sonication, in about 60% of the flask volume of *Diluent*. Dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25.0-cm; 5- μ m packing L1

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: 20 min

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between triprolidine hydrochloride and triprolidine hydrochloride

Z-isomer, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of triprolidine hydrochloride ($C_{19}H_{22}N_2 \cdot HCl$) in the portion of Triprolidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Triprolidine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= concentration of Triprolidine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis ▲*USP39*

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Delete the following:

-

• **Heavy Metals, Method II** 〈 231 〉

: NMT 20 ppm • (Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

Absorbent: 0.25-mm Layer of chromatographic silica gel mixture

Standard stock solution: 1 mg/mL of USP Triprolidine Hydrochloride RS in chloroform

Standard solution A: 200 µg/mL of USP Triprolidine Hydrochloride RS in chloroform from *Standard stock solution*

Standard solution B: 150 µg/mL of USP Triprolidine Hydrochloride RS in chloroform from *Standard stock solution*

Standard solution C: 100 µg/mL of USP Triprolidine Hydrochloride RS in chloroform from *Standard stock solution*

Standard solution D: 50 µg/mL of USP Triprolidine Hydrochloride RS in chloroform from *Standard stock solution*

Standard Z-isomer stock solution: 1 mg/mL of USP Triprolidine Hydrochloride Z-Isomer

RS in chloroform

Standard Z-isomer solution A: 200 µg/mL of USP Triprolidine Hydrochloride Z-Isomer RS in chloroform from *Standard Z-isomer stock solution*

Standard Z-isomer solution B: 150 µg/mL of USP Triprolidine Hydrochloride Z-Isomer RS in chloroform from *Standard Z-isomer stock solution*

Standard Z-isomer solution C: 100 µg/mL of USP Triprolidine Hydrochloride Z-Isomer RS in chloroform from *Standard Z-isomer stock solution*

Standard Z-isomer solution D: 50 µg/mL of USP Triprolidine Hydrochloride Z-Isomer RS in chloroform from *Standard Z-isomer stock solution*

[Note—These solutions correspond to 2.0% (*Standard solution A* and *Standard Z-isomer solution A*), 1.5% (*Standard solution B* and *Standard Z-isomer solution B*), 1.0% (*Standard solution C* and *Standard Z-isomer solution C*), and 0.5% (*Standard solution D* and *Standard Z-isomer solution D*), respectively, of the *Sample solution* concentration.]

Sample solution: 10 mg/mL of Triprolidine Hydrochloride in chloroform

Developing solvent system: Chloroform and diethylamine (95:5)

Application volume: 5 µL

Analysis: Separately apply the *Sample solution* and each of the eight *Standard solutions*, and develop the chromatograms, protected from light, in the *Developing solvent system*, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under long and short wavelength UV light. Compare the intensities of any secondary spots observed in the chromatogram of the *Sample solution* with those of the principal spots in the chromatograms of the *Standard solutions*.

Acceptance criteria: The intensity of the Z-isomer triprolidine hydrochloride spot (R_f value 1.2 relative to the R_f value for triprolidine hydrochloride) of the *Sample solution* corresponds to NMT 2.0%, and the sum of the intensities of all secondary spots of the *Sample solution* corresponds to NMT 3.0%.

▲Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 10 µg/mL each of USP Triprolidine Hydrochloride RS and USP Triprolidine Hydrochloride Z-Isomer RS in *Diluent*

Sample solution: 0.5 mg/mL of Triprolidine Hydrochloride in *Diluent* prepared as follows. Transfer a suitable amount of Triprolidine Hydrochloride to a suitable volumetric flask and dissolve, by sonication, in about 60% of the flask volume of *Diluent*. Dilute with *Diluent* to volume.

System suitability

Sample: *Standard solution*

[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between triprolidine hydrochloride and triprolidine hydrochloride Z-isomer

Relative standard deviation: NMT 1.8% for each peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of triprolidine hydrochloride Z-isomer in the portion of Triprolidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of triprolidine hydrochloride Z-isomer from the *Sample solution* r_S = peak response of triprolidine hydrochloride Z-isomer from the *Standard solution* C_S = concentration of USP Triprolidine Hydrochloride Z-Isomer RS in the *Standard solution* (mg/mL) C_U = concentration of Triprolidine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Triprolidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of any unspecified impurity from the *Sample solution* r_S = peak response of triprolidine hydrochloride from the *Standard solution* C_S = concentration of USP Triprolidine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = concentration of Triprolidine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tripolidine	1.0	—
Tripolidine hydrochloride Z-isomer	1.5	2.0
Any individual unspecified impurity	—	0.10
Total impurities	—	3.0

▲USP39

SPECIFIC TESTS

- **Water Determination, Method I** (921): 4.0%–6.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Triprolidine Hydrochloride RS

USP Triprolidine Hydrochloride Z-Isomer RS

▲(Z)-2-[3-(1-Pyrrolidinyl)-1-*p*-tolylpropenyl]pyridine monohydrochloride.

C₁₉H₂₂N₂·HCl 314.85

▲USP39

BRIEFING

Ursodiol Compounded Oral Suspension, *USP 38* page 5722. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compdial-nomenclature>). See also *Compounded Preparations* in General Chapter *Labeling* 〈 7 〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:**Ursodiol**

▲▲Compounded▲USP39

Oral Suspension

DEFINITION**Change to read:**

Ursodiol

▲Compounded▲USP39

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of ursodiol (C₂₄H₄₀O₄).

Prepare Ursodiol

▲Compounded▲USP39

Oral Suspension 50 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈 795 〉).

Ursodiol tablets ^a equivalent to	5 g
Vehicle: a 1:1 mixture of Ora-Sweet ^b (sugar-free) and Ora-Plus ^b , a sufficient quantity to make	100 mL
^a Urso 250-mg tablets, Axcan Pharma U.S. Inc., Birmingham, AL. ^b Paddock Laboratories, Minneapolis, MN.	

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make an ursodiol liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

• Procedure

Mobile phase: Methanol and 0.01 M dihydrogen potassium phosphate buffer (75:25).

Adjust with dilute phosphoric acid to a pH of 5.25. Filter, and degas.

Standard solution: 1.25 mg/mL of USP Ursodiol RS in methanol

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 1.25 mg/mL of ursodiol from Oral Suspension and methanol, and centrifuge.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 201 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 15 μL

System suitability

Sample: *Standard solution*

[Note—The retention time for ursodiol is about 7 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ursodiol (C₂₄H₄₀O₄) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of ursodiol in the *Standard solution* (mg/mL)

C_U = nominal concentration of ursodiol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** 〈 791 〉: 4.0–5.0

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at ~~controlled cold temperature~~

▲2°–8° ▲USP39

or at controlled room temperature.

Change to read:

- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded when stored at ~~controlled cold temperature~~

▲2°–8° ▲USP39

or controlled room temperature

- **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

- **USP Reference Standards** 〈 11 〉

USP Ursodiol RS

BRIEFING

Valacyclovir Compounded Oral Suspension, *USP 38* page 5725. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compdial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* 〈 7 〉 published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Valacyclovir

▲▲Compounded▲USP39

Oral Suspension

DEFINITION**Change to read:**

Valacyclovir

▲Compounded▲*USP39*

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of valacyclovir ($C_{13}H_{20}N_6O_4$).

Prepare Valacyclovir

▲Compounded▲*USP39*

Oral Suspension 50 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile*

Preparations 〈 795 〉).

Valacyclovir tablets ^a equivalent to	5 g
Vehicle: a 1:1 mixture of Ora-Sweet ^b (regular or sugar-free) and Ora-Plus ^b , a sufficient quantity to make	100 mL
<p>^a Valtrex 500-mg tablets, Glaxo Wellcome Inc., Research Triangle Park, NC.</p> <p>^b Paddock Laboratories, Minneapolis, MN.</p>	

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a valacyclovir liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY● **Procedure**

Mobile phase: Acetonitrile and 5.0 mM sodium acetate (1:99). Adjust with 6 N hydrochloric acid to a pH of 3.0. Filter and degas.

Diluent: 5.0 mM sodium acetate, adjusted with 6 N hydrochloric acid to a pH of 3.0

Standard solution: Dissolve an appropriately weighed amount of USP Valacyclovir Hydrochloride RS in *Diluent* to be equivalent to 0.2 mg/mL of valacyclovir.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.2 mg/mL of valacyclovir from Oral Suspension and *Diluent*. Pass through a nylon filter of 0.22- μ m pore size.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; 10- μ m packing L1

Flow rate: 3.0 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

[Note—The retention time for valacyclovir hydrochloride is about 14 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of valacyclovir ($C_{13}H_{21}ClN_6O_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of valacyclovir in the *Standard solution* (mg/mL)

C_U = nominal concentration of valacyclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** (791): 3.2–4.3

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Package in tight, light-resistant

▲glass▲*USP39*

containers. Store at ~~controlled-cold temperature~~

▲2°–8° .▲*USP39*

Change to read:

- **Beyond-Use Date:** NMT 14 days after the date on which it was compounded when stored at ~~controlled-cold temperature~~

▲2°–8° ▲*USP39*

- **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

- **USP Reference Standards** (11)

USP Valacyclovir Hydrochloride RS

BRIEFING

Verapamil Hydrochloride Compounded Oral Solution, *USP 38* page 5766. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* (7) published in *USP 38–NF 33*. A list of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Verapamil Hydrochloride

▲Compounded▲*USP39*

Oral Solution

DEFINITION

Change to read:

Verapamil Hydrochloride

▲Compounded▲*USP39*

Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$).

Prepare Verapamil Hydrochloride

▲Compounded▲*USP39*

Oral Solution 50 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* { 795 }).

Verapamil Hydrochloride powder	5 g
Vehicle for Oral Solution (regular or sugar-free), <i>NF</i> , a sufficient quantity to make	100 mL

Add *Verapamil Hydrochloride powder* and about 40 mL of *Vehicle* to a mortar, and mix. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring the preparation to final volume, and mix well.

ASSAY

● **Procedure**

Solution A: 0.01 N sodium acetate solution containing 33 mL/L of acetic acid

Mobile phase: Acetonitrile, 2-aminoheptane, and *Solution A* (50: 0.5: 50). Filter, and degas.

Standard solution: 500 µg/mL of USP Verapamil Hydrochloride RS in *Mobile phase*

Sample solution: Agitate containers of Oral Solution for 30 min on a rotating mixer,

remove a 5-mL sample, and store in a clear glass vial at -70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix on a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 0.5 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

[Note—The retention time for verapamil hydrochloride is about 4.8 min.]

Suitability requirements

Relative standard deviation: NMT 0.7% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of verapamil hydrochloride in the *Standard solution* (μg/mL)

C_U nominal concentration of verapamil hydrochloride in the *Sample solution* (μg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** { 791 } : 3.8–4.8

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature or in a cold place.

Change to read:

- **Beyond-Use Date:** NMT 60 days after the date on which it was compounded

▲when stored at controlled room temperature or in a cold place▲*USP39*

- **Labeling:** Label to indicate the *Beyond-Use Date*.
- **USP Reference Standards** { 11 }

USP Verapamil Hydrochloride RS

BRIEFING

Verapamil Hydrochloride Compounded Oral Suspension, *USP 38* page 5767. The Compounding Expert Committee and the Nomenclature, Safety, and Labeling Expert Committee propose to revise the title of this and other compounded monographs to identify that it is a compounded preparation, consistent with the *Nomenclature Guidelines* (<http://www.usp.org/usp-nf/development-process/compendial-nomenclature>). See also *Compounded Preparations* in general chapter *Labeling* { 7 } published in *USP 38–NF 33*. A list

of all compounding monograph titles that will be revised is posted online at <http://www.usp.org/usp-nf/notices/compounding-monograph-titles>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C142655

Comment deadline: March 31, 2015

Change to read:

Verapamil Hydrochloride

▲▲Compounded▲▲*USP39*

Oral Suspension

DEFINITION

Change to read:

Verapamil Hydrochloride

▲Compounded▲*USP39*

Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$).

Prepare Verapamil Hydrochloride

▲Compounded▲*USP39*

Oral Suspension 50 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile*

Preparations 〈 795 〉).

Verapamil Hydrochloride	5 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, (regular or sugar-free), <i>NF</i> , and Vehicle for Oral Suspension, <i>NF</i> , a sufficient quantity to make	100 mL

If using tablets, comminute to a fine powder using a suitable mortar, or add *Verapamil Hydrochloride* powder. Add about 40 mL of the *Vehicle* in small portions, and mix to obtain a uniform paste. Transfer the mortar contents, stepwise and quantitatively, to a calibrated bottle. Add the *Vehicle* in portions to rinse the mortar, add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY

● **Procedure**

Solution A: 0.01 N sodium acetate solution containing 33 mL/L of acetic acid

Mobile phase: Acetonitrile, 2-aminoheptane, and *Solution A* (50: 0.5: 50). Filter, and degas.

Standard solution: 500 µg/mL of USP Verapamil Hydrochloride RS in *Mobile phase*

Sample solution: Agitate containers of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at -70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and

mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 0.5 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

[Note—The retention time for verapamil hydrochloride is about 4.8 min.]

Suitability requirements

Relative standard deviation: NMT 0.7% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of verapamil hydrochloride in the *Standard solution* (μg/mL)

C_U nominal concentration of verapamil hydrochloride in the *Sample solution* (μg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** 〈 791 〉: 3.8–4.8

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a cold place.

Change to read:

- **Beyond-Use Date:** NMT 60 days after the date on which it was compounded

▲when stored at controlled room temperature or in a cold place▲*USP39*

- **Labeling:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.

- **USP Reference Standards** 〈 11 〉

USP Verapamil Hydrochloride RS

BRIEFING

Zaleplon, *USP 38* page 5825. Based on the comments received, it is proposed to revise the

monograph as follows:

1. Replace the test for *Organic Impurities* procedure with a different gradient elution liquid chromatographic procedure because the existing procedure is not selective enough to separate an additional process impurity from a different manufacturing process. The proposed new procedure is capable of separating and quantifying six different process impurities from two different manufacturing processes. The liquid chromatographic procedure is based on the validations performed with the YMC Pack C8 brand of L7 column in which zaleplon elutes at about 11 min.
2. Introduce a new Reference Standard to support the proposed new *Organic Impurities* procedure. The proposed new Reference Standard, USP Zaleplon Related Compound C RS, forms a critical pair with USP Zaleplon Related Compound A RS.

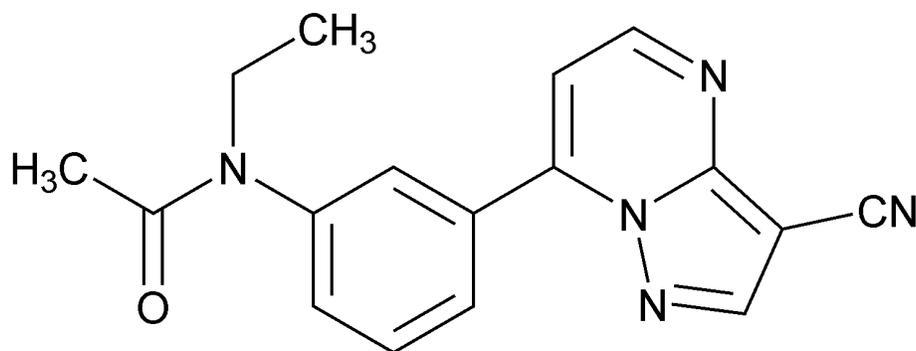
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R. Ravichandran.)

Correspondence Number—C112937

Comment deadline: March 31, 2015

Zaleplon



$C_{17}H_{15}N_5O$ 305.33

Acetamide, *N*-[3-(3-cyanopyrazolo[1,5- α]pyrimidin-7-yl)phenyl]-*N*-ethyl- ;
3'-(3-Cyanopyrazolo[1,5- α]pyrimidin-7-yl)-*N*-ethylacetanilide [151319-34-5].

DEFINITION

Zaleplon contains NLT 98.0% and NMT 102.0% of zaleplon ($C_{17}H_{15}N_5O$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: 0.3 g/L of ammonium formate in water. Adjust with formic acid to a pH of 4.0.

Mobile phase: Acetonitrile and *Buffer* (7:18)

Diluent: Acetonitrile and water (1:1)

System suitability solution: 0.5 mg/mL of USP Zaleplon RS and 0.5 µg/mL each of USP Zaleplon Related Compound A RS and USP Zaleplon Related Compound B RS in *Diluent*

Standard solution: 50 µg/mL of USP Zaleplon RS in *Diluent*

Sample solution: 50 µg/mL of Zaleplon in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 4-mm × 10-cm; 3-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: Two times the retention time of zaleplon

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for zaleplon and zaleplon related compound B are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 2.0 between zaleplon and zaleplon related compound B, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zaleplon (C₁₇H₁₅N₅O) in the portion of Zaleplon taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zaleplon from the *Sample solution*

r_S = peak response of zaleplon from the *Standard solution*

C_S = concentration of USP Zaleplon RS in the *Standard solution* (µg/mL)

C_U = concentration of Zaleplon in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Delete the following:

-

• **Heavy Metals, Method II** 〈 231 〉

: NMT 20 ppm (Official 1-Dec-2015)

• **Residue on Ignition** 〈 281 〉: NMT 0.2%

Change to read:

- **Organic Impurities**

Diluent: Acetonitrile and water (1:1)

Solution A: Use the *Buffer* in the *Assay*.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
11.0	68	32
17.0	60	40
30.0	60	40
31.0	80	20
35.0	80	20

System suitability solution: Prepare as directed in the *Assay*.

Standard solution: 0.5 µg/mL of USP Zaleplon RS in *Diluent*

Sample solution: 0.5 mg/mL of Zaleplon in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 4.6 mm × 25 cm; 5 µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 14.0 between zaleplon-related compound A and zaleplon, and NLT 2.0 between zaleplon and zaleplon-related compound B; *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Zaleplon taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*

r_S = peak response of zaleplon from the *Standard solution*

C_S = concentration of USP Zaleplon RS in the *Standard solution* (mg/mL)

C_U = concentration of Zaleplon in the *Sample solution* (mg/mL)

F = relative response factor for the corresponding impurity peak (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cyanopyrazolamine ^a	0.18	1.0	0.15
Zaleplon related compound A ^b	0.58	0.76	0.15
Zaleplon	1.0	—	—
Zaleplon related compound B ^c	1.08	0.92	0.15
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

^a 3-Aminopyrazole-4-carbonitrile.
^b (E)-N-[3-[3-(Dimethylamino)acryloyl]phenyl]-N-ethylacetamide.
^c N-[3-(3-Cyanopyrazolo[1,5-c]pyrimidin-5-yl)phenyl]-N-ethylacetamide.

▲Solution A: 1.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
15	60	40
25	45	55
26	80	20
35	80	20

Diluent: Acetonitrile and water (250:750)

System suitability solution: 0.5 mg/mL of USP Zaleplon RS and 0.5 µg/mL each of USP Zaleplon Related Compound A RS, USP Zaleplon Related Compound B RS, and USP Zaleplon Related Compound C RS in *Diluent*

Standard solution: 0.5 µg/mL of USP Zaleplon RS in *Diluent*

Sample solution: 500 µg/mL of Zaleplon in *Diluent*

Chromatographic system

(See *Chromatography* < 621 >, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between zaleplon related compound A and zaleplon related compound C; NLT 2.0 between zaleplon and zaleplon related compound B, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Zaleplon taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U

= peak response of any individual impurity from the *Sample solution*

r_S

= peak response of zaleplon from the *Standard solution*

C_S

= concentration of USP Zaleplon RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= concentration of Zaleplon in the *Sample solution* ($\mu\text{g/mL}$)

F

= relative response factor for the corresponding impurity peak (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cyanopyrazolamine ^a	0.15	0.50	0.15
Zaleplon related compound A	0.54	0.36	0.15
Zaleplon related compound C ^b	0.57	0.80	0.1
Desethylzaleplon ^{b,c}	0.70	1.1	0.1
Zaleplon	1.0	—	—
Zaleplon related compound B	1.11	0.65	0.15
Zaleplon oxopropenyl analog ^{b,d}	1.59	0.51	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

^a 3-Aminopyrazole-4-carbonitrile.

^b Process impurity; may not be found in all manufacturing processes.

^c *N*-[3-(3-Cyanopyrazolo[1,5-*a*]pyrimidin-7-yl)phenyl]acetamide.

^d (*E*)-*N*-[3-(3-Cyano-6-{3-[3-(*N*-ethylacetamido)phenyl]-3-oxoprop-1-en-1-yl}pyrazolo[1,5-*a*]pyrimidin-7-yl)phenyl]-*N*-ethylacetamide.

▲USP39

SPECIFIC TESTS

- **Water Determination, Method I** 〈 921 〉: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in light-resistant containers, and store at room temperature.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Zaleplon RS USP Zaleplon Related Compound A RS *(E)*-*N*-{3-[3-(Dimethylamino)acryloyl]phenyl}-*N*-ethylacetamide.C₁₅H₂₀N₂O₂ 260.33USP Zaleplon Related Compound B RS *N*-[3-(3-Cyanopyrazolo[1,5-*α*]pyrimidin-5-yl)phenyl]-*N*-ethylacetamide.C₁₇H₁₅N₅O 305.33

▲USP Zaleplon Related Compound C RS

7-[3-(*N*-Ethylacetamido)phenyl]pyrazolo[1,5-*a*]pyrimidine-3-carboxamide.C₁₇H₁₇N₅O₂ 323.35▲USP39**Stage 4 Harmonization**

This section contains monographs or chapters undergoing harmonization by the Pharmacopeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (**Stages 1** through **7**, as defined below). Stage 4 drafts are available for comments. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a

harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF*, this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

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《 671 》 *Containers—Performance Testing: Use of Water-Filled Test Containers for Water Vapor Transmission Rate Determinations*

Dwain L. Sparks^a and Seung-yil Yoon, PhD^{a,b}

ABSTRACT

This *Stimuli* article proposes the use of water as an alternative test-sample fill medium for water-vapor transmission rate (WVTR) determinations described in general chapter *Containers—Performance Testing* 《 671 》 for container-closure systems for solid oral drug products. The use of water as the test-sample fill medium eliminates problems with the handling of desiccants and also maintains a constant vapor-pressure difference across the container wall for the duration of the study, thereby reducing bias and variability in the WVTR determinations. Use of water-filled test containers is already provided in the chapter 《 671 》 method for the determination of weight loss for *Multiple-Unit Containers* and *Unit-Dose Containers for Liquids*, but has not been extended to the determination of the WVTR of bottle and blister container-closure systems for solid oral drug products.

INTRODUCTION

Traditionally, the water-vapor transmission rate (WVTR) of container-closure systems for solid oral drug products has been determined by using desiccant-filled test containers stored in environmental chambers to create a difference in relative humidity (RH) or vapor pressure inside versus outside the test containers. Use of desiccants can be problematic, however, because an inadequate amount of desiccant or incompletely dried desiccant may result in variability of the vapor-pressure differences between the inside and outside of the container during the study. This potential variability of the vapor-pressure difference can both bias and increase variability in WVTR determinations.

Eli Lilly and Company has proposed that the water-filled container method be adopted as an industry standard, by inclusion in general chapter 《 671 》, for WVTR determinations of container-closure systems for solid oral drug products. The authors anticipate that readers will incorporate this alternative method into their WVTR testing program.

USE OF DESICCANTS FOR WVTR DETERMINATION

According to the WVTR methods described in chapter *Containers—Performance Testing* 《 671 》, test containers are to be filled with sufficient anhydrous desiccant to maintain an inside RH close to 0% during the study. Desiccant-filled test containers are held in an environmental chamber, and weight determinations are made over time. Under constant environmental conditions, plastic [e.g., polyethylene, polypropylene, polyvinylchloride (PVC), and polychlorotrifluoroethylene (PCTFE)] container walls reach a steady state of water concentration after a preconditioning period. The inside RH remains constant during testing if adequate anhydrous desiccant is present. The weight gain of the desiccant is determined at

this steady-state condition and is translated into a permeation rate for the test container-closure system. In this *Stimuli* article, %RH is used to express the environmental chamber conditions and the headspace inside the test containers.

To obtain reliable WVTR determinations, humidity must remain constant inside and outside the test container throughout the study. Results from desiccant-filled test containers do not account for potential variability introduced if the internal RH of test containers neither starts at 0%, nor remains at 0% during the study. A recent *Stimuli* article describes improvements in desiccant preparation that are designed to maintain the internal RH below 10% throughout the study. However, this internal RH range (0%–10%) translates to variability in the internal RH. In contrast, a test container filled with water will maintain a constant RH (100%) from the beginning to the end of the WVTR study, thereby reducing potential variability.

USE OF WATER AS AN ALTERNATIVE FILL MEDIUM

To address the challenges involved in the use of desiccant-filled test containers, it is proposed to use the water-filled container method as an alternative approach for the determination of WVTR of container-closure systems for solid oral drug products. Desiccant-filled test containers stored in a 40°/75% RH chamber achieve a vapor-pressure difference of 65%–75% if the internal RH created by the desiccant ranges from 0% to 10%. On the other hand, water-filled test containers stored in an environmental chamber at 40°/25% RH achieve and maintain a constant vapor pressure difference of 75%.

Experience with Water-Filled Test Containers

In practice, multiple benefits have been realized by implementing WVTR testing using water as the test medium for blister samples. With water, the variability of the fill amount does not result in variability of internal RH, and no interaction with container walls has been observed. The stability of the internal RH afforded by water facilitates holding the samples or reusing them at a later time, or in multiple studies (e.g., different storage conditions using the same samples). Additional measurements or extensions of studies are possible without concern about depleting the water.

In addition, potential exposure of desiccant to moisture during filling and handling before testing is eliminated with the water-filled container method, and physical damage to foil lidding caused by desiccant is also eliminated. This includes elimination of special handling requirements to protect desiccant-filled blister samples from excessive exposure to moisture before testing. Also, samples filled with aqueous dye can be used for leak testing before or after WVTR testing. Use of dye enhances visual detection of gross leaks during the study.

Sample preparation techniques are important for filling blisters with water. The best practice for delivering water into the cavities on the packaging line was developed using a manual syringe filled with water, allowing delivery of a few drops of water to each cavity without causing sealing problems.

Amount of Water for Test Containers

For blisters, approximately 10–100 mg of water (about 1–10 drops), depending upon expected WVTR, is adequate for all blister types at 40°/25% RH. As an example, consider the preparation of a low-barrier WVTR package, e.g., a PVC water-filled blister as shown in *Table 1*. The expected WVTR is about 1.5 mg water/(day × cavity) at 40° and a 75% RH vapor-pressure

difference. The initial amount of water required to create 100% RH at 40° in this blister (approximately 0.5 cc volume) is 0.025 mg of water, on the basis of absolute humidity. For a typical WVTR study of a low-barrier blister, 2 days are allowed for equilibration, followed by a study period of 15 days for obtaining 3–5 weight determinations. This is a total exposure time, or permeation time, of 17 days. According to *Equation 1*, a minimum of 25.5 mg of water is needed.

$$1.5 \text{ mg water}/(\text{day} \times \text{cavity}) \times 17 \text{ days} + 0.025 \text{ mg} = 25.5 \text{ mg water/cavity} \quad (1)$$

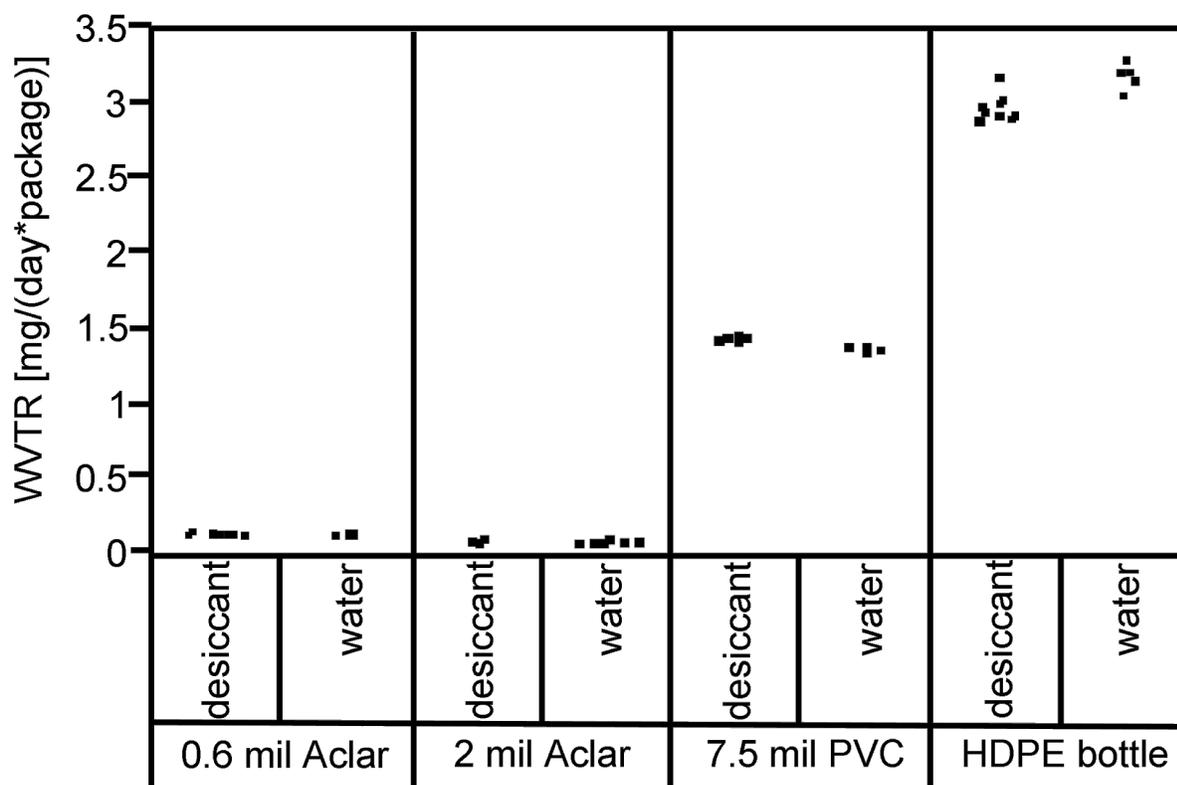
The recommended amount of water for this blister system is about 50 mg. *Equation 1* can be used to determine the minimum amount of water for all blister types, and this amount should then be multiplied by 2–3 to achieve an excess. For bottles, a 10%–25% fill volume of water will create and maintain 100% RH during the entire study.

Comparison of Results for Water-Filled and Desiccant-Filled Test Containers

Observed differences in WVTR results for water-filled test containers versus desiccant-filled test containers are practically insignificant. Several sets of data are provided in *Table 1*. The raw data for each set are shown graphically in *Figure 1*.

Table 1. Summary of WVTR Studies Comparing Desiccant-Filled and Water-Filled Samples

Sample description	Fill medium	Chamber conditions	Number of test containers	Average WVTR
7.5 mil PVC	Molecular sieves	40°/75% RH	10	1.46 mg/(day × cavity)
7.5 mil PVC	Water	40°/25% RH	5	1.39 mg/(day × cavity)
0.6 mil PCTFE	Calcium chloride	40°/75% RH	10	0.161 mg/(day × cavity)
0.6 mil PCTFE	Water	40°/25% RH	6	0.162 mg/(day × cavity)
2 mil PCTFE	Molecular sieves	40°/75% RH	10	0.104 mg/(day × cavity)
2 mil PCTFE	Water	40°/25% RH	10	0.105 mg/(day × cavity)
325 mL HIS ^a HDPE ^b Bottle	Calcium chloride	40°/75% RH	10	2.97 mg/(day × package)
325 mL HIS HDPE Bottle	Water	40°/25% RH	5	3.20 mg/(day × package)
^a HIS, heat induction sealed. ^b HDPE, high-density polyethylene.				



Sample type (desiccant-filled vs. water-filled) within package type

Figure 1. Graphic representation of raw data for *Table 1*.

SUMMARY AND CONCLUSIONS

WVTR can be measured in terms of desiccant weight gain or water weight loss for a container-closure system held in an environment with constant temperature and RH. Test containers filled with water provide a constant vapor-pressure difference across test container walls, offering potential improvements in WVTR testing compared to desiccant-filled containers where the vapor pressure inside may neither start nor remain at 0% RH. Also, use of water-filled containers mitigates many of the challenges inherent in the preparation and handling of desiccant and desiccant-filled containers. Eli Lilly and Company has proposed that the water-filled container method be included in general chapter $\langle 671 \rangle$ and be adopted as an industry standard for WVTR determinations of container-closure systems for solid oral drug products.

ACKNOWLEDGMENTS

The authors would like to acknowledge Lilly colleagues for their contributions to this work: Juan Arandia, Tamara Edelman, Allan Hoepers, Timothy Kramer, Jacob Lewis, David Pena, Michael Skibic, and Randy Thackrey. Also, the authors acknowledge that the contributions by Mocon and the members of the USP $\langle 671 \rangle$ Expert Panel and the PQRI Container/Closure Systems Working Group continue to be an important and valuable resource for the science of permeation testing and for the utilization of WVTR values for drug product registrations.

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Collaborative Study of the Variability of Drug Release Results from Nicotine Gums using Two Apparatus Designs

William Brown,^{a,d} Jayachandar Gajendran,^b Walter Hauck,^c and Johannes Krämer^b

ABSTRACT The differing types of orally administered dosage forms require different apparatus designs for testing drug release. Performance tests used to characterize medicated chewing gums have been described in the *European Pharmacopoeia*. Although the apparatus designs have been described, no report of the variability of results from those apparatus designs has been published. This *Stimuli* article provides the results of a multiple-laboratory collaborative study of this variability. The study evaluates the variability of results from both the gums and the apparatus design used. A nicotine polacrilex gum product, which is not available commercially, was evaluated in this study. The results show that controls need to be applied to the procedures and apparatus designs. A comparison of results from the two apparatus designs is given in this *Stimuli* article.

INTRODUCTION

The scientific rationale for the application of compendial performance testing for medicated chewing gums has been discussed elsewhere (1). Currently, USP does not describe general performance testing procedures for medicated chewing gums. Two apparatus designs for testing medicated gums are described in the *European Pharmacopoeia (Ph. Eur.)* (2). The *Ph. Eur.* nomenclature is followed in this *Stimuli* article.

The feasibility of the use of both apparatus A and apparatus B designs has been demonstrated for the drug release testing of medicated chewing gums by using marketed, multi-source, nicotine chewing gums (3). Gajendran et al. (3) demonstrated acceptable drug release testing methodology that was verified by an alternative in vitro-in vivo correlation approach (3, 4). An understanding of the source and degree of variability associated with the test performance, within and between the laboratories, is a necessary precursor to consideration of inclusion of new compendial performance test procedures (5). Overall, the variability of performance testing results can be affected by: unit-to-unit variability of the samples; variability in the performance of the test apparatus; and variability related to the laboratory procedures, operator, and analytical procedures. The USP Dosage Forms Expert Committee requested this study to test the suitability and performance of the apparatus designs used for performance testing of medicinal gum products and to detect potential sources of variability of results for the available medicated chewing gum performance test apparatus. The results of this study are reported here.

STUDY DESIGN

A multiple-laboratory study was conducted using two commercially available nicotine gum products from the U.S. market for which no compendial performance test is required. A compressed gum formulation was also manufactured expressly for the study. A call for participants identified six laboratories that had at least one of the apparatus designs described

in *Ph. Eur. 2.9.25 Dissolution Test for Medicated Chewing Gums*. Participants were required to furnish data from two runs of six units, conducted by two analysts, as a measure of the intermediate precision of the results. Sample identity was not disclosed to the participants. The participating laboratories are listed in *Table 1*. Laboratories were responsible only for testing and results from the apparatus designs in their laboratory and did not need to test both apparatus A and B designs. Five of the participating laboratories provided data from apparatus B, and one laboratory also provided data from apparatus A.

Table 1. Collaborative Study Participants

Laboratory	Country
Fertin Pharma A/S	Denmark
McNeil	Sweden
PHAST	Germany
USP Dosage Forms Performance Laboratory	USA
Watson Laboratories, Inc.	USA

MATERIALS AND METHODS

PROTOCOL

Samples: 2-mg nicotine chewing gums Commercial 1 (*Product A*), Commercial 2 (*Product B*), and development formulation (*Product C*)

Medium: Simulated saliva, pH 6.2; 12 mM monobasic potassium phosphate, 40 mM sodium chloride, and 1.5 mM calcium chloride, adjusted to pH 6.2 ± 0.05 with sodium hydroxide solution

Test procedure: Six units of the sample were tested (one per cell) in 40 mL of simulated saliva, pH 6.2 (no degassing). The test was conducted at $37^{\circ} \pm 0.5^{\circ}$. Three-mL samples were taken at 5, 10, 20, 30, 45, and 60 min with *Medium* replacement by an equivalent volume of preheated fresh *Medium* for times <60 min. Samples were filtered through an Acrodisc LC-13 mm PVDF membrane filter (or equivalent) of 0.45- μ m pore size with the first 1 mL of filtrate discarded.

For apparatus B, two circular plastic nets (Nylon PA6; 1.4-mm aperture; 0.405-mm wire diameter) were used in each cell as described in *Ph. Eur. 7.5*.

Table 2. Test Parameters for Apparatus A and Apparatus B

	Distance between Horizontal Pistons (mm)	Masticatory Frequency (strokes/min)	Distance from Vertical Piston to Bottom (mm)	Twisting Angle of Jaws ($^{\circ}$)
Apparatus A	0.5	40	3	—
Apparatus B	—	40	1.4	20

hplc method parameters

Standard solutions: 46.5, 29.8, and 2.33 μ g/mL

Sample solution: The *Sample solution* response was mapped to the standard curve calculated from the concentration and responses of the injected *Standard solutions*.

Chromatographic system

Column: Xterra RP 18; 150 × 4.6/3.9 mm, 5 µm or equivalent

Mobile phase: 0.04 M potassium phosphate monobasic, pH 6.5, methanol (98:2 v/v), 30°

Flow rate: 1.2 mL/min

Injection volume: 50 µL

Detection wavelength: 260 nm

statistical methods

Evaluating the results from apparatus A

A complete statistical analysis for apparatus A was not possible due to the limited data available. A limited comparison of results from apparatus A and apparatus B submitted by a single laboratory is provided.

Evaluating the results from apparatus B

Each of the six sampling times was analyzed separately. By doing so, there was no need to model either the amount released or the variances as a function of time. For each sampling time-product combination, the data were analyzed as an analysis of variance (ANOVA) of random effects, with laboratory and analyst nested within laboratory as random effects. Initial analyses were conducted in the untransformed (mg) scale and after log transformation. Log transformation for all three products was used because the distribution of residuals better followed a normal distribution.

For presentation, results from the log scale analyses were back-transformed to the original scale. Thus, the average reported is a geometric mean. The residual variance from the ANOVA is the estimated repeatability variance in the log scale, and the sum of the three components (between-laboratory, between-analyst, and residual) is the estimated reproducibility variance. For reporting, these were converted to the percentages of the coefficient of variance (%CVs) in the original scale using the log-normal formula.

RESULTS AND DISCUSSION

Five laboratories participated in the study, and each laboratory was to conduct the experiment with *Ph. Eur.* apparatus A or B design but could participate only if the laboratory had one of the two apparatus designs. For each apparatus, each laboratory was to conduct six runs by each of two analysts for each of three products (*A*, *B*, and *C*). The inclusion of two analysts was to capture intermediate precision information. All laboratories followed the protocol for apparatus B, except that laboratory 6 ran *Product C* with only one analyst and laboratory 3 did not test *Product C*.

Laboratory 1 provided data for the three products from apparatus A with the exception that only one analyst performed testing for *Product A*.

Evaluation of Results from Apparatus B

The first step was to examine the data sets for unusual values and trends; two data sets were identified. First, many values from lab 3 were >2 mg, the labeled nicotine content of all three products (see *Figures 1* and *2*). Data from this lab were dropped from further analysis. Second, one run from lab 6 for *Product C* was out of line with all of the other data from that lab and the

other labs (see *Figure 3*). In the ANOVA, these six values were the six largest studentized residuals. This run was dropped from further analyses, leaving data available for analyses from 48 runs for each time point for each of *Products A* and *B* and from 41 runs for each time point for *Product C*.

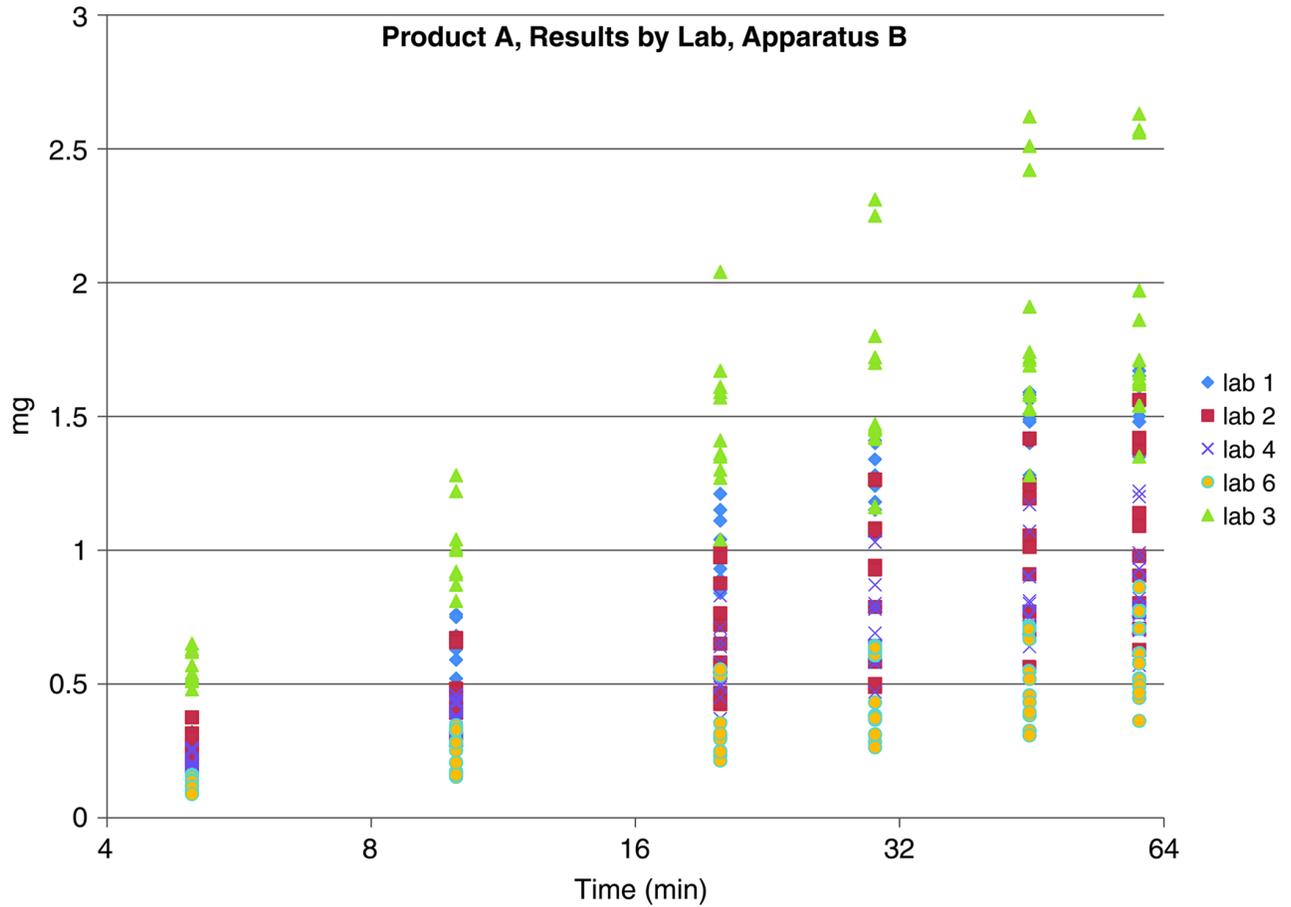


Figure 1. *Product A* data.

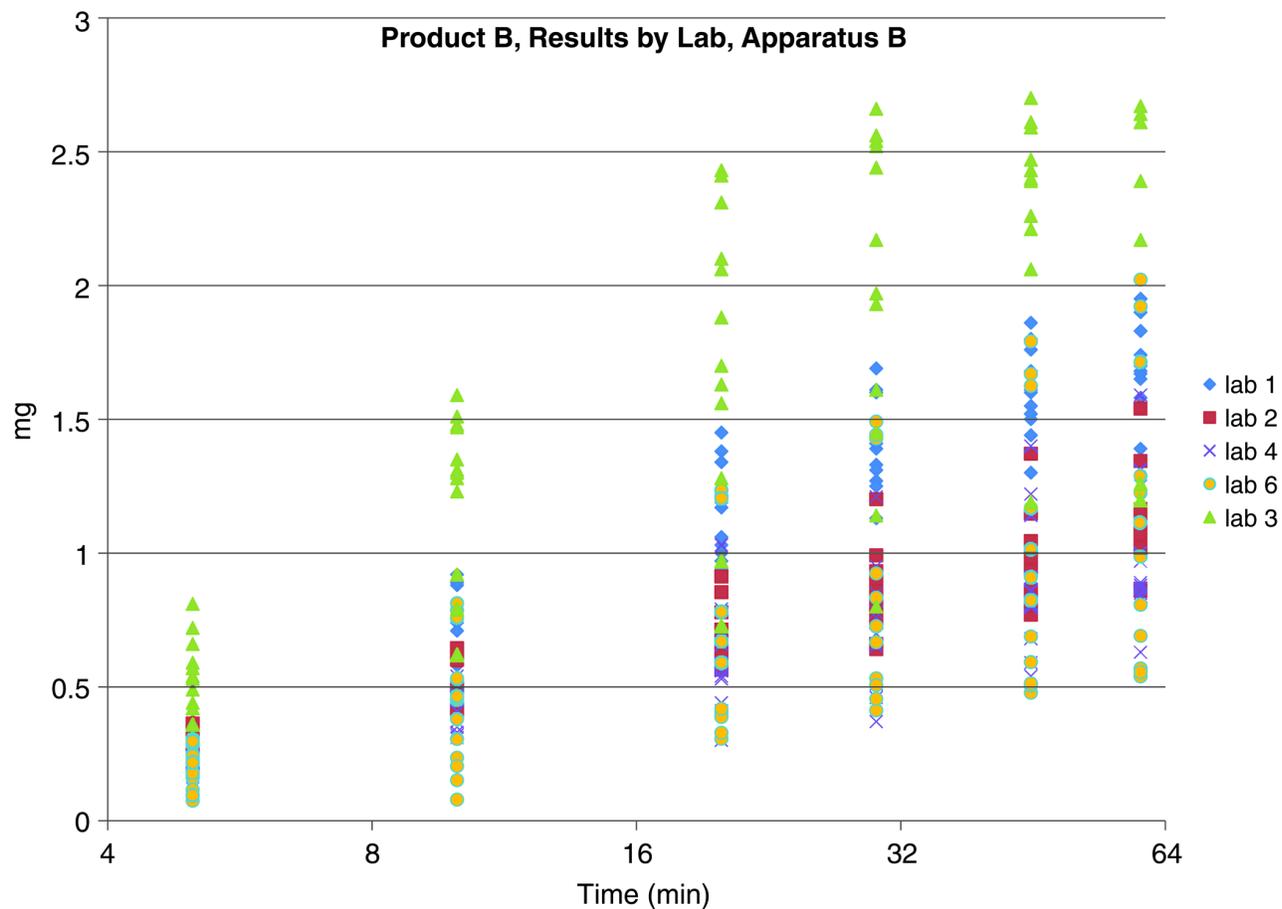


Figure 2. Product B data.

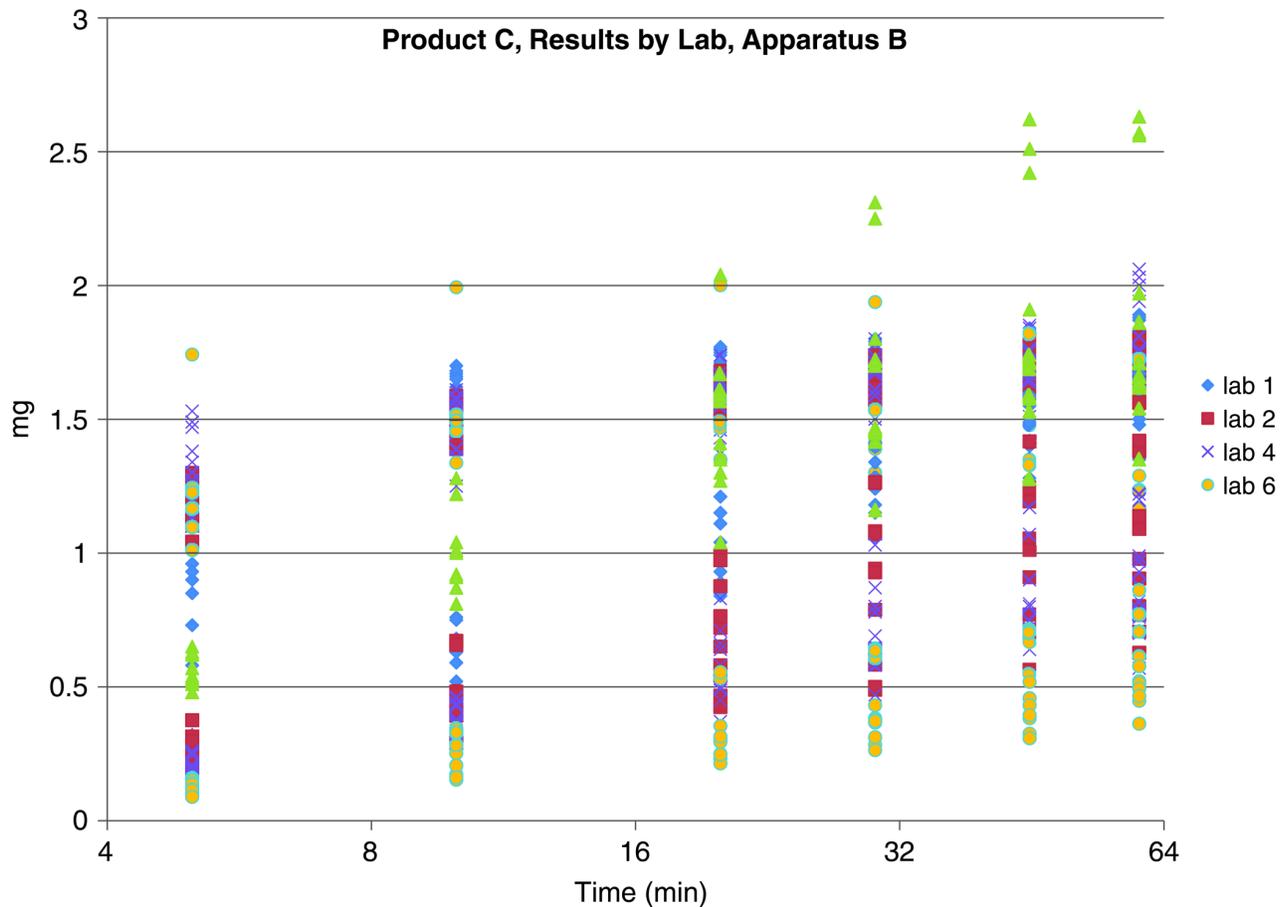


Figure 3. *Product C* data.

Tables 3, 4, and 5 present the geometric mean of amount recovered, repeatability %CVs, and reproducibility %CVs, respectively. Of note is how *Product C* differs from the other two products. *Product C* was developed for this study, whereas *Products A* and *B* are commercial products. The product literature for nicotine polacrilex gums indicates decreasing in vivo release after 30 min of chewing (6). *Product C* had much faster recovery, with the average amount recovered at 5 min equaling or exceeding the averages at 60 min for the other two products. Also, the variability of results for *Product C* is much less, although some of the variability may be due to being farther on the recovery asymptote than the other two products. However, at similar levels of recovery for *Products B* and *C*, *Product C* had considerably lower variability. At 5 min, *Product C* had released 1.20 mg of nicotine, while 1.03 and 1.16 mg of nicotine were released for *Product B* after 45 and 60 min, respectively. The repeatability %CV for *Product C* at 5 min was 12.0%, while for *Product B* the repeatability at 45 and 60 min was 21.5% and 21.2%, respectively. The reproducibility %CV for *Product C* at 5 min was 19.6%, while for *Product B* the reproducibility at 45 and 60 min were 42.4% and 40.6%, respectively. The reproducibility of results has a combined source in the apparatus, method, analyst, laboratory, and the gum sample. The repeatability of results for *Product C* is much less than for *Products A* and *B*. Although this is not a surprising observation for time points after 20 min where the release rate decreases to zero, it is also true for the 10-min timepoint and to a lesser degree at the 5-min timepoint. This serves as an indicator that the contribution from the apparatus design to the reproducibility of the results is not excessive. Other sources, such as the inherent variability of in vitro performance for the marketed products, may have a greater effect.

Table 3. Average Recovered^a for the Three Nicotine Gum Products (mg) Tested with Apparatus B

Product	Time (min)					
	5	10	20	30	45	60
A	0.19	0.37	0.59	0.71	0.83	0.91
B	0.22	0.46	0.71	0.88	1.03	1.16
C	1.20	1.54	1.62	1.67	1.69	1.73

^a Average reported is a geometric mean. All samples were 2 mg each.

Table 4. Repeatability of the %CVs for the Three Nicotine Gum Products Tested with Apparatus B

Product	Time (min)					
	5	10	20	30	45	60
A	18.3%	23.2%	25.3%	24.3%	22.5%	21.8%
B	23.3%	29.9%	23.1%	24.0%	21.5%	21.2%
C	12.0%	4.8%	4.4%	4.7%	4.7%	4.8%

Table 5. Reproducibility of the %CVs^a for the Three Nicotine Gum Products Tested with Apparatus B

Product	Time (min)					
	5	10	20	30	45	60
A	41.0%	51.5%	56.7%	56.4%	54.0%	51.5%
B	39.1%	53.8%	47.9%	44.5%	42.4%	40.6%
C	19.6%	7.1%	7.3%	9.6%	13.2%	17.6%

^a Includes repeatability, within-laboratory (between-analyst), and between-laboratory components.

Comparison of Apparatus A and Apparatus B Designs

One participating laboratory provided results from both apparatus designs, enabling a comparison of nicotine release using the two apparatus designs. The releases for both *Products A* and *B* were higher when apparatus A was used than was observed for apparatus B. *Figure 4* compares the average profiles from those products. In this limited study, the variability of results from the two apparatus designs seems to be different, with variance estimates higher for apparatus B than for apparatus A at all test time points and for times after 5 min, at least by a factor of 2 (*Table 6*).

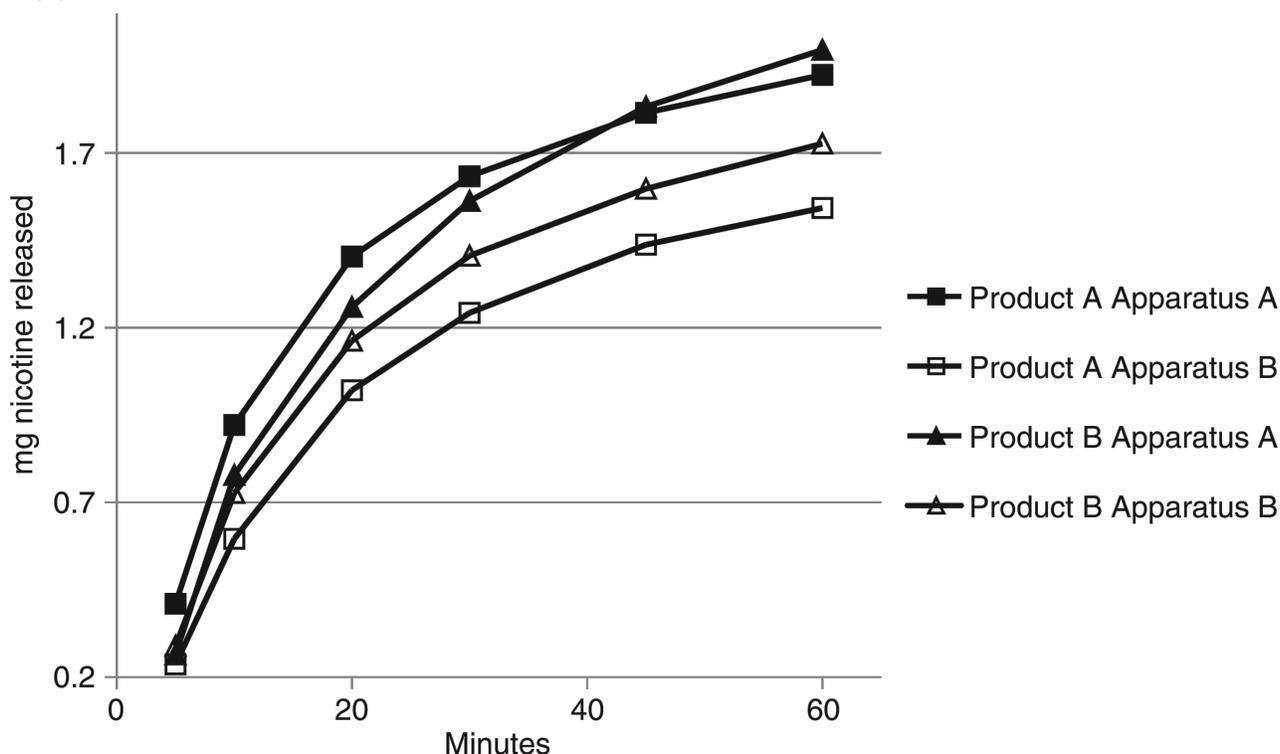


Figure 4. Comparison of nicotine release from *Products A* and *B* with apparatus A and apparatus B.

Table 6. Variance Ratios of Results for *Products A* and *B* Tested with Apparatus A and Apparatus B

Time (min)	Variance Ratios (Apparatus B to Apparatus A)					
	5	10	20	30	45	60
<i>Product A</i> ^a	3.2	2.9	2.8	3.5	4.0	4.8
<i>Product B</i>	1.4	7.8	10.5	11.5	14.7	21.0

^a Only 6 units of *Product A* were tested using apparatus A.

Conditioning the Sample

Several participants reported that thermal conditioning was required to test the samples in apparatus B. Freshly introduced samples resisted the vertical reciprocating action of the plates, but conditioning the samples in the heated medium for 5 min softened the samples, allowing testing to progress.

CONCLUSIONS

Medicated gum performance in vitro represents variability that can be studied and is potentially useful for the control of marketed products. Interlaboratory reproducibility is fairly high, roughly two-fold greater than the repeatability. This is an indication that a performance verification test might be justified and that continuing refinement of operating parameters will be of value. The repeatability of results from the marketed products is an indication that in vitro performance controls may add value in a strategy of control of the marketed product quality. The marketed products (*Products A* and *B*) released their nicotine content incompletely (45%–60%) over the 60-min test time with both test apparatus designs. This may be a reflection of

designed drug release mechanisms. Packaging inserts instruct the consumer to chew the gum until a tingling sensation is observed and, at that point, to place the gum in the buccal pouch until the sensation abates. The continuing renewal of the nicotine release is achieved by renewed chewing but decreases over time. The instructions indicate that the gum should be removed from the mouth after 30 min.

The specially manufactured product (*Product C*) released more rapidly, with a plateau (80%) established after 20 min of the test. The rapid release might offer an opportunity for a performance verification exercise that can be completed in a short period of time that would be convenient for laboratory quality systems. The less variable performance of *Product C*, at a point in the drug release profile where release is comparable to the marketed products, indicates that *Product C* may be a suitable candidate for reference standard use.

The recognition of the need to precondition the gum samples at the temperature of the test represents an improvement in any general procedure. This observation, along with other improvements, will be incorporated in the draft of chapter *Mucosal Drug Products—Product Performance Tests* (1004) that will be proposed in a future issue of *Pharmacopeial Forum*.

The comparison of apparatus A and apparatus B is limited due to the availability of apparatus A. On the basis of this limited experience, three observations are made: 1) apparatus A seems to enable a more rapid release of nicotine from the marketed products sampled than was seen for apparatus B; 2) the results from apparatus A appear to be less variable than results from apparatus B; and 3) with the results from apparatus A as a model, refinement of the procedures associated with apparatus B could improve the variability of results.

In summation, the contribution of the apparatus to the reproducibility of results is less than the variability contributed by the laboratory and the gum sample. Both apparatus A and apparatus B show promise for in vitro release testing of medicated gums.

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STIMULI TO THE REVISION PROCESS

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Medicines Marketed under the Food and Drug Administration Over-the-Counter Drug Monograph System Regulations: Strategy for Developing Compendial Quality Standards

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ABSTRACT This *Stimuli* article explains how the United States Pharmacopeia (USP) proposes to provide better coverage and control of drug product quality through a process that better parallels and supports the Food and Drug Administration Over-the-Counter Drug Monograph System (FDA OTC System). Rationale is provided for using general chapters to organize related tests and procedures in one place for simplified reference from related individual monographs. This *Stimuli* article provides a review of the OTC medicines, USP monograph coverage, and challenges for the development of drug product quality standards. A strategy for developing reference procedures for *Identification*, *Assay*, and *Organic Impurities* will be discussed.

1. Introduction and Background

- 1.1 Scope
- 1.2 Role in Law
- 1.3 Efforts to Date

2. Proposal for Creation of Standards

- 2.1 Identification and Assay Test Procedures
- 2.2 Impurity Tests
- 2.3 Reference Standards

3. General Considerations

- 3.1 Using General Chapters to Achieve Efficiencies in Creating a USP OTC Product Monograph System
- 3.2 Grouping Strategy of Drug Substances

4. Conclusion

1. INTRODUCTION AND BACKGROUND

The United States Pharmacopeial Convention (USP) has begun an initiative to establish and modernize drug product monographs that are applicable to medicines legally marketed under the system established by the FDA under the Federal Food, Drug, and Cosmetic Act (FDCA) for drugs that satisfy all of the requirements in 21 CFR Part 330, their respective therapeutic category monographs, and all other legal requirements (collectively, the FDA OTC Drug Monograph System, which is referred to as the FDA OTC System throughout). Drug products defined in this category are often described as being “generally recognized as safe and effective” (GRASE) and do not require FDA pre-market approval through the pathways of New

Drug Application (NDA)/Abbreviated New Drug Application (ANDA)/Biologics License Application (BLA). This category of drug products includes hundreds of individual drug substances marketed in the form of thousands of drug products. Considering the branding and labeling representations for these products, and not-yet marketed potential combinations, the number of versions marketed legally is considerably greater.

USP already has compendial monographs for many of the "active ingredients" referenced in the FDA OTC System. However, currently USP has not published compendial monographs for most of the OTC drug products currently on the US market and faces the challenge of establishing monograph standards for a broader range of these existing and potential "drug products". Because OTC products are deemed to be safe and effective as a class, the FDA does not require pre-marketing review and approval of quality specifications for OTC products. Each manufacturer establishes the necessary specifications and quality assurance controls for each product in compliance with current Good Manufacturing Practices (cGMPs). When available, USP standards play a critical role in quality assurance. Accordingly, USP is determined to assure that each of its applicable drug substance monographs is kept up-to-date and to provide even better compendial coverage of the legally marketed OTC drug products. Furthermore, USP is prioritizing the development and revision of monographs for as many of the FDA OTC combinations/drug products as is practicable. Certain common OTC drug products are already covered by a USP drug product monograph [e.g., Acetaminophen, Dextromethorphan Hydrobromide (HBr), Doxylamine Succinate, and Pseudoephedrine Hydrochloride (HCl) Oral Solution], but most marketed products currently lack an applicable compendial drug product monograph.

Recently, with the support and urging of FDA and other stakeholders, USP has made compendial standards for FDA OTC System medicines a major focus to ensure improved and ultimately complete coverage of quality standards for this large, consumer-accessible market. USP believes that the optimal way to provide better coverage and control of drug product quality may be through a streamlined monograph development process that parallels the efficiencies of the FDA OTC System by using general chapters to organize related tests and procedures in one place for simplified reference in related individual monographs. This *Stimuli* article outlines current thinking and provides an expanded discussion for the associated monographs and general chapters proposed in this *Pharmacopeial Forum (PF)*.¹

1.1 Scope

The objective of this initiative is to develop a strategy for establishing public quality standards for all OTC GRASE drug products that come to market through the FDA OTC System. *Figure 1* summarizes common pathways for the entry of drug products into commerce. Quality standards for *Prescription Drug Products* (Rx) and OTC products that come to market via the NDA/ANDA/BLA process are established using the specifications emanating from the FDA approval process. The current focus (highlighted in *Figure 1*) is on OTC products without pre-approved specifications, i.e., products covered by the FDA OTC System. To meet this objective, USP is engaged with the on-going challenge of developing and sustaining up-to-date monographs, particularly for complex multi-active ingredient drug products, that provide suitable quality standards while still allowing manufacturers the flexibility for innovation and the continued improvement of drugs in commerce.

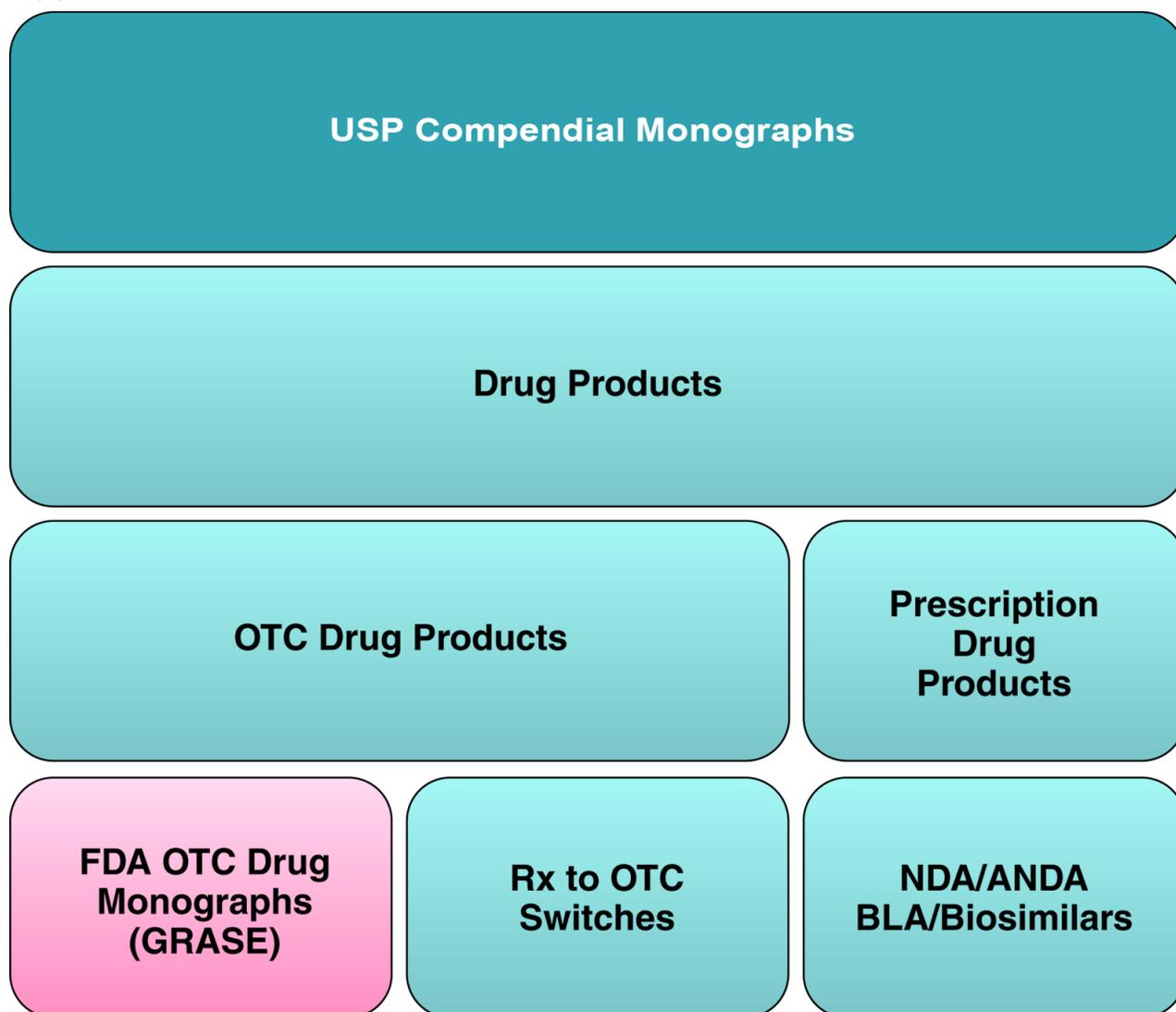


Figure 1. Focus on public quality standards for OTC products that come to market under the FDA OTC System (21 CFR Part 330). Rx = prescription.²

USP drug substance monographs effectively serve as a precondition for coverage in the FDA OTC System under FDA regulations [see e.g., 21 CFR §330.10(a)(2) §VII; §330.14(i)]. At the drug product level (which includes allowed combinations of drug substances), USP currently has about 500 monographs for FDA OTC System drug products. This represents a fraction of OTC products on the market. The OTC product landscape is constantly changing with innovation and because frequent reformulation is permitted within the regulatory flexibility provided by the FDA OTC System. This fast-changing environment makes it virtually impossible for USP to develop and maintain monographs for these products through its traditional approach. The initiative presented in this *Stimuli* article is to define new approaches to develop procedures for identification, assay, and specified impurities. Many drug products exist in the marketplace for which these new procedures may be applicable, and it is the intent of USP to modernize existing or establish new monographs for these products. While the basic tenet of this initiative is to address the drug products that come onto the market through means other than NDA/ANDA regulatory pathways, the outcome could help inform improved approaches to the development of conventional USP quality monographs. Comments are solicited from stakeholders to both improve the content of the current proposal and to gain feedback to

better align future development efforts for this initiative. For further background, see FDA letters submitted to USP^{3,4}, as well as USP comments submitted to FDA.⁵

1.2 Role in Law

USP is determined to better fulfill its compendial role in law and also provide improved support of the FDA OTC System by promptly modernizing existing standards and developing approaches, such as those outlined in this article, to provide more complete coverage of needed drug product monographs. As USP recently noted in a response to the FDA's request for public comments on the FDA OTC System, USP's compendial quality standards in the *United States Pharmacopeia–National Formulary (USP–NF)* support FDA's efforts to provide timely access to safe and effective drugs, and these compendial resources have a role in both law and federal regulations. First, where there is an applicable *USP–NF* compendial standard for either a drug ingredient or drug product, USP plays a role under the adulteration and misbranding provisions of the FDCA (§501 and §502). Second, FDA has also provided a role for applicable *USP–NF* standards in the regulations implementing the FDA OTC System (for example, see e.g., 21 CFR Part 330).

Potentially applicable USP standards include monographs for drug substances, drug products, and other ingredients or excipients. USP standards apply whether the drug is marketed as an OTC (non-prescription) product subject to one of the 21 CFR Part 330 FDA OTC System regulations or a prescription-only product marketed pursuant to a BLA, NDA, or ANDA. Thus, if the USP-designated nonproprietary name (official title) is used, or if the drug complies with monograph Identity,⁶ the official USP title must be used; otherwise, the drug will be deemed misbranded.⁷ If applicable compendial quality standards are not met, the drug will be deemed adulterated.

Note that as USP develops additional compendial drug product monographs applicable to drugs in the FDA OTC System, should there be any different title in an applicable monograph the existing nonproprietary names of those products would need to be revised. Before that is done, the USP Nomenclature Expert Committee would act to designate a name, and the proposed monograph would be posted in *PF* for comment, allowing opportunity for public comment on not only the proposed monograph but the monograph title (i.e., nonproprietary or established name) as well. USP has no role in enforcement; enforcement of USP standards is the responsibility of the FDA and other government authorities in the U.S. and elsewhere.

Beyond USP's general role in law, in terms of FDA OTC System regulations there are specified roles for USP drug substance monographs. The required format for submission of safety and effectiveness data (for classifying OTC drugs as GRASE; see 21 CFR §330.10(a)(2) §VII) specifies that there either be an official applicable *USP–NF* drug substance (active ingredient) quality monograph, or a proposed standard under development at USP for inclusion into *USP–NF*. Time and Extent applications [see 21 CFR §330.14(c)(1)(iii)] may reference the current *USP–NF* to help satisfy the basic information requirements for a "condition" (defined as an active ingredient, or a combination of active ingredients). Finally, as a condition of marketing, any active ingredient included in an OTC monograph "must be recognized in an official *USP–NF* drug monograph that sets forth its [compendial] standards of identity, strength, quality, and purity." [See 21 CFR §330.14(h&i).]

cGMP laws and regulations also play a role in ensuring the quality of OTC drug substances and drug products. In addition to the USP-related authorities in the adulteration provision of the FDCA §501(b), FDA is authorized in §501(a)(2)(B) to deem a drug adulterated if the methods or facilities used for manufacturing, processing, or packaging "do not conform to or are not

operated or administered in conformity with current good manufacturing practice” to assure that the drug meets overall FDCA requirements for safety and also satisfies requisite identity and standards for strength, quality, and purity. In addition, the regulations in 21 CFR Parts 210 and 211 set forth the cGMP requirements for finished pharmaceuticals including OTC drug products. The cGMPs require manufacturers to meet these quality standards, even in the absence of a compendial monograph.

FDA feedback on the *PF 39(1) Stimuli* article supports USP's efforts to establish compendial monographs for those OTC drug products that do not now have *USP* monographs and to modernize those (drug substance and drug product) *USP* monographs that are outdated. An important, generally recognized attribute of compendial standards is that they are public, whereas the manufacturer's standards and practices, including those used to meet FDA-required cGMP, are private and therefore of limited availability and use to anyone other than regulators who have access to such proprietary information.

1.3 Efforts to Date

USP's compendial mission is to engage in a continuous process of assessing and updating standards to ensure that the product specifications reflect changing science. Prioritization for modernization is based on factors such as prevalence of use, evolving measurement techniques and technologies, requests from FDA or other stakeholders, and emergent public health issues. USP has continuously revised standards since its inception, but only in the last decade has USP enlisted a comprehensive prioritization strategy for modernizing the entire set of monographs. For instance, USP posted on its website a list of monographs in need of modernization in May 2010 (*Figure 2*) and has made several periodic updates as more information regarding prioritization has been received from the FDA and other stakeholders.

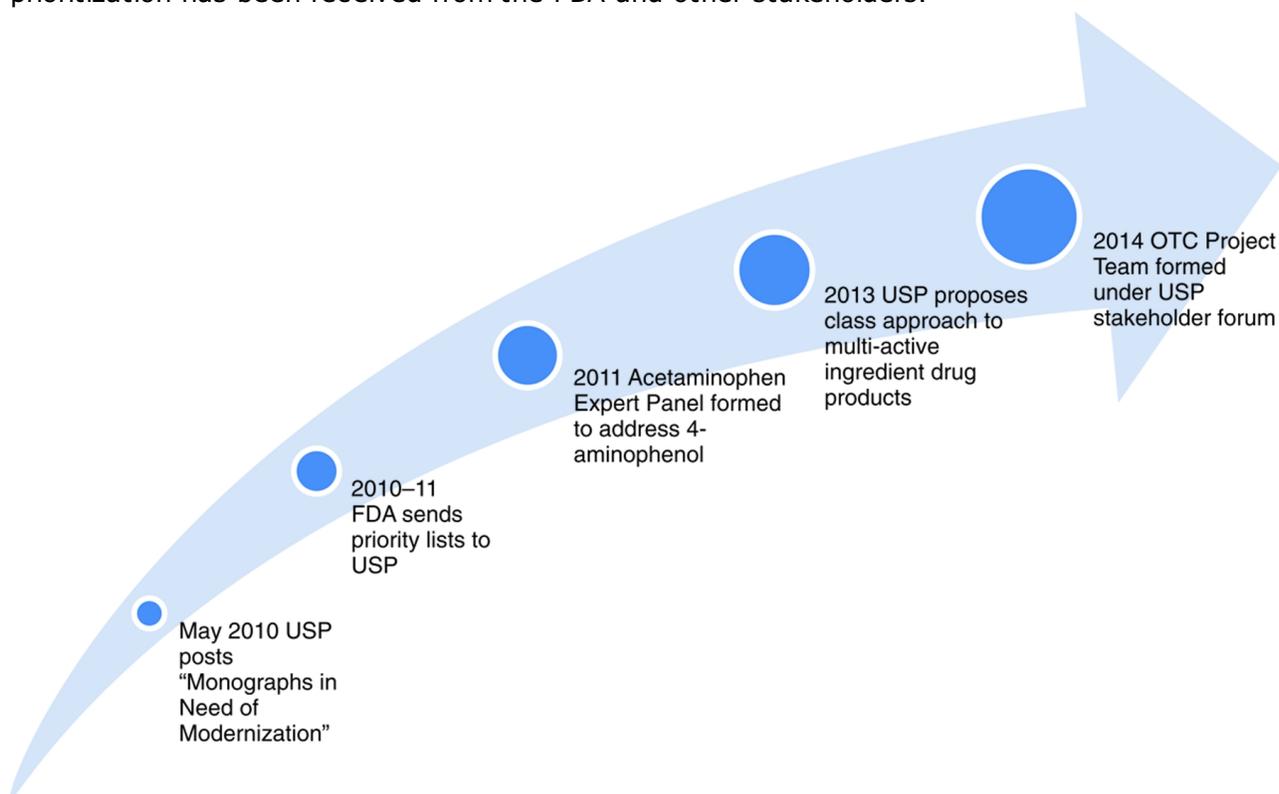


Figure 2. Progress in monograph modernization, 2010–2014.

The FDA sent letters to USP^{3,4} that brought attention to a list of drugs for which monograph modernization should be prioritized. The letters emphasize that USP should conduct a review and prioritize the drug product quality monographs. Several OTC drugs were specifically called out: acetaminophen, chlorpheniramine maleate, pseudoephedrine (HCl and sulfate), phenylephrine HCl, dextromethorphan HBr, and diphenhydramine (HCl and citrate). This provided the impetus for USP to make the fitness of OTC quality compendial monographs a major focus. Current approaches for developing compendial standards for OTC drug products are not efficient for addressing the many unique challenges. To successfully advance USP's role and ensure the preponderance of adequate quality compendial standards for this large and accessible market, a unique approach is needed.

A *Stimuli* article published in *PF 39(1)* entitled *USP's Monographs in Support of FDA's OTC Monograph System: Modernization Opportunities* introduced the concept of a class monograph and detailed the motivation for USP to explore this approach as well as others. The approach presented in that article described a class monograph including quality specifications and performance-based source-independent reference procedures for measuring conformance with the specifications. An example of a class monograph covering the FDA OTC Monograph for Nighttime Sleep-Aid Drug Products⁸, was highlighted. Commenters were generally supportive of USP's initiative to facilitate development of an approach that could provide useful drug product quality standards for use by both industry and regulators. However, the design of the proposed approach relied on the principles of a performance-based method model, which to date, does not have the proven regulatory track record equivalent to a traditional source-verified reference procedure. Feedback received from FDA and other USP stakeholders also highlighted challenges that USP would face if it undertook the development of the class monograph approach. Consideration of these various inputs has led to the current proposed approach. In this *Stimuli* article, we propose publishing individual compendial drug product monographs for products covered under the FDA OTC System, relying on procedures published in general chapters suitable for supporting multiple products which conform to logical combinations of drug substances.

2. PROPOSAL FOR CREATION OF STANDARDS

A systematic focused and simplified approach of developing compendial identification, assay, and specified impurity procedures is proposed. The procedures may be capable of analyzing single active or multi-active ingredient drug products. The proposed approach limits the scope of evaluating organic impurities to only the specified impurities. Development of an approach to address the organic impurity protocol is a priority of USP, but no formal proposals have emerged; more effort on this topic is necessary on the part of USP and stakeholders. The inclusion of drug product performance test requirements is currently being evaluated by USP; no formal proposals have emerged, and more effort on this topic will also be needed. The approaches developed may have applications in either particular monographs or general chapters. The tests and procedures must be capable of meeting specificity and sensitivity requirements for multi-active products, specified and unspecified impurities, and functional excipients. The compendial procedures must also consider the anticipated frequent reformulation and innovation on the part of manufacturers and allow for needed flexibility. Such a compendial standard would provide several advantages:

- A better means of monitoring the quality of products in the marketplace
- A means of establishing more rapid coverage of new GRASE combinations with USP monographs without creating barriers to innovation

- Advancing USP's initiative to modernize the public standards
- Definition of basic but meaningful quality standards for new compendial OTC drug product monographs
- The flexibility for using USP's traditional processes of monograph revision/establishment for drug products that are not suited for inclusion in the general chapter approach

It is understood that the procedures developed and presented based on this approach may not be optimum or suitable for all formulations. However, this approach offers timely delivery of basic core quality standards. USP has different mechanisms to address the challenge of any remaining concerns on a case-by-case basis including the following:

- Regular revisions (revisions based on submission of supporting data) can be submitted by sponsors and utilized to update or replace existing procedures.
- Different specifications (tests, procedures and acceptance criteria) can be considered and added, as a separate procedure, to individual monographs (directly, or indirectly by calling out specifications in a general chapter), or in the form of separate procedures under the flexible monograph policy.
- Alternative approaches as discussed in the *General Notices* (GN) can be used for many purposes, although only USP tests and specified reference materials [USP Reference Standards (RS)] can be used to conclusively demonstrate conformance with USP standards (see *GN 6.30. Alternative and Harmonized Methods and Procedures*, and *GN 5.80 USP Reference Standards*).

2.1 Identification and Assay Test Procedures

For compendial *Identification* and *Assay* tests, specificity becomes a challenge for multi-active ingredient and complex formulations. A clear understanding of the combinations of drug substances, dosage forms, and possible matrices is paramount to achieve a procedure that meets compendial use requirements. Procedure development challenges may also influence the number and types of procedures that will be necessary for some drug products. USP's goal is to minimize the number of procedures needed for a specific product or for a family of closely-related products. The commercial success of high-peak capacity methodologies, such as ultra high performance liquid chromatography (UHPLC) columns, core-shell HPLC columns, and other methodologies, make this approach more attractive today than even a few short years ago. A monograph *Identification* test can make use of the high selectivity of the separation procedure and state-of-the-art detection technologies (e.g., photo-diode array UV detectors). This approach for identification would minimize the number of additional procedures that might otherwise be necessary to comply with compendial requirements.

2.2 Impurity Tests

Test procedures for specified impurities will be developed where needed. In cases where degradation products or toxic impurities and their limits are known, the development of test procedures can be performed in a straightforward manner. A strategy to develop general procedures for each specified impurity in the many drug products will be undertaken. This is in alignment with the work of the Acetaminophen Expert Panel and Small Molecules 2 Expert Committee, which oversaw the development of a general procedure for *4-Aminophenol in Acetaminophen-containing Drug Products* { 227 }, an FDA priority.³ This procedure is official for single-active ingredient products containing acetaminophen, and now is being applied to multi-

active drug products containing acetaminophen in the revision appearing in this *PF*.⁹ Stakeholders may submit information to USP on limits for specified impurities. Comprehensive impurity testing protocols (e.g., unspecified impurities, total impurities) will require additional effort and will be addressed in the future.

2.3 Reference Standards

USP Reference Standards (RSs) are included as comparison standards in most OTC drug monographs; the approach for drug product monograph development discussed in this *Stimuli* article is not expected to generate many new reference standards. However, new reference standards for use in demonstrating procedure system suitability may be introduced for convenience.

3. GENERAL CONSIDERATIONS

3.1 Using General Chapters to Achieve Efficiencies in Creating a USP OTC Product Monograph System

Two approaches were considered by USP for publication of general procedures to simplify and hasten the development of compendial standards for drug products in the FDA OTC System: 1) class monographs, and 2) monographs calling out general chapters. The proposal outlined in the *Stimuli* article in *PF 39(1)* was defined as a 'class' or 'product group' monograph. The concept of a class monograph was assessed to find an efficient means to create drug product monographs for the large and changing array of OTC products as a solution to address the challenge raised both internally at USP and externally [by the FDA and the Consumer Healthcare Products Association (CHPA)]. Upon closer examination, the class monograph approach introduced more complications and provided fewer than expected benefits. Instead, it is anticipated that the required flexibility and efficiencies can be achieved by developing general chapters for identification, assay, and specified impurity procedures for reference by conventional product-specific monographs. This approach makes the referenced general chapter part of a compendial (monograph) requirement, thus avoiding potential confusion arising from applying compendial standards in class monographs (an unconventional and unproven approach) while retaining the expected usefulness of analytical procedures applicable to a range of products. Streamlining the number of analytical procedures needed to ensure the quality of a large class of related drug products would be of benefit to FDA and industry stakeholders. As those general chapters are completed, they will be available for monographs to reference as appropriate. A proposed structure for the general chapters is provided in *Table 1*.

Table 1. Proposed General Chapter Structure

Test	Contents	Chapter Number	Procedure Type
Assay	Organic actives	{ 321 } ^a	Group 1, 2, 3... procedures
	Inorganic actives	{ 323 } ^b	Individual or group 1, 2, 3... procedures
	Other actives (e.g., atypical, natural product, and complex actives)	{ 325 } ^b	Individual 1, 2, 3 procedures

Impurities	Known degradation products with specified limits	{ 327 } ^c	Individual impurity procedures
	Known toxic impurities	{ 327 }	Individual impurity procedures
	Elemental impurities	{ 232 } and { 233 } ^d	—
	Residual solvents	{ 467 } ^d	—
Preservatives	Anti-microbial agents	{ 341 } ^d	—
	Antioxidants	{ 342 } ^b	Group or individual procedures
	Other	{ 343 } ^b	Group or individual procedures

^a *Drug Product Assay Tests—Organic Chemical Medicines* { 321 }, proposed in this *PF*.

^b Future.

^c For acetaminophen-containing drug products, general chapter { 227 } is an example of an individual impurity procedure. New procedures for other specified impurities will be published in *Drug Product Impurity Tests* { 327 }, proposed in this *PF*.

^d No changes are expected to the *Official* chapters.

3.2 Grouping Strategy of Drug Substances

Analytical procedures capable of analyzing groups of FDA OTC System drug products will be developed whenever possible. The term “group” indicates that the procedure may be simultaneously applied for the analysis of multiple drug substances. This approach is expected to allow for a single procedure to be adapted to both single-active and multi-active ingredient drug quality products. Groupings will be largely based on the logical combinations of drug substances and their natural divisions along routes of administration and/or dosage form. Such compendial groupings may or may not happen to conform to FDA's existing regulatory categories in the FDA OTC System. Procedures for “individual” analytes (drug substances, specified impurities, or preservatives) may be more efficient in some cases and Table 1 outlines the proposal of each chapter's contents.

The *Assay* procedures in General Chapter *Drug Product Assay Tests—Organic Chemical Medicines* { 321 } will cover groups of drug substances (FDA OTC active ingredients) to reduce the total number of procedures necessary for multi-active ingredient products. Ideally, each drug product monograph will require a single Assay procedure rather than separate procedures for each individual drug substance. The development strategy is flexible to accommodate groupings based on the logical combinations of drug substances. Although some combinations of active ingredients may not be allowed by the FDA OTC System, the benefits of including them in a group procedure are that fewer procedures would be needed overall.

There are logical boundary conditions to finding the optimal number of procedures. For instance, some active ingredients span different therapeutic categories and are marketed together in combination with multiple other active ingredients through different routes of administration using a variety of dosage forms. A single procedure capable of handling the complexity of all of these factors adds complexity and would perhaps needlessly require additional selectivity. Dividing the number of drug substances along the lines of various routes

of administration or dosage forms may be done to reduce the burden of inactive ingredient interferences. Diphenhydramine is an example of an active ingredient that appears in both oral and topical formulations. If multiple procedures are deemed necessary, the appropriate procedure to be used will be cross-referenced in the monograph. Alternatively, reducing the number of drug substances that are analyzed by a single procedure may be appropriate. Acetaminophen is an example of a drug substance where smaller groupings with other drug substances may provide a benefit.

USP's goal is to establish an optimal number of procedures that will largely reflect the current state of legally marketed products and be derived as a result of specific analytical challenges during procedure development. Furthermore, these procedures may also be useful as a starting point for new drug products introduced to markets containing the same set of drug substances.

4. CONCLUSION

The approaches discussed in this *Stimuli* article provide a general means of updating *USP–NF* consistent with provisions of the FDCA and implementation of regulations. This *Stimuli* article offers a novel approach for developing drug product monographs, particularly those for multi-active ingredient OTC products, and provides a forum for further discussion about ways to modernize the compendial monographs. USP understands that these approaches require careful consideration, and encourages all interested parties and stakeholders to submit comments in response to this article. USP is committed to facilitating further discussion and considering all viewpoints on these matters.

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^c Vice Chair, USP Small Molecules 2 Expert Committee.

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¹ See *Aspirin Capsules*, *Brompheniramine Maleate Tablets*, *Chlorpheniramine Maleate Tablets*, *Brompheniramine Maleate Oral Solution*, *Drug Product Assay Tests—Organic Chemical Medicines* { 321 }, and *Drug Product Impurity Tests* { 327 }.

² Rx switches: refers to prescription drug products switched to non-prescription status.

³ FDA letter to USP dated November 16, 2010 (http://www.usp.org/sites/default/files/usp_pdf/EN/USPNF/key-issues/modernizationlistouderkirkseo.pdf).

⁴ FDA letter to USP dated August 22, 2011 (http://www.usp.org/sites/default/files/usp_pdf/EN/USPNF/key-issues/2011-08-22_fda_letter_to_usp--monograph_modernization_requests.pdf).

⁵ USP comments submitted May 9, 2014 to FDA Docket No. FDA-2014-N-0202, Re: "Over-The-Counter Drug Monograph System; Request for Comments," 79 Fed. Reg. 10168 (Feb. 24, 2014).

⁶ Note that monograph identity tests have a limited purpose: the determination of whether a particular monograph applies to the particular drug substance or drug product is the article that is named in *USP–NF*. Compendial identity tests, and related quality specifications, are not intended to be sufficient to establish biological or chemical identity, safety and effectiveness, or other attributes that are required for licensure by FDA or other regulatory authorities. Thus, compendial identity is not equivalent to regulatory identity; where two drugs share a compendial identity, this does not mean they are one and the same drug—only that they are subject to one and the same USP standard for quality. (See *GN 5.40*)

⁷ USP's nonproprietary naming role applies to all drugs with an applicable USP monograph, including drugs in the FDA OTC System. Reflecting USP's role in law, as specified in FDA OTC drug product labeling regulations, if a drug is recognized in *USP* or *NF*, "the official title of the drug or ingredient in such compendium" is the established name unless it has been designated in a notice and comment rulemaking under FDCA §508 [see e.g., 21 CFR §201.66(b)(5)]. No such names have been designated to date by FDA using §508.

⁸ Final monograph 21 CFR part 338 *NIGHTTIME Sleep-Aid Drug Products for Over-the-Counter Use*.

⁹ See *Acetaminophen and Caffeine Tablets*

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[L## Succinylcholine Chloride, Dionex IonPac CG19 \[NEW\] \(USP39-NF34\)](#)

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Description and Solubility of USP and NF Articles

[Description and Solubility of USP and NF Articles \[NEW\] \(USP39-NF34 1S\)](#)

[Description and Solubility - T](#)

[Description and Solubility - Z](#)**DIETARY SUPPLEMENT MONOGRAPHS**[Hesperidin \[NEW\] \(USP39-NF34 1S\)](#)[Krill Oil \(USP39-NF34 1S\)](#)[Ribose \[NEW\] \(USP39-NF34 1S\)](#)[meso-Zeaxanthin \(USP39-NF34 1S\)](#)**NF MONOGRAPHS**[Guar Gum \(USP39-NF34 1S\)](#)**USP MONOGRAPHS**[Acetaminophen and Caffeine Tablets \(USP39-NF34 1S\)](#)[Acetylcysteine Compounded Solution \[NEW\] \(USP39-NF34 1S\)](#)[Adapalene Gel \[NEW\] \(USP39-NF34 1S\)](#)[Alendronate Sodium \(USP39-NF34 1S\)](#)[Alprazolam Extended-Release Tablets \(USP39-NF34 1S\)](#)[Aminobenzoate Potassium \(USP39-NF34 1S\)](#)[Aminobenzoate Sodium \(USP39-NF34 1S\)](#)[Amitraz \(USP39-NF34 1S\)](#)[Amitraz Concentrate for Dip \(USP39-NF34 1S\)](#)[Aripiprazole \(USP39-NF34 1S\)](#)[Atomoxetine Hydrochloride \(USP39-NF34 1S\)](#)[Atomoxetine Capsules \(USP39-NF34 1S\)](#)[Carbamazepine Extended-Release Tablets \(USP39-NF34 1S\)](#)[Carbidopa and Levodopa Extended-Release Tablets \(USP39-NF34 1S\)](#)[Carbidopa and Levodopa Orally Disintegrating Tablets \(USP39-NF34 1S\)](#)[Cefuroxime Axetil \(USP39-NF34 1S\)](#)[Cefuroxime Axetil Tablets \(USP39-NF34 1S\)](#)[Cetylpyridinium Chloride \(USP39-NF34 1S\)](#)[Clemastine Fumarate Tablets \(USP39-NF34 1S\)](#)[Clomipramine Hydrochloride Compounded Oral Suspension, Veterinary \[NEW\] \(USP39-NF34 1S\)](#)[Desloratadine \[NEW\] \(USP39-NF34 1S\)](#)[Desloratadine Tablets \[NEW\] \(USP39-NF34 1S\)](#)[Desloratadine Orally Disintegrating Tablets \[NEW\] \(USP39-NF34 1S\)](#)[Dexchlorpheniramine Maleate Tablets \(USP39-NF34 1S\)](#)[Dihydroxyaluminum Sodium Carbonate \(USP39-NF34 1S\)](#)[Diphenhydramine Hydrochloride and Ibuprofen Capsules \[NEW\] \(USP39-NF34 1S\)](#)[Docusate Potassium \(USP39-NF34 1S\)](#)[Dronedaron Hydrochloride \[NEW\] \(USP39-NF34 1S\)](#)[Dronedaron Tablets \[NEW\] \(USP39-NF34 1S\)](#)[Duloxetine Hydrochloride \(USP39-NF34 1S\)](#)[Edetate Disodium \(USP39-NF34 1S\)](#)[Esmolol Hydrochloride \(USP39-NF34 1S\)](#)[Ethambutol Hydrochloride Compounded Oral Suspension \[NEW\] \(USP39-NF34 1S\)](#)

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[STIMULI TO THE REVISION PROCESS](#)

[Modernization of Identifications Tests in USP-NF](#)

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Pharmacopeial Forum is covered in *Current Contents/Life Sciences* and in the *Science Citation Index (SCI)*, in *International Pharmaceutical Abstracts*, and in *Current Awareness in Biological Sciences*.

The United States Pharmacopeial Convention comprises representatives from colleges and national and state organizations of medicine and pharmacy. It publishes the U.S. Pharmacopeia and National Formulary, the legally recognized compendia of standards for drugs and products of other health care technologies. The USP and NF include assays and tests for the determination of strength, quality, and purity and requirements for packaging and labeling. **Pharmacopeial Forum's Public Review and Comment Process**

This section presents an overview of the public review and comment process, conducted through *Pharmacopeial Forum (PF)*, for the development of official standards in the *United States Pharmacopeia* and the *National Formulary (USP–NF)*.

USP publishes *PF* on a bimonthly basis to provide an opportunity to review and comment on new or revised standards.

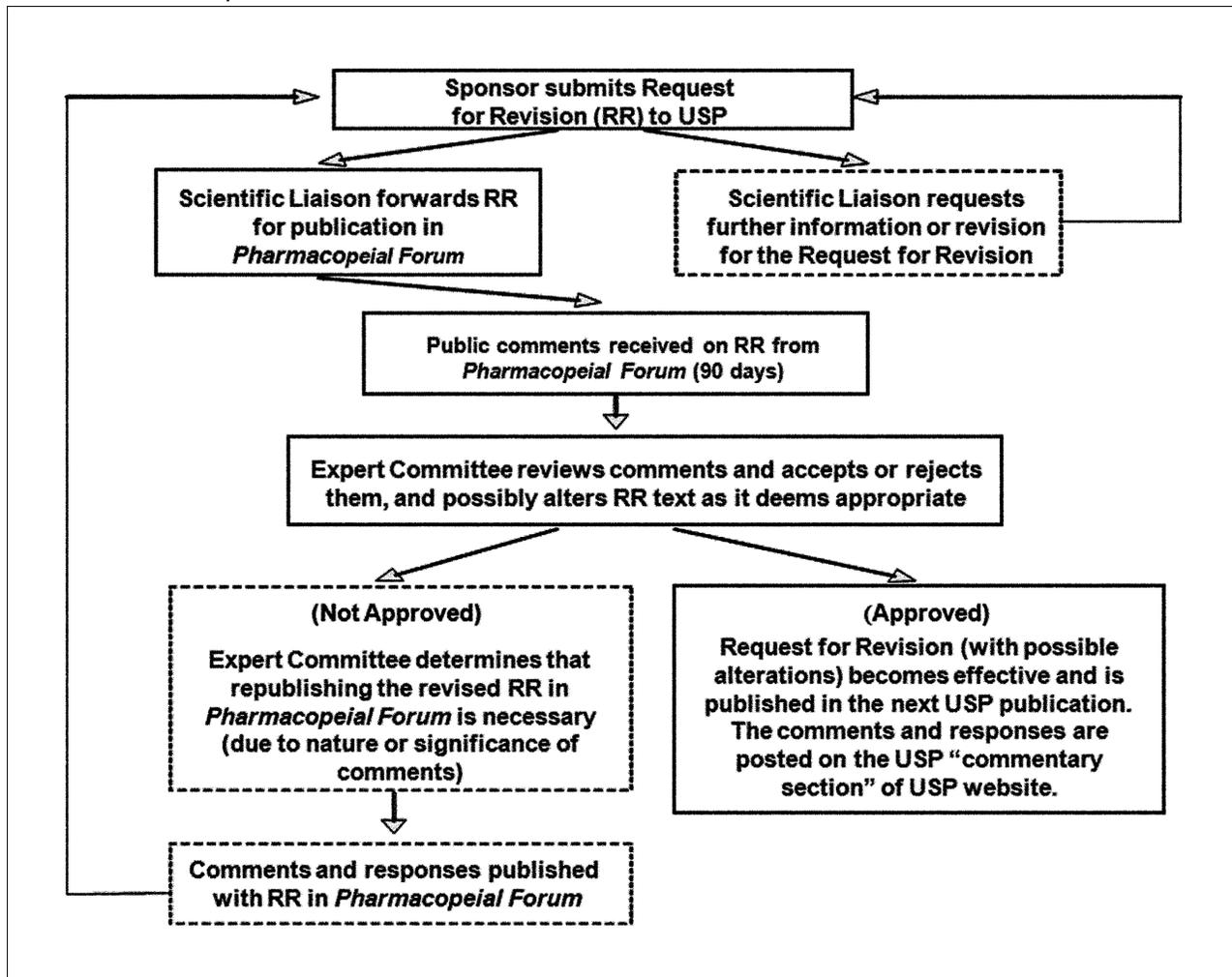
There are two types of proposed revisions in *PF*:

1. **Proposed Revisions**—New or revised standards targeted for adoption through USP's Standard Revision Process. USP's Revision Process calls for publication of a proposed revision in *PF* for a 90-day notice and comment period. After the comment period and subsequent review of comments and approval by the relevant USP Expert Committee, the official standard is published in the next available *USP–NF* or *Supplement*. If comments received are significant, or if the Expert Committee's consideration of comments results in significant additional changes, the Expert Committee may determine that republishing in *PF* is necessary prior to the revision becoming official. See the *In-Process Revision* section for current proposed revisions.

2. **Proposed Interim Revision Announcements**—New or revised standards that become official through an accelerated process in accordance with USP’s Guideline on Accelerated Revisions (available on the USP Web site). *Interim Revision Announcements (IRAs)* allow for a revision to become official prior to the next *USP–NF* or *Supplement*. *IRAs* are first presented for a 90-day public comment period in the *Proposed Interim Revision Announcement* section of the *PF*. Note that final *IRAs*, as well as *Errata*, and *Revision Bulletins*, which also are defined in the Accelerated Revision Guideline, appear only on the USP Web site.

USP welcomes comments and data on proposed revisions. A summary of comments received, along with USP’s responses, will be published in the *Revisions and Commentary* section of the USP website (<http://www.usp.org/USPNF/revisions/>).

The chart below shows the public review and comment process and its relationship to standards development.



Questions on the process should be addressed to the USP Executive Secretariat, United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852 (e-mail: execsec@usp.org).

HOW TO USE PF

The various sections of *PF* are briefly described below. A more detailed description of each section is provided at the beginning of that section. A general description of the types and

amount of information expected in a *Request for Revision* is available in the *Guideline for Submitting Requests for Revision to the USP-NF* on the USP Web site (www.usp.org/USPNF/submitMonograph/subGuide.html). Note that the Expert Committee listing and the Scientific Staff Directory also are located on the USP Web site (see below for links).

Proposed and Adopted Revisions to the USP-NF

Section	Content	How Readers Can Respond
Proposed Interim Revision Announcements	Proposals for <i>Interim Revision Announcements (IRAs)</i> that will be published as official <i>USP</i> or <i>NF</i> standards BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Expert Committee and Scientific Liaisons who handled the monograph or general chapter.	Review material and send comments within 90 days of the <i>PF</i> publication in which the standard was proposed (or per the comment deadline listed in the Briefing section). Direct comments to the USP Scientific Liaison using the contact information provided at the end of each <i>Proposed IRA</i>
In-Process Revision	Proposals for standards that will be published as official in a future <i>USP-NF</i> book or <i>Supplement</i> . BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Expert Committee and USP Scientific Liaisons who handle the monograph or general chapter.	Review material and send comments within 90 days of the <i>PF</i> publication in which the revision was proposed (or per the comment deadline listed in the Briefing section). Direct comments to the USP Scientific Liaison using the contact information provided at the end of each proposed revision. For general inquiries or in cases where a Scientific Liaison is not identified, use the general USP telephone number 301-881-0666 or fax number 301-998-6839 or stdsmonographs@usp.org .

Section	Content	How Readers Can Respond
Stage 4 Harmonization	Revision proposals from the Pharmacopoeial Discussion Group (PDG), which comprises the European Pharmacopoeia, the Japanese Pharmacopoeia, and USP. The Stage 4 draft and the briefing are published in the forum of each pharmacopoeia for public comment. The draft is published in its entirety. BRIEFING: Scientific rationale for the potential inclusion or change or for the proposed change.	Review material and provide comments to the USP Scientific Liaison using the contact information provided at the end of each Stage 4 Harmonization. Individuals who wish to correspond with the European and Japanese Pharmacopoeias concerning monographs in the Stage 4 period of international harmonization should address their comments to the coordinating pharmacopoeia, with a copy to USP. PhEur Secretariat Ms. Lynn Kelso-Eleuterio Central Secretariat European Pharmacopoeia Department European Directorate for the Health Care Council of Europe 7, Allée Kastner CS 30026 67081 Strasbourg France Tel: +33 (3) 88 41 31 48 Fax: +33 (3) 88 41 27 71 lynn.kelso@edqm.eu JP Secretariat Mr. Issei Takayama Technical Officer Pharmaceuticals and Medical Devices Agency (PMDA) Shin-kasumigaseki Building 3-3-2, Kasumigaseki, Chiyoda-ku Tokyo, 100-0013 Japan Phone: +81-3-3506-9431 Fax: +81-3-3506-9440 takayama-issei@pmda.go.jp
Stimuli to the Revision Process	Articles on standards development topics authored by the USP Council of Experts, USP staff, or other interested parties on which USP desires public input prior to further development.	Review material and provide comments to the recipient indicated (usually footnoted in each <i>Stimuli</i> article).

Other Sections

Expert Committees

A listing of the 2010–2015 Expert Committees that work on the development of USP compendial standards

(<http://www.usp.org/aboutUSP/governance/councilOfExperts/expertCommittees.html>)

Staff Directory

Names and contact information of key USP scientific staff members who work on the development of USP compendial standards

(<http://www.usp.org/USPNF/devProcess/contactScientists.html>)

Proposed Interim Revision Announcements

This section includes proposals for *Interim Revision Announcements (IRAs)* that will be published as official *USP* or *NF* standards. There is a 90-day comment period for these proposals (see <http://www.usp.org/USPNF/pf/pfRedesign.html> for the *PF* comment schedule). Readers should review material in this section and provide comments to the Scientific Liaison using the contact information appearing at the end of each proposed *IRA*. Information on how to comment can be found under the "Participation" section of www.usp.org. It is important to send comments promptly so that the Expert Committee members can consider reader's input as they are deciding whether to advance standards to official status. The approved official text will be published under *IRAs* in the "New Official Text" section of USP's Web site.

Each proposal is preceded by a Briefing that indicates the proposed revisions.

Symbols—New text is enclosed in symbols and set off from the current official text as shown in the following example:

◆new text◆

Where the symbols appear together with no enclosed text, such as

◆◆

, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the date the proposal, if approved, will become official as an *IRA*. For example, ◆(IRA 1-Apr-2011)

In-Process Revision

This section contains proposals for adoption as official *USP* or *NF* standards (either proposed *new* standards or proposed *revisions* of current *USP* or *NF* standards). These may be any of the following: (1) proposed revisions placed directly under *In-Process Revision*, or (2) modifications of revisions previously proposed under *In-Process Revision*. Readers should review material in this section and provide comments to the Scientific Liaison using the contact information appearing at the end of each proposal. Information on how to comment can be found under the "Participation" section of www.usp.org. It is important to send comments promptly, using the comment deadline listed after each title.

Briefings Each Proposal is preceded by a Briefing in the following format:

Briefing
Name of Item , citations of the most recent USP publications in which this item appeared. Rationale for the revision. Other relevant information. (For example, if a chromatographic method is being proposed, column specifications and retention times for compounds of interest.) Finally, the Expert Committee acronym (the name of the Scientific Liaison who handled the particular monograph or general chapter, and the USP tracking correspondence number, as shown in the example below:
(Expert Committee Acronym: Liaison Name.)
Correspondence Number—CXXXXX

Symbols Proposed revisions are shown with language proposed for deletion or replacement crossed out. All *USP-NF* revisions use the following symbols that indicate the final destination of the official text:

◆new text◆

if slated for an *IRA*;

▲new text ▲

if slated for *USP–NF*;

■new text ■

if slated for a *Supplement to USP–NF*. The same symbols indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as

* *

, or

■ ■

, or

▲▲

, it means that text has been deleted and no new text was proposed to replace it.

In all revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA*, *Supplement*, or the *USP* or *NF* as the publication where the revision will appear if approved. For example, ■

2S (*USP 34*) indicates that the proposed revision is slated for the *Second Supplement to USP 34*, and ▲

USP35 and ▲*NF30* indicates that the revisions are proposed for *USP 35* and *NF 30*, respectively.

Official Title Changes Where the specification "***Monograph title change***" is found, it indicates that the official title stated after that specification will be substituted for the former title in the appropriate places throughout that monograph once this revision becomes official.

BRIEFING

⟨ 3 ⟩ **Topical and Transdermal Drug Products—Product Quality Tests**, *USP 38* page 71 and *PF 40(2)* [Mar.–Apr. 2014]. On the basis of comments received to the proposed revision published in *PF 40(2)*, the following revisions are being proposed to this chapter:

1. *Description*: Include a visual assessment for the possible presence of cold flow.
2. Replace sachets with packets throughout the text.
3. Replace single-dose and multi-dose with single-unit and multiple-unit, respectively, throughout the text.
4. *Apparent Viscosity*: Include a clearer definition and techniques.
5. *Uniformity in Containers*: Revise *Acceptance Criteria for Products Packaged in Tubes*.
6. *Specific Tests* for transdermal delivery systems: Revise the physical properties to be evaluated and remove specific instructions to assess cold flow, allowing the use of any suitable procedure.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCDF: M. Marques.)

Correspondence Number—148324

Comment deadline: May 31, 2015

〈 3 〉 TOPICAL AND TRANSDERMAL DRUG PRODUCTS—PRODUCT QUALITY TESTS

Change to read:

INTRODUCTION

Topically applied drug products fall into two general categories: those applied to achieve local action and those applied to achieve systemic effects after absorption through the skin into the blood circulation. Local action can occur at or on the surface of the application site (e.g., stratum corneum ~~ocular epithelium~~

■ ■ 1S (USP39)

), in the underlying tissues (e.g., epidermis and/or dermis), and on

■ in ■ 1S (USP39)

subcutaneous tissues (e.g., muscle or joint). Topically applied drug products include but are not restricted to creams, gels, ointments, pastes, suspensions, lotions, foams, sprays, aerosols, solutions, and transdermal delivery systems (TDS, ~~also known as patches~~

■ ■ 1S (USP39)

). The definitions and descriptions of these dosage forms, and brief information on their composition and/or manufacturing process, can be found in *Pharmaceutical Dosage Forms* 〈 1151 〉.

Procedures and acceptable criteria for testing topically applied drug products can be divided into those that assess general product quality attributes and those that assess product performance. The product quality attributes include the following: description, identification, assay (strength), impurities, physicochemical properties, uniformity of dosage units, water content, pH, apparent viscosity, microbial limits, antimicrobial preservative content, antioxidant content, sterility (if applicable), and other tests that may be product specific. Product performance testing assesses drug release and other attributes that affect drug release from the finished dosage form.

~~Although most topically applied drug products are semisolids, liquids, or suspensions, TDS are physical devices that are applied to the skin and vary in their composition and method of fabrication.~~

■ ■ 1S (USP39)

TDS release their active ingredients by different mechanisms. They can be passive or active. This chapter covers only the tests related to passive TDS.

Change to read:

PRODUCT QUALITY TESTS FOR TOPICALLY APPLIED

■ **TOPICAL AND TRANSDERMAL** ■ 1S (USP39)

DRUG PRODUCTS

Universal Tests

Universal tests (see *ICH Guidance Q6A—Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances*, available at www.ich.org) are listed as follows and are applicable to all topically applied drug products.

Description: A qualitative description of the drug product should be provided. The acceptance criteria should include the final acceptable appearance of the finished dosage form and packaging. A visual examination should identify changes in color, adhesive migration (i.e., cold flow; see *Cold Flow Test*) for TDS, separations, crystallization, and others that are specific to the drug product. The description should specify the content or the label claim of the article.

■ For TDS, a visual examination should also be done to assess potential use issues with the product. The examination should include an assessment of the difficulty of removing the TDS from the pouch (e.g., due to adhesive migration adhering the system to the pouch), inability to remove the TDS from the pouch without damage to the system, and adhesive residue remaining on the pouch after removal of the TDS. ■ 1S (USP39)

This is not a compendial test but is part of the manufacturer's specification for the drug product.

Identification: Identification tests are discussed in *General Notices and Requirements, 5.40*. Identification tests should establish the identity of the drug or drugs present in the article and should discriminate between compounds of closely related structures that are likely to be present. Identity tests should be specific for the drug substance(s) (e.g., infrared spectroscopy). Near infrared (NIR) or Raman spectrophotometric methods also could be acceptable for the identification of the drug product (for additional information, see *Near-Infrared Spectroscopy* 〈 1119〉 and *Raman Spectroscopy* 〈 1120〉). Identification solely by a single chromatographic retention time is not specific.

Assay: A specific and stability-indicating test should be used to determine the strength (content) of the drug product.

■ This assay requirement can be satisfied for topical products containing antibiotics by a standard microbiological method (see *Antibiotics—Microbial Assays* 〈 81〉). ■ 1S (USP39)

In cases when the use of a nonspecific assay (e.g., *Titrimetry* 〈 541〉) is justified, other supporting analytical procedures should be used to achieve overall specificity.

Impurities: Process impurities, synthetic byproducts, impurities associated with the adhesive (e.g., residual monomers), residual solvents (see *Residual Solvents* 〈 467〉), heavy metals (see *Heavy Metals* 〈 231〉), and other inorganic and organic impurities may be present in the drug substance and excipients used in the manufacture of the drug product and should be assessed and controlled. Impurities arising from the degradation of the drug substance and those arising during the manufacturing process of the drug product also should be assessed and controlled.

Specific Tests

In addition to the *Universal Tests* listed previously, the following specific tests should be considered on a case-by-case basis:

Uniformity of dosage units: This test is applicable for TDS and for

■ topical ■ 1S (USP39)

dosage forms packaged in single-dose

■ **unit** ■ 1S (USP39)

containers,

■ **such as sachets** ■ 1S (USP39)

(see *Uniformity of Dosage Units* 〈 905 〉).

Water content: A test for water content should be included when appropriate (see *Water Determination* 〈 921 〉). This test is generally formulation dependent. Therefore, it is not included in the compendial drug product monograph but is part of the manufacturer's specification for the drug product.

Microbial limits: Microbial examination of nonsterile drug products is performed according to the methods given in general chapters *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* 〈 61 〉 and *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* 〈 62 〉, unless the formulation itself is demonstrated to have antimicrobial properties. Acceptance criteria for nonsterile pharmaceutical products based on total aerobic microbial count and total combined yeasts and molds count are given in *Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use* 〈 1111 〉.

Antimicrobial preservative content: Acceptance criteria for antimicrobial preservative content in ~~multidose~~

■ **multi-unit** ■ 1S (USP39)

products should be established. They should be based on levels of antimicrobial preservative necessary to maintain the product's microbiological quality at all stages throughout its proposed usage and shelf life (see *Antimicrobial Effectiveness Testing* 〈 51 〉).

Antioxidant content: If antioxidants are present in the drug product, tests of their content should be established unless oxidative degradation can be detected by another test method such as impurity testing. Acceptance criteria for antioxidant content should be established. They should be based on the levels of antioxidant necessary to maintain the product's stability at all stages throughout its proposed usage and shelf life.

Sterility: Depending on the use of the dosage form (e.g., ~~ophthalmic preparations,~~

■ 1S (USP39)

products that will be applied to open wounds or burned areas), sterility of the product should be demonstrated as appropriate (see *Sterility Tests* 〈 71 〉).

pH: When applicable, topically applied drug products should be tested for pH. ~~at the time of batch release and at designated stability time points for batch-to-batch monitoring.~~

■ 1S (USP39)

Because some topically applied drug products contain very limited quantities of water or aqueous phase, pH measurements may not always be warranted. This test is generally formulation dependent. Therefore, it is not included in the compendial drug product monograph but is part of the manufacturer's specification for the drug product.

Particle size:

■ When the finished product contains suspended solid drug substance, the product should be examined for particle size. ■ 1S (USP39)

The particle size of the active drug substance(s) in topically applied drug products is usually determined

■ established ■ 1S (USP39)

and controlled at the formulation development stage. However, topically applied drug products should be examined for evidence of particle

■ size ■ 1S (USP39)

alteration (i.e.,

■ drug polymorphic form, ■ 1S (USP39)

appearance of particles, ~~changes in particle form,~~

■ ■ 1S (USP39)

size, shape, ~~habit,~~ or

■ morphology, agglomeration, or ■ 1S (USP39)

aggregation) of the drug substance that may occur during the course of product processing and storage. ~~Such examinations should be conducted at the time of batch release and at designated stability test time points for batch-to-batch monitoring because changes that are visually (macro and microscopically) observable would likely compromise the integrity and/or performance of the drug product.~~

■ ■ 1S (USP39)

These types of tests are generally formulation dependent. Therefore, such tests are not included in compendial monographs but are part of the manufacturer's specification for the drug product.

■ **Crystal formation:** When

■ the drug substance is dissolved in ■ 1S (USP39)

~~the finished product, does not contain a suspended active drug substance but rather contains a dissolved active drug substance~~

■ ■ 1S (USP39)

the product should be

■ microscopically ■ 1S (USP39)

~~examined for evidence of crystal formation of the active drug substance. Such examinations should be done microscopically and conducted at the time of batch release and at designated stability test time points.~~

■ ■ 1S (USP39)

This test is generally formulation dependent. Therefore, it is not included in the compendial drug product monograph but is part of the manufacturer's specification for the drug product. It is recommended that the potential for the drug product to form crystals of drug substance be examined during product development using conditions of stress. ■ 1S (USP39)

Change to read:**SPECIFIC TESTS FOR OPHTHALMIC DRUG PRODUCTS**

~~Ophthalmic dosage forms must meet the requirements of *Sterility Tests* $\langle 71 \rangle$. If the specific ingredients used in the formulation do not lend themselves to routine sterilization techniques, ingredients that meet the sterility requirements described in $\langle 71 \rangle$, along with aseptic manufacture, may be used. Multiple-use ophthalmic preparations must contain a suitable substance or mixture of substances to prevent growth of, or to destroy, microorganisms accidentally introduced during the use of the product (see *Added Substances* in *Ophthalmic Ointments* $\langle 771 \rangle$), unless otherwise directed in the individual monograph or unless the formula itself is bacteriostatic and/or the delivery system promotes bacteriostasis. The finished ophthalmic preparation must be free from large particles and must meet the requirements for *Leakage* and for *Metal Particles* in $\langle 771 \rangle$. The immediate containers for ophthalmic preparations shall be sterile at the time of filling and closing. It is mandatory that the immediate containers for ophthalmic preparations be sealed and tamper-proof so that sterility is ensured at the time of first use.~~

■ See *Ophthalmic Products—Quality Tests* $\langle 771 \rangle$. ■ 1S (USP39)

Change to read:**SPECIFIC TESTS FOR TOPICALLY APPLIED SEMISOLID DRUG PRODUCTS**

■

Minimum Fill

~~This test applies only to multiple-dose containers, such as tubes and jars (see *Minimum Fill* $\langle 755 \rangle$).~~

■ Single-unit and multiple-unit containers must meet minimum fill requirements as established by testing described in *Minimum Fill* $\langle 755 \rangle$. ■ 1S (USP39)

~~For single-dose~~

■ unit ■ 1S (USP39)

~~containers, such as sachets, see chapter~~

■ where the test for ■ 1S (USP39)

Uniformity of Dosage Units $\langle 905 \rangle$

is applied, the test for general chapter $\langle 755 \rangle$ is not required. ■ 1S (USP39)

Apparent Viscosity

Viscosity is a measure of a formulation's resistance to flow and is an assessment of the

~~rheological properties of the dosage form (e.g., semisolid dosage form). Because only Newtonian fluids possess a measurable viscosity that is independent of shear rate, semisolid pharmaceutical dosage forms that are non-Newtonian products exhibit an apparent viscosity.~~

■ of a rheological property of a semisolid dosage form. The term "apparent viscosity" applies to non-Newtonian fluids, which compose the majority of semisolid pharmaceutical dosage forms.

■ 1S (USP39)

~~The apparent viscosity of semisolid drug products should be tested at the time of batch release and initially at designated stability test time points to set specifications for batch-to-batch and shelf-life monitoring.~~

■ 1S (USP39)

Measurement procedures should be developed as outlined in

• *Viscosity—Capillary Methods* 〈 911 〉 • (CN 1-May-2015)

■ *Viscosity—Rotational Methods* 〈 912 〉, and *Viscosity—Rolling Ball Method* 〈 913 〉. ■ 1S (USP39)

For semisolids that show thixotropy and/or irreversible changes in viscosity after shearing, specific attention should be given to sample preparation procedures to minimize variability in the measurement of apparent viscosity caused by variable shear histories (e.g., mixing speed and temperature, filling operation, and sample handling). Furthermore, for some products it may be warranted to have apparent viscosity specifications at more than

■ one stage of the manufacturing process or with more than ■ 1S (USP39)

one set of

■ test ■ 1S (USP39)

conditions (e.g., bulk in-process stage, final packaged product, high and low shear rates, and different temperatures).

~~Apparent viscosity specifications based on data obtained during product development and shelf-life testing should be established for batch release and throughout the proposed shelf life.~~

■ An acceptable range for apparent viscosity should be established based on data obtained during product development and based on statistical assessment of multiple product batches over the product's shelf life. ■ 1S (USP39)

~~The apparent viscosity test is formulation and/or process dependent. Therefore, it is not included in compendial drug product monographs but is part of the manufacturer's specification for the drug product. Furthermore, the specifications for apparent viscosity of semisolid dosage forms at batch release and during stability testing may be different. Although the apparent viscosity of the finished drug product at the time of batch release must conform to the product development specifications, for stability testing, the apparent viscosity specifications for the drug product should be based on statistical assessment of the product over its shelf life.~~

■ 1S (USP39)

■ Apart from single-point viscosity measurements, more advanced rheological techniques (flow, oscillatory, creep, and stress relaxation testing) can be applied to develop a mechanistic understanding of a formulation and its structure. These techniques may be useful for development of the product using the principles of Quality by Design or for comparative

physicochemical characterization of the test and reference formulations in the case of a biowaiver argument in a generic drug application. However, these techniques are not generally suitable for routine quality testing. Common parameters derived from rheological testing of semisolid pharmaceutical dosage forms that may be useful for characterization and comparison are the storage modulus, loss modulus, relaxation modulus, compliance, thixotropic index, and yield stress.

Acceptance criteria are product specific and defined to assure that the apparent viscosity of each batch of semisolid drug product is within the range defined by the product design and is consistent between batches based on the product development specifications and statistical assessment of multiple product batches over the product's shelf life. ■ 1S (USP39)

Uniformity in Containers

Typically applied semisolid drug products may show physical separation during manufacturing processes and during their shelf life. To ensure the integrity of the drug product, it is essential to evaluate the uniformity of the finished product. ~~at the time of batch release and throughout its assigned shelf life.~~

- This test applies only to multiple-dose
- unit ■ 1S (USP39)

containers, such as tubes and jars. ~~For single-dose containers, such as sachets, see chapter *Uniformity of Dosage Units* (905).~~

- 1S (USP39)

This test does not apply to more fluid topical drug products in multiple-unit containers, such as emulsions, lotions, two-phase gels, or topical suspensions, where the labeling directs the user to mix the product (e.g., shake well) before use. ■ 1S (USP39)

PRODUCTS PACKAGED IN TUBES

~~Within-tube content uniformity can be assessed in the following manner.~~

~~Carefully remove or cut off the bottom tube seal and make a vertical cut from the bottom to the top of the tube. Carefully cut the tube around the upper rim, open the two flaps, and lay the flaps open to expose the product.~~

~~Inspect the product visually for the presence of phase separation, change in physical appearance and texture, and other properties described in the product test for *Description*. If there is no observable phase separation or change in physical appearance and texture, and if the product meets the *Description* acceptance criteria, proceed as described in the following sections. If the product exhibits phase separation and/or change in physical appearance or texture, the product fails the tube content uniformity test.~~

~~The procedures described as follows can be modified depending on the sensitivity of the quantitative procedure used to determine the amount of the drug substance(s) present in the formulation.~~

~~**For multiple-dose products that contain 5 g or more**~~

Procedure 1

1. Using a single tube, after visually inspecting the product, remove an appropriate amount of product from the top, middle, and bottom portions of the tube. The sample size should be sufficient for at least one quantitative determination of the active ingredient(s). Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results using *Acceptance criteria A*.
2. If the product fails *Acceptance criteria A*, test three additional tubes from the same batch following step 1 described above, and evaluate all 12 test results using *Acceptance criteria B*.

Procedure 2

1. Using two tubes, after visually inspecting the product, remove an appropriate amount of product from the top, middle, and bottom portions of each tube. The sample size should be sufficient for at least one quantitative determination of the active ingredient(s). Determine the amount of the active ingredient(s) in each portion of the tube using any appropriate validated quantitative procedure, and evaluate the test results using *Acceptance criteria A*.
2. If the product fails *Acceptance criteria A*, test two additional tubes from the same batch following step 1 described above, and evaluate all 12 test results using *Acceptance criteria B*.

For multiple-dose products that contain less than 5 g of product

1. Test the top and bottom portions of two tubes using *Procedure 1* or *Procedure 2* as described above. Evaluate the test results using *Acceptance criteria A*.
2. If the product fails *Acceptance criteria A*, test two additional tubes from the same batch following step 1 described above, and evaluate all eight test results using *Acceptance criteria B*.

Tube (container) content uniformity test acceptance criteria: In determining the relative standard deviation (RSD) from multiple tubes, first determine the variance from the three measurements for each tube and average across the tubes. The RSD is calculated using this average variance:

Acceptance criteria A—All results are within the product assay range, and the RSD is NMT 6% or as specified in the product specification or in the compendial monograph. If the RSD is greater than 6%, use *Acceptance Criteria B*.

Acceptance criteria B—All results are within the product assay range, and the RSD of the 12 assay results is NMT 6% or as specified in the product specification or in the compendial monograph.

■ **Visual uniformity:** Carefully remove or cut off the bottom tube seal and make a vertical cut from the bottom to the top of the tube. Carefully cut the tube around the upper rim, open the two flaps, and lay the flaps open to expose the product. Repeat this procedure for a total of three tubes.

■ **1S (USP39)**

Inspect the product visually for the presence of phase separation, change in physical appearance and texture (e.g., color change, crystallization, lumping), and other properties described in the product specification for *Description*. If there is no ~~observable~~

■ significant ■ 1S (USP39)

phase separation or significant

■ ■ 1S (USP39)

change in physical appearance and texture, and if the product meets the *Description* criteria, the product passes the test. If the product exhibits

■ significant ■ 1S (USP39)

phase separation and significant

■ Or ■ 1S (USP39)

change in physical appearance or texture, the product fails the test.

Uniformity of active ingredient(s):

The procedures described as follows can be modified depending on the sensitivity of the quantitative procedure used to determine the amount of the drug substance(s) present in the formulation.

For multiple-dose

■ unit ■ 1S (USP39)

tubes that contain 5 g or more of product

Stage 1

1. Using ~~three tubes~~

■ a single tube, ■ 1S (USP39)

after performing the test for *Visual uniformity*, remove an appropriate amount of the product from the top (i.e., cap end), middle, and bottom (i.e., seal end) portions of the tube. The sample size should be sufficient for at least one quantitative determination of the active ingredient(s)

■ and should not exceed the maximum dose recommended by the product labeling for a single application. ■ 1S (USP39)

2. Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the single tube using the *Stage 1 Acceptance Criteria*.

3. The *Stage 1 Acceptance Criteria* are met if:

- None of the ~~nine results (i.e., three each from three tubes)~~ are outside of the product assay range by $\pm 5.0\%$ (e.g., if the product assay range is 90.0%–120.0%, the range

~~will be 85.0%–125.0%),~~

■ three results are outside of the product assay range, ■ 1S (USP39)

and

◦ The maximum difference in the amount of active ingredient(s) determined within ~~each tube,~~ for each of the three tubes tested, is NMT ~~10.0%~~. For example, if the three measurements within a tube are ~~87.0%, 95.2%, and 89.7%,~~ the maximum difference would be ~~8.2% (i.e., 95.2% – 87.0% = 8.2%).~~

■ the tube is NMT 10.0%. For example, if the three measurements within the tube are 97.0%, 95.2%, and 99.7%, the maximum difference would be 4.5% (i.e., 99.7%–95.2% = 4.5%).

■ 1S (USP39)

4. Proceed to *Stage 2* testing if ~~NMT one of the nine test results is outside of the product assay range by ±5.0%, and none are outside the product assay range by ±15.0%, and the maximum difference of the amount of active ingredient(s) measured within each tube is NMT 15.0%;~~

■ none of the test results are outside the product assay range by NMT 5.0% (e.g., if the product assay range is 90.0%–120%, the range will be 85.0%–125.0%), and the maximum difference of the amount of active ingredient(s) measured within the tube is NMT 10.0%.

5. Proceed to *Stage 3* testing if NMT one of the three test results is outside of the product assay range by ±5.0%, and none are outside the product assay range by ±15.0%, and the maximum difference of the amount of active ingredient(s) measured within the tube is NMT 15.0%. ■ 1S (USP39)

Stage 2

1. Test an additional ~~seven~~

■ two ■ 1S (USP39)

tubes for *Visual uniformity* and *Uniformity of active ingredient(s)* for a total of three samples from ~~10~~

■ each from 3 ■ 1S (USP39)

tubes.

■ 2. Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the three tubes using the *Stage 2 Acceptance Criteria*. ■ 1S (USP39)

3. The *Stage 2 Acceptance Criteria* are met if:

- The *Visual uniformity* test is met for all tubes; and
- ~~29 of 30 test results are within the product assay range by ±5.0%, and none are outside of the product assay range by ±15.0%; and~~

- None of the nine results (i.e., three each from three tubes) are outside of the product assay range by NMT 5.0%, and ■ $1S$ (USP39)

- The maximum difference of the amount of active ingredient(s) measured within each tube, for each of the ~~10 tubes tested~~, is NMT ~~15.0%~~

- three tubes tested, is NMT 10.0%.

- Proceed to Stage 3 testing if NMT one of the nine test results is outside of the product assay range by $\pm 5.0\%$, and none are outside the product assay range by $\pm 15.0\%$, and the maximum difference of the amount of active ingredient(s) measured within each tube is NMT 15.0%.

Stage 3

1. Test an additional seven tubes for *Visual uniformity* and *Uniformity of active ingredient(s)* for a total of three samples from 10 tubes.

2. Determine the amount of active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the 10 tubes using the *Stage 3 Acceptance Criteria*.

3. The *Stage 3 Acceptance Criteria* are met if:

- The *Visual uniformity* test is met for all tubes; and
- 29 of the 30 test results are within the product assay range by $\pm 5.0\%$, and none are outside of the product assay range by $\pm 15.0\%$; and
- The maximum difference of the amount of active ingredient(s) measured within each tube, for each of the 10 tubes tested, is NMT 15.0%.

■ $1S$ (USP39)

For multiple-dose

■ *unit* ■ $1S$ (USP39)

tubes that contain less than 5 g of product

Stage 1

1. Using

~~three tubes~~

- a single tube, ■ $1S$ (USP39)

after performing the test for *Visual uniformity*, remove an appropriate amount of product from the top (i.e., cap end) and bottom (i.e., seal end) portions of the tube. The sample size should be sufficient for at least one quantitative determination of the active ingredient(s)

- and should not exceed the maximum dose recommended by the product labeling for a single application. ■ $1S$ (USP39)

2. Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the tube using the *Stage 1 Acceptance Criteria*.

3. The *Stage 1 Acceptance Criteria* are met if:

- ~~None of the six test results (i.e., two each from three tubes) are outside of the product assay range by $\pm 5.0\%$ (e.g., if the product assay range is 90.0%–120.0%, the product assay range $\pm 5\%$ is 85.0%–125.0%), and~~
 - Neither result is outside of the product assay range, and ■ 1S (USP39)

- The difference between the amount of active ingredient(s) determined for the two samples within each tube, for each of the three tubes
 - the tube ■ 1S (USP39)

tested is NMT 10.0%. For example, if the two measurements within a tube were 95.2% and 89.7%, the difference would be 5.5%.

4. Proceed to *Stage 2* testing if ~~NMT one of the six tests results is outside of the product assay range by $\pm 5.0\%$, and none are outside of the product assay range by $\pm 15.0\%$, and the maximum difference of the amount of active ingredient(s) measured within each tube is NMT $\pm 5.0\%$~~

■ neither of the test results are outside the product assay range by $\pm 5.0\%$ (e.g., if the product assay range is 90.0%–120.0%, the range will be 85.0%–125.0%), and the difference between the amounts of active ingredient(s) measured within the tube is NMT 10.0%.

5. Proceed to *Stage 3* testing if NMT one of the test results is outside of the product assay range by $\pm 5.0\%$, and none are outside the product assay range by $\pm 15.0\%$, and the difference between the amounts of active ingredient(s) measured within the tube is NMT 15.0%.

■ 1S (USP39)

Stage 2

1. Test an additional ~~seven~~

■ two ■ 1S (USP39)

tubes for *Visual uniformity* and *Uniformity of active ingredient(s)* for a total of two samples each from ~~10~~

■ 3 ■ 1S (USP39)

tubes.

■ 2. Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the three tubes using the *Stage 2 Acceptance Criteria*. ■ 1S (USP39)

3. The *Stage 2 Acceptance Criteria* are met if:

- The *Visual uniformity* test is met for all tubes; and
- ~~19 of 20 test results are within the product assay range by $\pm 5.0\%$, and none are outside of the product assay range by $\pm 15.0\%$; and~~
 - None of the six test results (i.e., two each from three tubes) are outside of the product assay range by $\pm 5.0\%$; and ■ 1S (USP39)

- The difference between the amount of active ingredient(s) determined for the two samples within each tube, for each of the ~~10 tubes tested~~, is NMT ~~15.0%~~
■ three tubes tested is NMT 10.0%.
- Proceed to Stage 3 testing if NMT one of the six test results is outside of the product assay range by $\pm 5.0\%$ and none are outside of the product assay range by $\pm 15.0\%$, and the difference between the amounts of active ingredient(s) measured within each tube is NMT 15.0%.

Stage 3

1. Test an additional seven tubes for *Visual uniformity* and *Uniformity of active ingredient(s)* for a total of 20 samples, two samples apiece from each of 10 tubes.
2. Determine the amount of active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the 10 tubes using the *Stage 3 Acceptance Criteria*.
3. The *Stage 3 Acceptance Criteria* are met if:
 - The Visual uniformity test is met for all tubes; and
 - 19 of 20 test results are within $\pm 5.0\%$ of the product assay range, and none are outside of the product assay range by $\pm 15.0\%$; and
 - The difference between the amount of active ingredient(s) determined for the two samples within each tube, for each of the 10 tubes tested, is NMT 15.0%.

■ 1S (USP39) ■ 1S (USP39)

PRODUCTS PACKAGED IN CONTAINERS OTHER THAN TUBES

For semisolid products packaged in a container other than a tube when the sampling method presented previously cannot be used, other sampling methods are acceptable, such as the following one described for a jar.

1. Select a suitable syringe of sufficient length to extend to the bottom of the container.
2. Remove and set aside the syringe plunger, and cut off the bottom of the syringe barrel. Sampling should take place from a location to the left/right of the mid-line of the jar surface to preserve an undisturbed region on the other side for any additional investigation (see *Figure 1*).

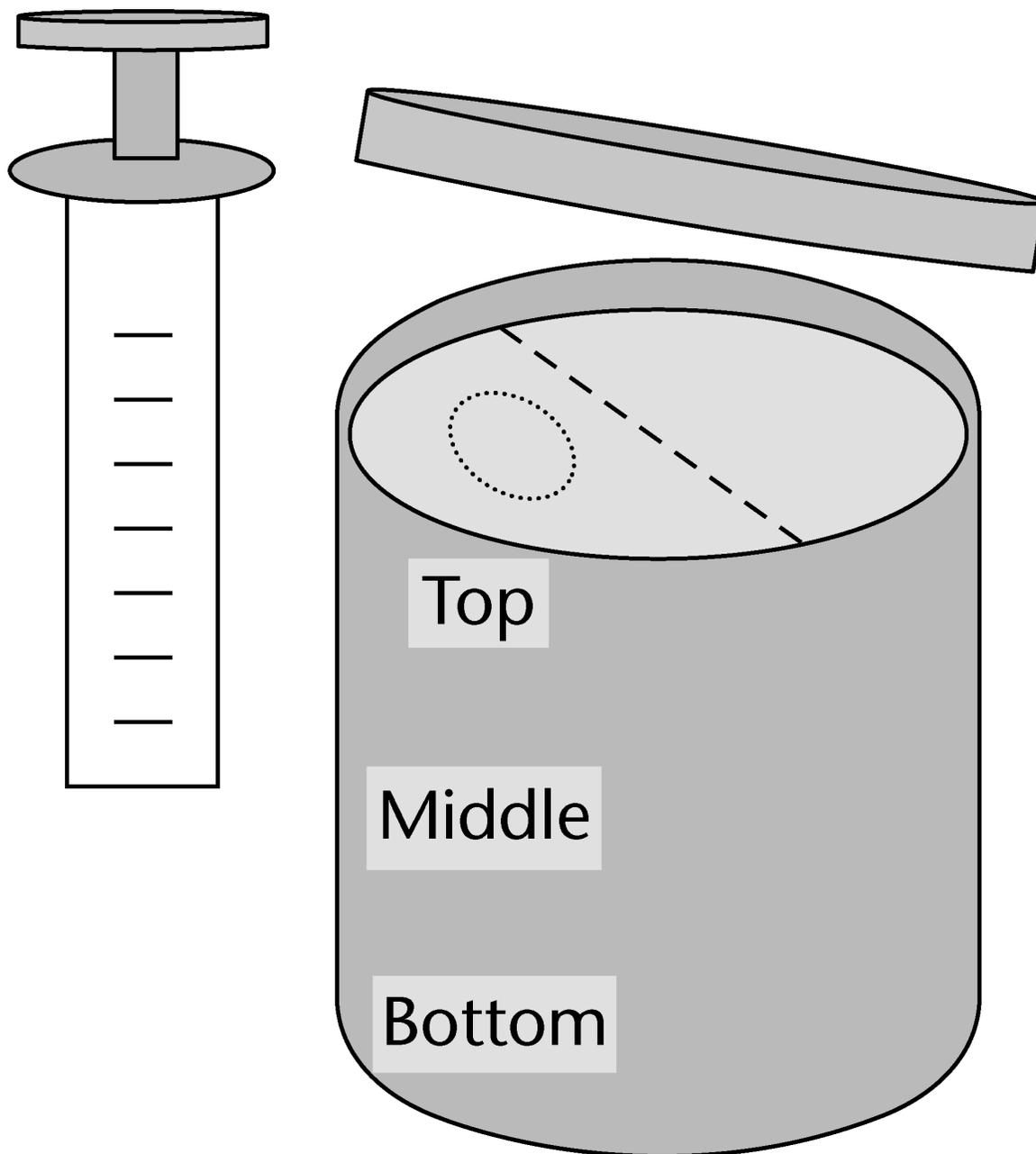


Figure 1. Sampling from a jar container.

3. Slowly push the syringe barrel into the container until it reaches the bottom. Then twist the syringe barrel containing the sample core, and remove the syringe from the container.
4. Insert the syringe plunger into the barrel, and carefully extrude the sample core onto a clean surface in three equal portions to represent the top, middle, and bottom portions of the container.
5. Remove an appropriate sample representative of the middle section of the top, middle, and bottom portions of the container samples, and test according to the instructions outlined in *Products Packaged in Tubes*.

Change to read:

SPECIFIC TESTS FOR TRANSDERMAL DELIVERY SYSTEMS

~~TDS or patches~~

■ TDS ■ 1S (USP39)

are formulated with an adhesive layer to ensure intimate contact with the skin to allow the delivery of the desired dose of drug. Adhesives in TDS must permit easy removal of the release liner before use, adhere properly to human skin upon application, maintain adhesion to the skin during the prescribed period of use, and permit easy removal of the TDS at the end of use without leaving a residue or causing damage to the skin or other undesirable effect(s). Additionally, adhesives must be able to maintain the performance of the TDS throughout the shelf life of the drug product.

~~Three types of TDS adhesion tests are generally used: peel adhesion test (from a standard substrate), release liner peel test, and tack test.~~

■ Testing of the physical properties of the TDS generally include peel adhesion, release liner peel, tack, cold flow, shear, and crystal formation (crystal formation is discussed above in *Product Quality Tests for Topical and Transdermal Drug Products, Specific Tests*). The peel adhesion, release liner peel, and tack tests measure the adhesion properties of the TDS. Each of these tests measures the force required to separate the TDS from another surface. The cold flow and shear measure the cohesive properties of the TDS. These latter tests measure the resistance to flow of the adhesive matrix.

■ 1S (USP39)

Acceptance criteria are product specific and defined to assure that adhesion of each batch of TDS is within the range defined by the product design and is consistent between batches based on the product development specifications ~~or~~

■ and ■ 1S (USP39)

statistical assessment of multiple product batches over the product's shelf life.

■ In addition to physical testing, this section also discusses the *Leak Test* applicable to form-fill-seal (reservoir or pouched)-type TDS.

■ 1S (USP39)

Peel Adhesion Test

This test measures the force required to remove (peel away) a TDS attached to a standard substrate surface (e.g., polished stainless steel). The TDS is applied to the substrate using specified techniques for application and is conditioned at a specified temperature and time. Then the TDS is peeled away from the substrate with an instrument that allows control of peel angle (e.g., 90° or 180°) and peel rate (e.g., 300 mm/min), and the peel force is recorded. This procedure is repeated using a minimum of five independent samples. The product fails the test if the mean peel force is outside the acceptable range determined during product development ~~and/or~~

■ ■ 1S (USP39)

based on statistical assessment of multiple product batches over the product's shelf life.

Release Liner Peel Test

This test measures the force required to separate the release liner from the adhesive layer of

the TDS. The test is performed with a finished product sample. The test sample is conditioned using specific procedures (temperature and time). Then, the release liner is pulled away from the TDS with an instrument that allows for control of peel angle (e.g., 90° or 180°) and peel rate, and the peel force is recorded. This procedure is repeated using a minimum of five independent samples. The product fails the test if the mean peel force is outside the acceptable range determined during product development and/or

■ ■ 1S (USP39)

based on statistical assessment of multiple product batches over the product's shelf life.

Tack Test

Several methods of tack tests have been developed. Examples include the *Probe Tack Method* and the *Rolling Ball Method*. It is up to the TDS manufacturer to decide which one

■ tack test ■ 1S (USP39)

is more

■ most ■ 1S (USP39)

appropriate for each drug product.

PROBE TACK METHOD

This test measures the force required to separate the tip of the test probe from the adhesive layer of the TDS. This test uses an instrument designed to create a bond between the tip of the stainless steel test probe (of defined geometry) and the TDS using a controlled force (light pressure) and specified test conditions (i.e., rate, contact time, contact pressure, and temperature). Then while controlling the rate of probe removal, the test measures the profile of force required to separate the probe tip from the TDS and the maximum force required to break the bond (tack). This procedure is repeated using a minimum of five independent samples. The product fails the test if the mean test result (force profile(s) and/or tack) is outside the acceptable range determined during product development and/or

■ ■ 1S (USP39)

based on statistical assessment of multiple product batches over the product's shelf life.

ROLLING BALL METHOD

This test measures the distance traveled by a defined ball on the adhesive layer of the TDS under defined conditions, as a parameter dependent on the tack properties of the adhesive layer. This test uses a setup designed to roll a ball (with defined material, weight, size, and surface) from a ramp (with defined angle and length) onto the adhesive layer (with defined orientation) under specified test conditions (temperature) (see *ASTM D3121* for more details). The distance traveled by the ball on the adhesive layer is measured using a suitable measuring device. This procedure is repeated using a minimum of five independent samples. The product fails the test if the mean distance traveled is outside the acceptable range determined during product development and/or

■ ■ 1S (USP39)

based on statistical assessment of multiple product batches over the product's shelf life.

Cold Flow Test

Cold flow is the migration of adhesive matrix beyond the edge of the TDS backing
 ■ and through the slit in the release liner, ■ 1S (USP39)

that may occur during the course of product processing and storage. Cold flow is an inherent property of transdermal systems due to the use of pressure-sensitive adhesives that flow when force is applied (i.e., if the adhesive matrix did not flow, the TDS would not stick). The magnitude of the cold flow is generally dependent on the product formulation, storage conditions, and storage time. Cold flow can be
 ■ should be qualitatively and ■ 1S (USP39)

quantitatively measured as an amount (i.e., the region of cold flow can be carefully isolated from the transdermal system and weighed or assayed), an area (i.e., the region of cold flow can be imaged, with suitable magnification, and the area of the cold flow measured using image analysis software), or, most commonly, as a distance (i.e., the distance the adhesive has migrated beyond the edge of the TDS backing, typically NMT a few millimeters, can be measured with suitable magnification; this method is described in further detail below).
 ■ by a suitable method. It is up to the TDS manufacturer to determine the most suitable cold flow test for each drug product. Acceptance criteria are product specific and defined to assure that cold flow of each batch of TDS is within the range defined by the product design and is consistent between batches based on the product development specifications and statistical assessment of multiple batches over the product's shelf life. ■ 1S (USP39)

Assessing cold flow through a combination of qualitative and quantitative methods may be needed. The method(s) chosen to assess cold flow must demonstrate discerning capabilities and be scientifically justified throughout the shelf life of the drug product.

DISTANCE METHOD

Samples to be tested should be carefully handled so as not to disturb the cold flow (if present). If the TDS to be tested is not adhered to the pouching material, carefully remove the TDS without disturbing the cold flow. If the TDS to be tested is adhered to the pouching materials, carefully cut around the perimeter of the pouch so that all edges of the pouch are open. Then carefully peel the front and back of the pouch apart, exposing the TDS, which may be adhered to the inside of the front or back pouch film. A minimum of three TDS should be prepared for testing.

With the aid of magnification (~20–100×) and appropriate lighting, a measurement of the distance the adhesive matrix has migrated beyond the edge of the TDS backing should be taken. A minimum of four measurements should be taken from each TDS. The positions on the TDS where the measurements are to be taken should be evenly spaced and predefined. For example, with the TDS oriented with the printing, measurements could be taken at the 3:00, 6:00, 9:00, and 12:00 positions. Measurements may be taken with the use of a graduated eye piece, through the use of a camera with suitable software, or other appropriate approach.

The product fails if the overall average measured cold flow is outside the acceptable range determined during product development and based on statistical assessment of multiple product batches over the product's shelf life.

~~Note that the test for cold flow provides a direct measure of the cohesive properties of the adhesive matrix, which is a quality attribute that is correlated to shear. Although the shear test has some value during product development as a rudimentary predictor of the potential for cold flow, with the inclusion of cold flow as a product quality test, shear is not recommended as a test to control the quality of the finished product.~~

■ ■ 1S (USP39)

Shear Test

The shear test measures the cohesive strength of a TDS. It can be measured under either static (this method is described in further detail below) or dynamic conditions. Shear testing may not be feasible for all TDS; the presence of multiple layers of adhesive in the system, the presence of a membrane or scrim, or the use of an emulsion adhesive system may result in the inability to achieve cohesive failure. TDS that are constructed with a peripheral adhesive ring or form-fill-seal TDS may not be suitable for this test.

■ It is up to the TDS manufacturer to decide if a shear test is appropriate, and if so, which shear test is most appropriate, for each drug product. Acceptance criteria are product specific and defined to assure that shear of each batch of TDS is within the range defined by the product design and is consistent between batches based on the product development specifications and statistical assessment of multiple product batches over the product's shelf life. ■ 1S (USP39)

Static Shear Test

For the static shear test, the time required to remove a standard area of the TDS from the substrate (i.e., stainless steel test panel) under a standard load (e.g., 250 g) is measured. The TDS is applied to a test panel,

that is at an angle 2° from the vertical

■ ■ 1S (USP39)

and the sample is subjected to a shearing force by means of a given weight suspended from the TDS. The test apparatus holds the test panels at

■ 0° ■ 1S (USP39)

2° from vertical to assure that the TDS will not experience peeling action when the weight is attached. Dwell time, weight used, type of test panel, mode of failure, and sample size should be noted; the time taken for the TDS sample to detach from the test panel is reported. This procedure is repeated using a minimum of five independent samples yielding valid results (see below). The product fails the test if the mean shear force

■ (i.e., arithmetic mean or geometric mean as determined by the manufacturer) ■ 1S (USP39)

is outside the acceptable range determined during product development and based on statistical assessment of multiple product batches over the product's shelf life.

Determination of the validity of static shear test:

Cohesion is the preferred

■ desired ■ 1S (USP39)

failure mode for the static shear test. Cohesive failure is indicated when adhesive is left on the TDS and on the stainless steel test panel. In contrast, an adhesive failure occurs when: 1) the TDS strips cleanly from the stainless steel panel, leaving no visible adhesive on the stainless steel panel; 2) the adhesive transfers to the stainless steel test panel, leaving no adhesive on the TDS; or 3) the TDS delaminates at an interface (e.g., between a membrane and an adhesive layer, or between the two different adhesive layers of a bilayer product). If adhesive failure is indicated, the sample test is invalid. In addition, if the TDS breaks or tears before detaching from the stainless steel test panel or the TDS slides out of the clamp ~~before detaching from the stainless steel test panel,~~

■ ■ 1S (USP39)

or the hanging weight does not hang freely, the sample test is invalid. ■ 1S (USP39)

Leak Test

This test is applicable only for form-fill-seal (reservoir or pouched)-type TDS. Form-fill-seal TDS must be manufactured with zero tolerance for leaks because of their potential for dose dumping if leaking occurs.

In-process control methods to examine TDS for leakers or potential leakers are needed and require considerable development on the part of TDS manufacturers.

IN-PROCESS TESTING

During the manufacturing process, the presence of leakage (or potential for leakage) because of TDS perforation, cuts, and faulty seals resulting from failures such as air bubbles, gel splash, or misalignment of a TDS's backing and release liner layers must be examined. Unless automated process analytical technology is implemented, in-process testing to identify these defects should be performed using the following test procedures.

Visual inspection:

- A specified number of TDS, defined on the basis of batch size, should be examined randomly.
- Each sampled TDS should be thoroughly visually inspected for leakage.
- The product fails if any of the TDS examined are detected with a leak.

Seal integrity: Transdermal system seals should be stress tested to ensure that the application of pressure does not force seals to open, thereby leading to leakage.

- A specified number of TDS, defined on the basis of batch size, should be randomly examined.
- Each sampled TDS should be thoroughly visually inspected for leakage.
- Each sampled TDS is placed on a hard, flat surface and overlaid with a weight so that it is subjected to 13.6 kg. The weight should be left in place for 2 min. Upon removal of the weight, the TDS should be visually inspected for leakage.

- The product fails if the number of TDS detected with a leak is greater than the acceptable limit established by the manufacturer.

Packaged product testing: TDS may leak after they have been individually placed in the primary packaging material as a result of the packaging operation itself or by user opening of the packaging. Therefore, TDS should be tested for leakage after they have been manufactured and packaged in their primary packaging material.

- A specified number of TDS, defined on the basis of batch size, should be randomly tested after they have been placed in their primary packaging material.
- The sampled TDS should be removed from their packaging and thoroughly visually inspected for leakage.
- Each sampled TDS should then be uniformly wiped with a solvent-moistened swab. Both the backing side and the release liner side of the TDS should be wiped. The inside surface of the pouch should also be wiped. The swab(s) is then extracted and assayed for the drug.
- The product fails if the total amount of drug from the TDS, and the corresponding pouch, exceed the acceptable limit established by the manufacturer.

BRIEFING

〈 55 〉 **Biological Indicators—Resistance Performance Tests**, *USP 38* page 103. The General Chapters—Microbiology Expert Committee proposes revisions to this chapter to align its content with the new series of *Sterilization of Compendial Articles* 〈 1229 〉 chapters. References to individual biological indicators monographs are proposed to be deleted and appropriate content moved to the proposed general chapter *Biological Indicators for Sterilization* 〈 1229.5 〉, also found in this issue of *Pharmacopeial Forum*.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCM: R. Tirumalai.)

Correspondence Number—C154737

Comment deadline: May 31, 2015

〈 55 〉 BIOLOGICAL INDICATORS—RESISTANCE PERFORMANCE TESTS

Change to read:

TOTAL VIABLE SPORE COUNT

~~For paper carrier biological indicators, remove three specimens of the relevant biological indicators from their original individual containers. Disperse the paper into component fibers by placing the test specimens in a sterile 250-mL cup of a suitable blender containing 100 mL of chilled, sterilized *Purified Water* and blending for a time known to be adequate to achieve a homogeneous suspension. It is not unusual for blending times of 15 minutes or more to be required for optimal recovery. Transfer a 10-mL aliquot of the suspension to a sterile, screw-capped 16 × 125-mm tube. For *Biological Indicator for Steam Sterilization, Paper Carrier*, heat the tube containing the suspension in a water bath at 95° to 100° for 15 minutes (heat shock), starting the timing when the temperature reaches 95°. For *Biological Indicator for Dry Heat*~~

Sterilization, Paper Carrier, and for Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier, heat the tube containing the suspension in a water bath at 80° to 85° for 10 minutes, starting the timing when the temperature of the spore suspension reaches 80°. Cool rapidly in an ice water bath at 0° to 4°. Transfer two 1-mL aliquots to suitable tubes, and make appropriate serial dilutions in sterilized *Purified Water*, the dilutions being selected as calculated to yield preferably 30 to 300 colonies, but not less than 6, on each of a pair of plates when treated as described below. Where the biological indicator has a low spore concentration, it may be necessary to modify the dilution series and to use more plates at each dilution. Prepare a separate series of plates for each aliquot. Place 1.0 mL of each selected dilution in each of two 15 × 100-mm Petri dishes. Within 20 minutes, add to each plate 20 mL of *Soybean Casein Digest Agar Medium* that has been melted and cooled to 45° to 50°. Swirl to attain a homogeneous suspension, and allow it to solidify. Incubate the plates in an inverted position at 55° to 60° for *Biological Indicator for Steam Sterilization, Paper Carrier*, and at 30° to 35° for *Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier* and for *Biological Indicator for Dry Heat Sterilization, Paper Carrier* or at the optimal recovery temperature specified by the manufacturer. Examine the plates after 24 and 48 hours, recording for each plate the number of colonies; and use the number of colonies observed after 48 hours to calculate the results. Calculate the average number of spores per specimen from the results, using the appropriate dilution factor. The test is valid if the log number of spores per carrier at 48 hours is equal to or greater than the log number after 24 hours in each case. For *Biological Indicator for Steam Sterilization, Self-Contained*, aseptically remove the three carriers from the container, and proceed as directed for *Biological Indicator for Steam Sterilization, Paper Carrier*.

For *Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Nonpaper Carriers*, aseptically remove the three carriers from their original packaging or container. Place each carrier in a suitable sterile container containing 100 mL of chilled *Purified Water*, and sonicate or shake on a reciprocal shaker for an appropriate time. Fifteen minutes or more may be required for optimal recovery. A previous study should be conducted that ensures that the recovery method results in at least 50% to 300% recovery of the labeled spore viable count. Transfer a 10-mL aliquot of the suspension to a sterile, screw-capped 16 × 125-mm tube. Heat the tubes containing suspensions of *Bacillus atrophaeus*, *Bacillus subtilis*, and *Bacillus coagulans* at 80° to 85° for 10 minutes. Heat the tubes containing a suspension of *Geobacillus stearothermophilus* at 95° to 100° for 15 minutes. Start the timing when the lowest temperature of the stated temperature ranges is reached. Cool rapidly in an ice water bath at 0° to 4°. Transfer two 1-mL aliquots to suitable tubes, and make appropriate serial dilutions in *Purified Water*. The selected dilutions should be those that will preferably yield 30 to 300 colonies but not fewer than 6 on each pair of plates when treated as described below. When the biological indicator has a low spore concentration, it may be necessary to modify the dilution series and to use more plates at each dilution. Prepare a separate series of plates for each aliquot. Place 1.0 mL of each selected dilution in each of two 15 × 100-mm Petri dishes. Within 20 minutes add the aliquot to each plate containing 20 mL of agar that has been melted and cooled to between 45° and 50°. Swirl to attain a homogeneous suspension.

For *G. stearothermophilus*, *B. atrophaeus*, *B. subtilis*, and *B. coagulans*, use *Soybean Casein Digest Agar Medium* and incubate the plates in an inverted position aerobically at the following

respective temperatures for each microorganism: 55° to 60°, 30° to 35°, and 48° to 52°, or at the optimum temperature specified by the biological indicator manufacturer. Examine the plates after 24 and 48 hours. Record the number of colonies observed on each plate. Calculate the average number of spores per carrier from the results, using the appropriate dilution factor. The test is valid if the log number of spores per carrier at 48 hours is equal to or greater than the log number after 24 hours in each case.

For *Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Liquid Spore Suspensions*, using *G. stearothermophilus*, *B. atrophaeus*, *B. subtilis*, and *B. coagulans* as biological indicators, prepare an appropriate serial dilution of the original spore suspension in chilled *Purified Water* contained in a sterile, screw-capped 16 × 125-mm tube, and proceed with the viable spore count procedures specified under *Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Nonpaper Carriers*.

D-VALUE DETERMINATION

Conduct all the tests described in this section under aseptic conditions, using sterilized equipment for nonthermophilic microorganisms. D-value determination for *G. stearothermophilus* and *B. coagulans* can be performed in a controlled but unclassified environment.

Apparatus

The test equipment for the determination of microbial resistance is described in substantial detail in ISO 18472, *Sterilization of Health Care Products—Biological and Chemical Indicators—Test Equipment*.³ The details of individual Biological Indicator Evaluation Resistometers (BIERs) vary with the specifics of their design and the particular sterilization process in conjunction with which they are used. Provided that the performance of the BIER vessel meets the requirements of the ISO standard for exposure of the biological indicator, design differences are acceptable.

Procedure

Carry out the tests for D value at each of the applicable sets of sterilization conditions for which the packaged biological indicator under test is labeled for use. Take a sufficient number of groups of specimens of biological indicators in their original individual containers, each group consisting of not less than 5 specimens. The number of groups provides a range of observations from not less than one labeled D value below the labeled survival time through not less than one labeled D value above the labeled kill time. Place each group on a separate suitable specimen holder that permits each specimen to be exposed to the prescribed sterilizing condition at a specific location in the sterilizing chamber of the BIER. Check the BIER apparatus for operating parameters using specimen holders without specimens. Select a series of sterilizing times in increments from the shortest time for the specimens to be tested. The differences in sterilizing times over the series are as constant as feasible, and the difference between adjacent times is no greater than 75% of the labeled D value.

Test procedures for the use of BIER vessels for the evaluation of microbial resistance are defined in a series of ISO standards under the 11138 series.^{2,3,4,5} The appropriate standard should be followed for the biological indicator. The test methods and carriers used with the BIER may be adapted to the specifics of the biological indicator. The method and apparatus used for paper carriers may differ from those for other carriers and will be substantially different

from those used for suspensions of biological indicators.

The D value exposure conditions for alternative material carriers are the same as the conditions used to determine the D value for paper carriers. If the manufacturer's label permits usage of the biological indicator carrier with multiple sterilization methods, then data on D value, survival time, and kill time will need to be provided by the manufacturer for each sterilization method. It is possible that biological indicators inoculated onto carriers other than paper will be used for gaseous or vapor sterilization/decontamination methods such as vapor phase hydrogen peroxide and chlorine dioxide.

Standard physical conditions for the evaluation of biological indicators for use with vapor phase hydrogen peroxide or chlorine dioxide have not been defined. In the case of chlorine dioxide, concentration of the gas, relative humidity, and temperature are critical process control conditions that can be accurately measured. The manufacturer of biological indicators marketed for use with chlorine dioxide should state the conditions under which the D value determination was conducted so that the user can at least discern the resistance of a lot of biological indicators as compared to their own anticipated use conditions. The situation with vapor phase hydrogen peroxide is a more complex one. Various equipment manufacturers have proposed different decontamination or sterilization conditions. Thus, there is no standard process for the conduct of vapor phase hydrogen decontamination or surface sterilization. It follows, then, that there are no industry standard biological indicator evaluation methods for vapor hydrogen peroxide, and it has been reported that there may not be a direct correlation between vapor concentration and rate or even effectiveness of biological indicator inactivation. Additionally, it is difficult to accurately assess relative humidity, which is often defined as a critical process parameter, in the presence of vapor hydrogen peroxide. For these reasons it is more reasonable to consider resistance of biological indicators to be a relative or comparative measure from the manufacturer rather than a true D value. It follows that, depending upon equipment and processes employed, it may be impossible for an end user to duplicate the biological indicator resistance tests performed by the manufacturer.

For *Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Liquid Spore Suspensions*, conduct D value determinations for each of the microorganisms that are provided as a liquid spore crop suspension. The test is conducted using appropriate serial dilutions predicated upon the stated spore titer of the suspension in *Purified Water* in a sterile tube.

Where the suspension is placed on or in a substrate such as an elastomeric closure or formulated product, its resistance may differ from that determined in *Purified Water*. That difference may be significant to the usage of the biological indicators and appropriate measurements made prior to use in sterilization validation activities.

Recovery

After completion of the sterilizing procedure for *Biological Indicator for Dry Heat Sterilization, Paper Carrier*; *Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier*; or *Biological Indicator for Steam Sterilization, Paper Carrier*, whichever is applicable, and within a noted time not more than 4 hours, aseptically remove and add each strip to a suitable medium (see *Media* under *Sterility Tests* (71)) to submerge the biological indicator completely in a suitable tube. For each *Biological Indicator for Steam Sterilization, Self-Contained* specimen, the paper strip is immersed in the self-contained medium according to manufacturers' instructions, within

~~a noted time not more than 4 hours. Incubate each tube at the optimal recovery temperature specified by the manufacturer. Observe each inoculated medium-containing tube at appropriate intervals for a total of 7 days after inoculation. (Where growth is observed at any particular observation time, further incubation of the specimen(s) concerned may be omitted.) Note the number of specimens showing no evidence of growth at any time.~~

~~For *Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Nonpaper Carriers*, recovery of spores from the biological indicator carriers will follow recovery procedures described in the procedures under *Total Viable Spore Count*. D-value determination methods for paper carrier biological indicators may be used to calculate the D value for nonpaper carriers. Incubation conditions for the microorganisms that may be used for nonpaper biological indicators are described in the *Total Viable Spore Count* section.~~

~~For *Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Liquid Spore Suspensions*, the method of recovery following sterilization exposure conditions are those methods described in the *Total Viable Spore Count* section for liquid suspensions, and when a dry heat D-value determination is made from *B. atrophaeus* suspensions, the same recovery procedures as described under *Biological Indicator for Steam Sterilization, Paper Carrier* are followed.~~

~~Where *C. sporogenes* is used as a biological indicator, methods for preparation, inoculation, and recovery methods and media must be adapted to accommodate the use of this anaerobic sporeformer.~~

Calculation

~~The determination of D values of biological indicators can be performed using the Limited Spearman-Kärber, Survival Curve Method or Stumbo-Murphy-Cochran procedures.^{6,7,8} It is preferable to use the same method as that defined by the biological indicator manufacturer to determine D values. The use of a different method can result in differences that are more an artifact of the method than a variation in the performance of the biological indicator.~~

Survival Time and Kill Time

~~Take two groups, each consisting of 10 specimens of the relevant biological indicator, in their original, individual containers. Place the specimens of a group in suitable specimen holders that permit each specimen to be exposed to the sterilizing conditions at a specific location in the BIER chamber.~~

~~Expose the specimens for the required survival time, enter the chamber, and remove the holder(s) containing the 10 specimens. Repeat the above procedure immediately, or preheat if a substantial interval has elapsed, so as to subject the second holder(s) containing 10 specimens similarly to the first conditions, but for the required kill time.~~

~~The *Survival time and kill time* for all monographed biological indicators is described in the official monograph under the heading for each.~~

INTRODUCTION

A biological indicator (BI) is a well-characterized preparation of a specific microorganism with a known resistance to a specific sterilization process. The correct use of BIs in the development,

validation, and control of sterilization processes requires that their population and resistance be accurately known. The population and resistance can be selected to confirm the adequacy of individual sterilization process conditions for an article. The recommendations of *Sterilization of Compendial Articles* (1229) should be followed for effective BI usage. The methods described below can be used to establish population and resistance, such that the response of the BI to the subject sterilization process is appropriate. Although the BI manufacturers are required to maintain rigorous control of population and resistance using the number of replicates as specified below, the end users are not required to use the same number of replicates for verification of those determinations. Conduct all of the tests described in this chapter under appropriate microbiological laboratory conditions (see *Microbiological Best Laboratory Practices* (1117)).

TOTAL VIABLE SPORE COUNT

Sample Collection/Recovery

PAPER/FIBER INDICATORS

For paper/fiber carrier BIs, remove four test samples from their individual containers. Disperse the indicator into component fibers by placing the test samples in a sterile vessel containing 100 mL of chilled, sterilized *Purified Water* and blending to achieve a homogeneous suspension. For self-contained BIs, aseptically remove four BI carriers from their containers and proceed as directed above.

INDICATORS ON OTHER SUBSTRATES

For all other biological indicators, remove four samples from their individual containers. Place the test samples in a sterile vessel containing 100 mL of chilled, sterilized *Purified Water* and agitate to achieve a homogeneous suspension of the spores in the water.

SPORE SUSPENSIONS

For spore suspensions of BIs, prepare an appropriate serial dilution of the original spore suspension in chilled, sterilized *Purified Water* in a sterile container, and follow the viable spore count procedures as specified below. The requirements of the tests are met if the average number of viable spores per carrier is within 0.5 log of the labeled spore count per carrier.

Viable Cell Elimination

Transfer a 10-mL aliquot of the suspension to a sterile tube. For BIs using spores of *Geobacillus stearothermophilus*, *Bacillus coagulans*, and other thermophilic spore formers, heat the tube containing the suspension in a water bath at 95°–100° for 15 min (heat shock), starting the timing when the temperature reaches 95°. For BIs containing nonthermophilic spore formers, heat the tube containing the suspension in a water bath at 80°–85° for 10 min, starting the timing when the temperature of the spore suspension reaches 80°. Cool suspensions rapidly in an ice-water bath at 0°–4°. Transfer two 1-mL aliquots to suitable tubes, and make appropriate serial dilutions in sterilized *Purified Water*. Calculate the dilutions to yield preferably 30–300 colonies, but NLT 6, on each plate in a pair, when treated as described below. Where the BI has a low spore concentration, it may be necessary to modify the dilution series and to

use more plates at each dilution.

Prepare a separate series of plates for each aliquot. Place 1.0 mL of each selected dilution in each of two 15 × 100-mm Petri dishes. Within 20 min, add to each plate 20 mL of *Soybean-Casein Digest Agar Medium* that has been melted and cooled to approximately 45°. Swirl to attain a homogeneous suspension, and allow it to solidify. Incubate the plates in an inverted position at 55°–60° for thermophilic spore formers and at 30°–35° for nonthermophilic spore formers or at the optimal recovery temperature specified by the manufacturer. Examine the plates after 48 h, recording for each plate the number of colonies. Calculate the average number of spores per test sample from the results, using the appropriate dilution factor. When evaluating vendor-supplied BIs, the viable spore count shall be between 50% and 300% of the manufacturer's stated value.

D-VALUE DETERMINATION

Apparatus

The test equipment used for the determination of microbial resistance ("D-value") is described in substantial detail in ISO 18472, *Sterilization of Health Care Products—Biological and Chemical Indicators—Test Equipment* (1). The details of individual Biological Indicator Evaluation Resistometers (BIERs) vary with the specifics of their design and the particular sterilization process for which they are used. Provided that the performance of the BIER vessel meets the requirements of the ISO standard for exposure of the BI, design differences are acceptable. For single-phase sterilization processes where an acceptable BIER has not been defined, the D-value determination can be accomplished by adapting a BIER design intended for a sterilization process operating in the same phase. There are no current methods available for D-value determination for multiple-phase sterilization processes.

Procedure

Carry out the tests for D-value at sterilization conditions consistent with those intended for use. Use 20 replicate test sample BIs in their original individual containers, subjected to at least five exposure conditions for a total of 100 tests. The number of exposure conditions is chosen to provide a range of observations from NLT one labeled D-value below the expected survival time through NLT one labeled D-value above the expected kill time. Place each group on a separate suitable sample holder that permits each sample to be exposed to the prescribed sterilizing condition at a specific location in the sterilizing chamber of the BIER. Check the BIER apparatus for operating parameters using sample holders without test samples. Select a series of sterilizing times in increments from the shortest time for the samples to be tested. The differences in sterilizing times over the series are as constant as feasible, and the difference between adjacent times is NMT 75% of the expected D-value. Test procedures for the use of BIER vessels for the evaluation of microbial resistance are defined in a series of ISO standards under the 11138 series (2–5). The appropriate standard should be followed for the BI. The test methods and carriers used with the BIER may be adapted to the specifics of the BI. The method and apparatus used for paper carriers may differ from those for other carriers and will be substantially different from those used for suspensions of BIs.

The D-value exposure conditions for alternative material carriers are the same as the conditions used to determine the D-value for paper carriers. If a manufacturer's label permits usage of the

BI carrier with multiple sterilization methods, then data on D-value, survival time, and kill time will need to be provided by the manufacturer for each sterilization method.

For BIs that are spore suspensions, conduct D-value determinations for each of the microorganisms that are provided as a liquid spore crop suspension. The test is conducted using appropriate serial dilutions predicated upon the stated spore filter of the suspension in *Purified Water* in a sterile tube. Where the suspension is placed on or in a substrate such as an elastomeric closure or formulated product, its resistance may differ from that determined in *Purified Water*. That difference may be significant to the usage of the BIs and appropriate measurements made before use in sterilization validation activities.

Recovery

After completion of the sterilizing procedure for BIs and within a noted time (NMT 4 h), aseptically remove and add each BI to a suitable medium (see media in *Sterility Tests* (71)) to submerge the BI completely in a suitable tube. For self-contained BIs, the paper strip is immersed in the self-contained medium according to manufacturers' instructions, within a noted time NMT 4 h. For insoluble items inoculated with a spore suspension, aseptically transfer these items individually to a suitable medium (see media in (71)) to submerge the item completely in the medium (aseptic handling is not required when the suspension used is a thermophilic spore former). When a sealed aqueous filled container has been inoculated, test the units individually, as described within (71).

Incubate each tube at the optimal recovery temperature appropriate for the challenge microorganism. Observe each inoculated medium-containing tube at appropriate intervals for a total of 7 days after inoculation. Where growth is observed at any particular observation time, further incubation of the test sample(s) concerned may be omitted. Note the number of samples showing no evidence of growth at any time.

Where *Clostridium sporogenes* or another anaerobic microorganism is used as a BI, methods for preparation, inoculation, and recovery methods and media must be adapted to accommodate the use of this anaerobic spore former.

Calculation

The determination of D-values of BIs can be performed using the Limited Spearman-Kärber, Survival Curve Method, or Stumbo-Murphy-Cochran procedures (6–8). When the BI has been purchased, use the same method as that defined by the BI manufacturer to subsequently determine D-values. The use of an alternate method can result in differences that are more an artifact of the method than a variation in the performance of the BI.

Survival Time and Kill Time

Take two groups of BIs, each consisting of 10 test samples, in their original, individual containers. Place the samples of each group in suitable sample holders that permit each sample to be exposed to the sterilizing conditions at a specific location in the BIER chamber.

Expose the samples for the required survival time, enter the chamber, and remove the holder(s) containing the 10 test samples. Repeat the above procedure immediately, or preheat if a substantial interval has elapsed, so as to subject the second holder(s) containing 10 test samples similarly to the first conditions, but for the required kill time. Recover BIs as described

above. The *Survival Time and Kill Time* should be provided by the BI manufacturer and verified by the end user.

REFERENCES

1. ANSI/AAMI/ISO 18472:2006. Sterilization of health care products—biological and chemical indicators—test equipment. 1st ed. Arlington, VA: Association for the Advancement of Medical Instrumentation (AAMI); 2006.
2. ANSI/AAMI/ISO 11138-1:2006. Sterilization of health care products—biological indicators—part 1: general requirements. 2nd ed. Arlington, VA: Association for the Advancement of Medical Instrumentation (AAMI); 2006.
3. ANSI/AAMI/ISO 11138-2:2006. Sterilization of health care products—biological indicators—part 2: biological indicators for ethylene oxide sterilization processes. 3rd ed. Arlington, VA: Association for the Advancement of Medical Instrumentation (AAMI); 2006.
4. ANSI/AAMI/ISO 11138-3:2006. Sterilization of health care products—biological indicators—part 3: biological indicators for moist heat sterilization processes. 1st ed. Arlington, VA: Association for the Advancement of Medical Instrumentation (AAMI); 2006.
5. ANSI/AAMI/ISO 11138-4:2006. Sterilization of health care products—biological indicators—part 4: biological indicators for dry heat sterilization processes. 1st ed. Arlington, VA: Association for the Advancement of Medical Instrumentation (AAMI); 2006.
6. Pflug IJ. Syllabus for an introductory course in the microbiology and engineering of sterilization processes. 4th ed. St. Paul, MN: Environmental Sterilization Services; 1980.
7. Pflug IJ, Smith GM. The use of biological indicators for monitoring wet-heat sterilization processes. In: Gaughran ERL, Kereluk K, editors. Sterilization of medical products. New Brunswick, NJ: Johnson and Johnson; 1977. p. 193–230.
8. Holcomb RG, Pflug IJ. The Spearman-Kärber method of analyzing quantal assay microbial destruction data. In: Pflug IJ, editor. Microbiology and engineering sterilization processes. St. Paul, MN: Environmental Sterilization Services; 1979.

■ 1S (USP39)

¹ ~~ANSI/AAMI/ISO 18472:2006, Sterilization of Health Care Products—Biological and Chemical Indicators—Test Equipment. Association for the Advancement of Medical Instrumentation (AAMI), 1110 N. Glebe Road, Suite 220, Arlington, VA 22201-4795.~~

² ~~ANSI/AAMI/ISO 11138-1:2006, Sterilization of health care products—Biological indicators—Part 1: General requirements, 2nd ed. Association for the Advancement of Medical Instrumentation (AAMI), 1110 N. Glebe Road, Suite 220, Arlington, VA.~~

³ ~~ANSI/AAMI/ISO 11138-2:2006, Sterilization of health care products—Biological indicators—Part 2: Biological indicators for ethylene oxide sterilization processes, 3rd ed. Association for the Advancement of Medical Instrumentation (AAMI), 1110 N. Glebe Road, Suite 220, Arlington, VA.~~

⁴ ~~ANSI/AAMI/ISO 11138-3:2006, Sterilization of health care products—Biological indicators—Part 3: Biological indicators for moist heat sterilization processes. Association for the Advancement of Medical Instrumentation (AAMI), 1110 N. Glebe Road, Suite 220, Arlington, VA.~~

⁵ ~~ANSI/AAMI/ISO 11138 4:2006, Sterilization of health care products—Biological indicators—Part 4: Biological indicators for dry heat sterilization processes. Association for the Advancement of Medical Instrumentation (AAMI), 1110 N. Glebe Road, Suite 220, Arlington, VA.~~

⁶ ~~Pflug, I.J. *Syllabus for an Introductory Course in the Microbiology and Engineering of Sterilization Processes*, 4th ed. St. Paul, MN: Environmental Sterilization Services, 1980.~~

⁷ ~~Pflug, I.J., and G.M. Smith. The Use of Biological Indicators for Monitoring Wet Heat Sterilization Processes, in *Sterilization of Medical Products*, ed. E.R.L. Gaughran and K. Kereluk. New Brunswick, NJ: Johnson and Johnson, 1977, 193–230.~~

⁸ ~~Holcomb, R.G., and I.J. Pflug. The Spearman-Kärber Method of Analyzing Quantal Assay Microbial Destruction Data, in *Microbiology and Engineering Sterilization Processes*, ed. I.J. Pflug. St. Paul, MN: Environmental Sterilization Services, 1979.~~

BRIEFING

《 123 》 **Glucagon Bioidentity Tests**, *USP 38* page 198. This chapter revision proposal contains two options for manufacturers to test the bioidentity of their glucagon material. One option is the currently official *ex vivo* bioidentity test, and a second option is now offered that uses an *in vitro* cell-based procedure. The new test was validated and approved by the FDA for assuring potency of a particular manufacturer's approved product. In the future, USP plans to omit the rat method completely after multiple manufacturers have been able to demonstrate that the cell-based method is appropriate for all their glucagon products. This is part of USP's ongoing effort to phase out animal-based assays in favor of modern *in vitro* testing. Manufacturers are encouraged to contact USP if the new *in vitro* test is not suitable for their glucagon product or if they would like to submit an alternative validated and approved *in vitro* test for this purpose.

In addition, the following changes to the official text are proposed:

- The *Definition* section is now titled *Introduction* and irrelevant text for the purpose of the chapter was removed. This section was also modified to introduce the use of the new *in vitro* method and to clarify that only one suitable bioidentity test is required to meet the monograph requirement in *Glucagon for Injection*, the only USP monograph that cites this chapter. Note that this citation will remain as written in the monograph when the revised general chapter becomes official.

Additionally, minor editorial changes have been made to update the chapter to current USP style.

(BB1: M. Kibbey.)

Correspondence Number—C143303

Comment deadline: May 31, 2015

《 123 》 GLUCAGON BIOIDENTITY TESTS

INTRODUCTION

Change to read:

Glucagon is a polypeptide

■ peptide ■_{1S} (*USP39*)

hormone that increases blood glucose levels via release of liver glycogen stores. ~~and is~~

clinically used to treat hypoglycemia. Human, porcine, and bovine glucagon share an identical 29-amino acid sequence. Commercially available Glucagon was previously purified from bovine and porcine pancreas glands. Today, human glucagon is recombinantly produced (rGlucagon) with various microbial fermentation systems using the human amino acid sequence. The *USP-NF Glucagon for Injection* monograph defines glucagon identification tests. Glucagon bioidentity must be determined using a validated bioassay method approved by a competent authority. The bioassay must demonstrate that the manufacturing process produces Glucagon that has a biologic activity of NLT 0.80 USP Unit/mg of glucagon. This chapter describes a validated glucagon bioidentity test that measures glucose released from freshly prepared rat liver cells (hepatocytes) stimulated with Glucagon in vitro.

■ A robust and precise physicochemical chromatographic procedure is used in the Glucagon Assay to assign potency on a mass basis. Bioidentity is still required in the *USP-NF Glucagon for Injection* monograph, and two procedure options are presented here: an in vivo procedure based on release of glucose from freshly prepared rat liver cells (hepatocytes) stimulated with Glucagon ex vivo, or production of cyclic adenosine monophosphate (cAMP) in vitro in response to Glucagon stimulation of the USP Glucagon Receptor Cell Line RS. To meet the acceptance criteria of the bioidentity test, only one of these bioidentity tests is required. ■ 1S (*USP39*)

PROCEDURE

Change to read:

•

■ A. ■ 1S (*USP39*)

Primary Liver Cell Assay

■ Bioidentity Test ■ 1S (*USP39*)

[Note—All buffers are oxygenated, prepared with either *Sterile Water for Injection* or *Sterile Water for Irrigation*, warmed to 37°, and adjusted to a final pH of 7.4 unless otherwise indicated. At least two independent assays (replicates) must be performed utilizing two rat livers for each lot of Glucagon. *Figure 1* demonstrates the process used to generate one replicate value. A minimum of two replicates are combined according to the *Calculations* section. The concentration range of the *Standard preparations* and *Assay preparations* may be modified to fall within the linear range of the *Assay*, and the calculations can be adjusted accordingly. Alternatively, full curve analysis using validated nonlinear statistical methods can be used, provided that similarity is demonstrated when analysts compare the responses of the *Standard preparations* and *Assay preparations*.]

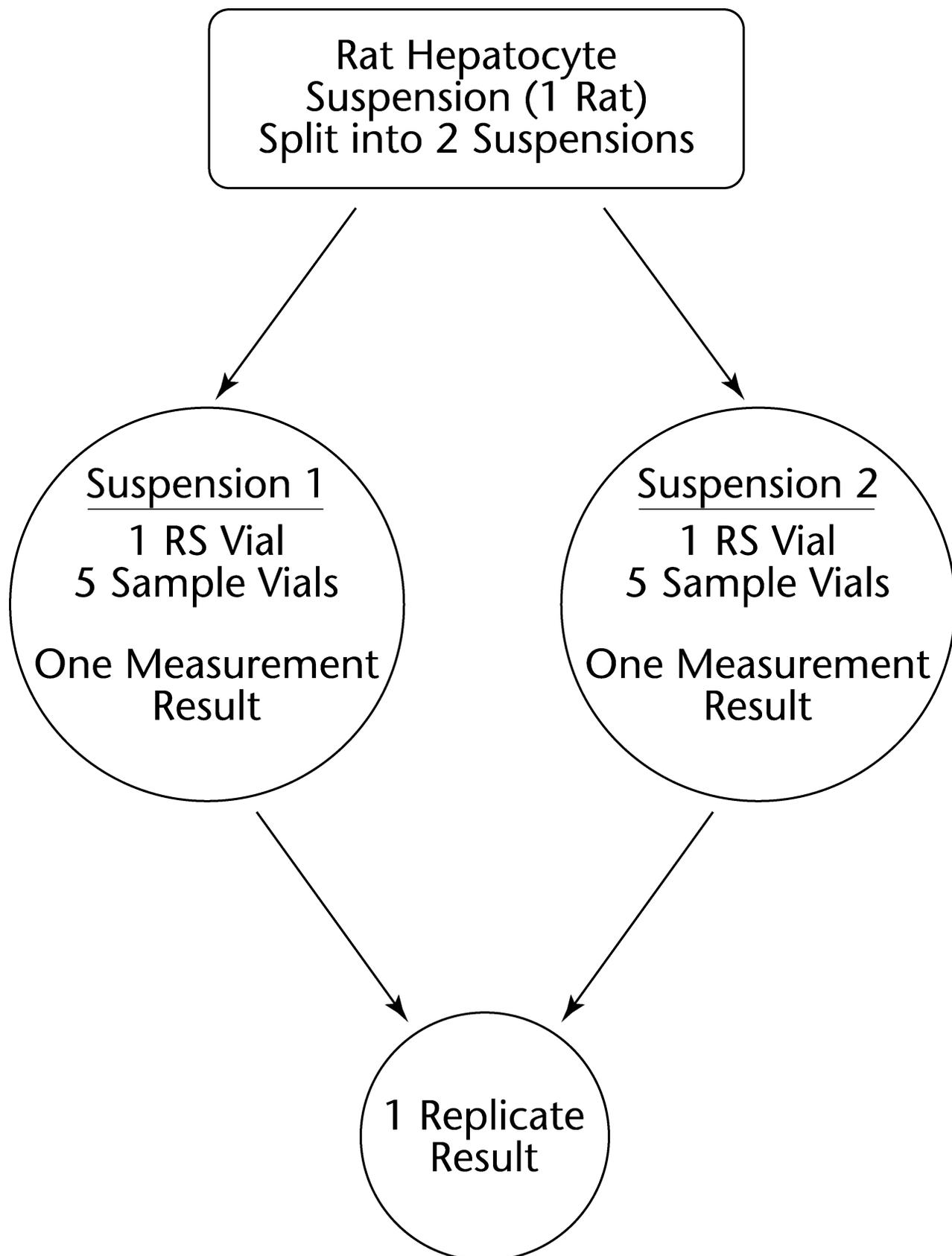


Figure 1. Rat hepatocyte assay method flow diagram (RS = Reference Standard).

Hepatocyte preparation

Calcium-free perfusion buffer with dextrose: Prepare a solution containing 7.92 g/L of

sodium chloride, 0.35 g/L of potassium chloride, 1.80 g/L of dextrose, 0.19 g/L of edetic acid (EDTA), and 2.38 g/L of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES).

Oxygenate before use.

Collagenase buffer: Prepare a solution containing 3.62 g/L of sodium chloride, 23.83 g/L of HEPES, 0.35 g/L of potassium chloride, 0.52 g/L of calcium chloride, and 1.8 g/L of dextrose. Adjust to a pH of 7.6. Immediately before perfusion, dissolve a quantity of collagenase in this solution to obtain a concentration of 0.02%–0.05%. The exact concentration of collagenase is determined empirically for each new lot of enzyme and is the amount that can consistently dissociate the tissue within 10 min of buffer entry and produce a viable cell concentration of $\text{NLT } 3 \times 10^6$ cells/mL.

Wash buffer: Prepare a solution containing 7.92 g/L of sodium chloride, 0.35 g/L of potassium chloride, 0.19 g/L of EDTA, 2.38 g/L of HEPES, 0.11 g/L of calcium chloride, and 0.06 g/L of magnesium sulfate.

Incubation buffer: Prepare a solution containing 6.19 g/L of sodium chloride, 0.35 g/L of potassium chloride, 0.22 g/L of calcium chloride, 0.12 g/L of magnesium sulfate, 0.16 g/L of monobasic potassium phosphate, 11.915 g/L of HEPES, and 10 g/L of bovine serum albumin (1% BSA). Adjust to a pH of 7.5.

Test animals: Male Sprague-Dawley rats are maintained on a standard rat chow diet, given water ad libitum, and allowed to adjust to their new housing before testing. On the morning of the test, select a healthy rat weighing approximately 300–400 g, and administer 100 Units of Heparin Sodium subcutaneously.

Procedure: [Note—Conduct this procedure in the morning to ensure that the rat has optimal glycogen in its liver and so that the procedure can be completed in 1 day.] Anesthetize the rat with an appropriate anesthetic. Open the abdominal cavity and isolate the portal vein. Insert an angiocatheter and tie into the portal vein at the general location of the lienal branch and then connect to a perfusion pump. Start the perfusion (25 mL/min) in situ with the previously warmed, oxygenated, *Calcium-free perfusion buffer with dextrose*. As the liver enlarges, cut the inferior vena cava to allow pressure equilibrium. [Note—About 300 mL of the perfusate is needed to clear the liver of red blood cells at a flow rate of 25–60 mL/min.] Then circulate *Collagenase buffer* at an appropriate flow rate so that the liver leaks perfusate out of the lobes in approximately 10 min (typically 25–60 mL/min). When the liver significantly increases in size, changes color and consistency, and starts to leak perfusate out of the lobes, change the system to the oxygenated prewarmed *Wash buffer*. About 100 mL of *Wash buffer* is needed to wash the liver of collagenase at a flow rate of 25 mL/min. Surgically remove the liver from the animal, and place in a prewarmed Petri dish containing a small amount of oxygenated *Wash buffer* (37^o). Gently comb the liver with a stainless steel, fine-toothed comb to free the hepatocytes. Filter and wash the hepatocytes with *Wash buffer*, through prewetted cheesecloth (3 layers thick, or through a 150- μm mesh polyethylene net) into a beaker. Transfer the cells to two centrifuge tubes and spin for about 1 min at 600 rpm. Discard the supernatant fractions and resuspend the two pellets in *Incubation buffer*. Combine the two pellets in a suitable container and add sufficient *Incubation buffer* to make 150 mL.

System suitability of cell preparation: The cell yield may vary because of the collagenase activity and the viability of the hepatocytes. To check cell viability and to determine viable cell concentration, dilute a 100- μL aliquot of the cell suspension with 400 μL of *Wash buffer* and 500 μL of isotonic 0.4% trypan blue solution. Load aliquots of the cell suspension into both chambers of a hemocytometer and count all eight quadrants. To meet system suitability

of the cell preparation method, a viable cell concentration of 3×10^6 cells/mL (acceptable range of 2.5×10^6 to 3.4×10^6 cells/mL) must be obtained to proceed with the bioassay. If the viable cell concentration exceeds the upper limit, additional *Incubation buffer* may be added to the cells to adjust the concentration to 3×10^6 cells/mL. In this case, the cells are counted again in a hemocytometer, as described above to verify the concentration. [Note—Viable cells are those cells that exclude the trypan blue.]

Glucose determination

Negative control solution: Prepare a solution containing 0.5% BSA using *Sterile Water for Injection* or *Sterile Water for Irrigation*.

Incubation flasks: Use specially prepared 25-mL conical flasks, the bottoms of which have been heated and pushed inward to form a conically raised center, or similar flasks that allow sufficient mixing when swirling. Place the *Incubation flasks* in an orbital shaker water bath at 35° .

Standard preparations: On the day of the assay, dissolve two vials of USP rGlucagon RS, accurately measured,

■ ■ 1S (USP39)

in 0.01 N hydrochloric acid or other suitable diluent (volume based on the potency of the Reference Standard lot) to obtain two solutions each containing 1 USP rGlucagon Unit/mL. All dilutions thereafter are made using *Negative control solution*. Accurately dilute measured volumes of each solution with *Negative control solution* to obtain an intermediate concentration of 400 $\mu\text{U/mL}$, and then dilute the intermediate to produce five concentrations: 200, 100, 50, 25, and 12.5 $\mu\text{U/mL}$ (*Standard preparations*). Pipette 0.1 mL of each *Standard preparation* into separate *Incubation flasks*. Pipette 0.1 mL of *Negative control solution* into each of two flasks (*Negative control solutions 1* and 2).

Assay preparations: Using accurately weighed quantities of Glucagon samples, proceed as directed for *Standard preparations* or, if testing *Glucagon for Injection*, reconstitute 10 vials by slowly adding the contents of the accompanying prefilled syringes containing an appropriate glucagon diluent. Gently mix each vial until the glucagon is dissolved. Using the same syringes, withdraw the contents of five vials and place the solutions in a 25-mL volumetric flask. Repeat for the second five vials, transferring the contents to a second 25-mL volumetric flask. Dilute each flask with 0.01 N hydrochloric acid to volume. Dilute an accurate amount of each solution with 0.5% BSA to yield a concentration of 400 $\mu\text{U/mL}$, and dilute the intermediate to produce five *Assay preparation* concentrations: 200, 100, 50, 25, and 12.5 $\mu\text{U/mL}$. Then proceed as directed for the *Standard preparations*.

Reference stock solution: Dry USP Dextrose RS, and then transfer 1.0 g, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with saturated benzoic acid solution to volume.

Reference solutions: Transfer suitable quantities of *Reference stock solution* to four flasks, and dilute with saturated benzoic acid solution to obtain *Reference solutions* having concentrations of 100, 500, 1000, and 1500 mg/L.

Potassium ferrocyanide solution: Dissolve 1.25 g of trihydrate potassium ferrocyanide in 125 mL of *Sterile Water for Injection*, or use an appropriate commercial source.

System suitability: Analyze the *Potassium ferrocyanide solution*, the *Reference solutions*, and an additional five replicates of either the 500- or 1000-mg/L *Reference solution* in an appropriate glucose analyzer. [Note—*Potassium ferrocyanide solutions* are only appropriate standards for glucose analyzers that measure glucose oxidase activity. The procedure can

also be performed using alternative platforms.] Prepare a standard curve using the *Reference solutions* as directed for the *Standard preparations*. The square root of the residual error mean square from the regression divided by the average of the response multiplied by 100% (line %RSD) must be NMT 2.0%. In addition, the response of the *Potassium ferrocyanide solution* must be NMT 30 mg/L, and the relative standard deviation must be NMT 2.0% for the replicate analyses of the middle *Reference solution*.

Procedure: Dispense 5 mL of *Hepatocyte Preparation* into the *Incubation flasks* in sequence from high glucagon concentration to low glucagon concentration, alternating the *Standard preparations* with the *Assay preparations*. Swirl the flasks in an orbiting water bath at 125 rpm at 30°–35° for approximately 30 min. Following incubation, remove 1.0-mL aliquots from each *Incubation flask*, transfer to labeled microcentrifuge tubes, and centrifuge at 13,000 rpm for 15 s. Place each supernatant fraction in a labeled sampling tube for a glucose analyzer, and determine the glucose concentration (mg/L) of each *Standard preparation* and *Assay preparation*. Measure the background reading of *Negative control solutions 1* and *2*, and calculate the average of the two responses.

To conform to the linear range of the instrument being used, analysts may find it necessary to adjust by dilution each of the *Standard preparations* and *Assay preparations*. Use a glucose analyzer that has demonstrated appropriate specificity, accuracy, precision, and linear response over the range of concentrations being determined. Determine the increase in glucose concentration for each *Standard preparation* and *Assay preparation* compared to the average value of the *Negative control solution*.

Calculations

Calculate the relative potency of the Glucagon samples using statistical methods for parallel-line assays, comparing the Reference Standard curve (from the *Standard preparations*) to the Glucagon sample curve (from the *Assay preparations*). No dose–response reversals may occur within a run for the 25, 50, or 100 µU/mL *Standard preparations* and *Assay preparations*.

[Note—Either the low- or high-dose level, but not both, may be excluded from the calculation in order to meet linearity requirements.] Because a minimum of two valid assays (rats) are required, the estimated potencies are combined using the procedures in *Design and Analysis of Biological Assays* (111), *Combination of Independent Assays*, and the width, L , of a 95% confidence interval for the estimated logarithm of the relative potency is calculated. If L is NMT 0.1938, the results are valid. If L is >0.1938, additional assays may be performed and combined until a valid L term results, and the relative potency is then calculated from all valid independent runs. Calculate the potency of the Glucagon samples in USP rGlucagon Units/mg by multiplying the relative potency result by the potency of USP rGlucagon RS. ~~It meets the requirement of bioidentity if the potency is NLT 0.80 USP rGlucagon Unit/mg.~~

■ **Acceptance criteria:** NLT 0.80 USP rGlucagon Units/mg ■ 1S (USP39)

Add the following:

■ • B. In Vitro Cell-Based Bioidentity Test

Medium A: Dulbecco's Modified Eagle Medium (DMEM) containing 4.5 mg/mL d-glucose and sodium pyruvate, 4 mM l-alanine-l-glutamine¹ or L-glutamine, 10% (v/v) fetal bovine serum,² and 0.5 mg/mL G418.³

Buffer A: Hank's balanced salt solution⁴ containing 5.3 mM potassium chloride, 0.4 mM potassium phosphate monobasic, 4.2 mM sodium bicarbonate, 137.9 mM sodium chloride, 0.3 mM sodium phosphate dibasic anhydrous, and 5.6 mM dextrose.

Cell culture preparation: Remove USP Glucagon Receptor Cell Line RS from cryostorage and immediately thaw at 37° until the cell suspension has just thawed. Aseptically transfer the cell suspension from the cryotube into a sterile test tube containing 10 mL of 37° *Medium A*. Mix and then pellet the cells by centrifugation for 5 min at 125 × *g*. Remove the supernate and resuspend the cells in fresh, 37° *Medium A*. Measure the quantity of cells contained in an aliquot of the suspension by suitable methods and adjust the cell concentration with *Medium A* such that the cell suspension is 0.5–5 × 10³ cells/cm² of tissue culture flask surface. Inoculate tissue culture flasks⁵ and store in a humidified incubator at 37° containing 5% carbon dioxide. Cells should be passaged 2–3 times/week when they are NMT 90% confluent but never trypsinized 2 d in a row. If cells are not ready for passaging after 3 d of culture, the medium should be replaced with fresh *Medium A*. Cells are passaged by first removing the medium from the cell flasks, followed by adding sufficient *Buffer A* prewarmed to room temperature to cover the surface of the flasks. The flasks are gently rocked to wash the cells. This wash fluid is discarded and then sufficient trypsin⁶ is added to the cell flasks to cover the surface, followed by gentle rocking and placement back in the incubator. After 3–5 min, the trypsinized cells are aseptically collected from the flasks and transferred to a sterile centrifuge tube, then a volume of *Medium A* is added that is about 2× the volume of trypsinized cells, and an aliquot is counted. The cell suspension is further diluted with fresh *Medium A* to a final cell concentration of 0.5–5 × 10³ cells/cm² of tissue culture flask surface. After a minimum of 8 passages post-thaw but NMT 25 passages, the cells can be used in the assay. The day before an assay, follow the cell passaging instructions above but resuspend the cells in fresh, 37° *Medium A* to a final cell concentration of 4–5 × 10⁴ cells/mL. [Note—Three identical, independent 96-well white plates⁷ are needed for measurement of two test samples (“A” and “B”) analyzed on each. All wells must be loaded with cells within 40 min.] Using constant, gentle mixing without foaming, each well is loaded with 0.1 mL of cell suspension and incubated overnight at 37° and 5% carbon dioxide (approximately 24 h ± 4 h) prior to starting the *Procedure*. [Note—All remaining solutions should be prepared on the day of the *Procedure*.]

Medium B: Kreb's salt solution containing 0.3% (v/v) human serum albumen, 25 mM HEPES, 1.7 mM IBMX, 0.2 mg/mL glucose, 650 KIU/mL aprotinin, and 0.0003% (v/v) polysorbate 80, pH 7.4.

Medium C: Kreb's salt solution containing 0.3% (v/v) human serum albumen, and 25 mM HEPES, pH 7.4.

Standard stock solutions: Reconstitute USP rGlucagon RS in *Water for Injection* to a concentration of 4 mg/mL by gently mixing on a rotator for 10 min or until completely clear. Dilute this material 1:1000 with *Medium B* to a concentration of 0.4 µg/mL and then 1:200 with *Medium B* to 20 ng/mL.

Standard solutions: Within an hour of use, dilute the 20 ng/mL *Standard stock solution* with *Medium B* to make a 1:5 concentration series of 4 ng/mL (R8), 0.8 ng/mL (R7), 160 pg/mL (R6), 32 pg/mL (R5), 6.4 pg/mL (R4), and 1.3 pg/mL (R3). A final dilution is made from R3 in *Medium B* to make 65 fg/mL (R2). R1 is a blank solution of *Medium B*. [Note—For these *Standard solutions*, as well as the *Sample solutions* and *cAMP standard solutions* described below, it may be helpful to prepare these dilutions in a dilution microplate that mimics the assay plate layout to easily and quickly transfer the materials

to the wells of the assay plate.]

Sample solutions: Reconstitute two independent preparations of glucagon from the same lot of material in *Water for Injection* to a concentration of 4 mg/mL. Prepare by diluting this material further with *Medium B* to prepare a concentration series similar to that described above for the *Standard solutions*. One preparation series is *Sample solution A8–A2* and the other preparation series is *Sample solution B8–B2*.

cAMP standard solutions: Prepare a 5 mM cAMP⁸ solution in water, and then prepare further solutions by dilution with *Medium B* as shown in *Table 1*.

Table 1: Preparation of cAMP Standard Solutions

Starting cAMP Solution	Fold Dilution with <i>Medium B</i>	Final cAMP Solution	Final cAMP Standard Solution Name
5 mM cAMP	1:10	0.5 mM cAMP	—
0.5 mM cAMP	1:20	25 μM cAMP	C1
25 μM cAMP	1:3.3	7.5 μM cAMP	C2
25 μM cAMP	1:10	2.5 μM cAMP	C3
7.5 μM cAMP	1:10	0.75 μM cAMP	C4
2.5 μM cAMP	1:10	250 nM cAMP	C5
0.75 μM cAMP	1:10	75 nM cAMP	C6
250 nM cAMP	1:10	25 nM cAMP	C7
75 nM cAMP	1:10	7.5 nM cAMP	C8
<i>Medium B</i> only	—	No cAMP	C9

Procedure: Remove the plates seeded with cells the day before, and discard the *Medium A* in each well. Wash the cells with 350 μL/well of *Medium C*, then discard the wash solution. Rapidly add 20 μL/well of diluted 1× anti-cAMP-acceptor beads.⁹ Add 30 μL/well of each *Standard solution*, *Sample solution*, or *cAMP standard solutions* as indicated in the plate layouts shown in *Tables 2* and *3*. [Note—The white plate for the *cAMP standard solutions* does not contain cells.] Cover the plate¹⁰, protecting it from light and evaporation, and incubate for 35–60 min in an incubator at 37^o with gentle shaking. Next, dispense 60 μL of diluted 1× donor beads containing biotin-cAMP probe in a 5 mM HEPES lysis buffer containing 0.1% (w/v) BSA, 0.3% (v/v) polysorbate 20, and a pH of 7.4.¹¹ Mix well, then cover¹² the plate again and wrap it with foil. [Note—The beads are light sensitive so plates should be kept dark or under green light conditions.] Gently rotate the plate on a shaker for at least 30 min at room temperature. Keep the plate in the dark, at room temperature, without shaking for 16–30 h before measuring the top luminescence from the plate wells on a suitable plate reader¹³ and detection mode (excitation wavelength of about 680 nm; emission of 520–620 nm).

Table 2. Schematic Representation of the Standard and Sample Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	R1	R2	A2	B2	R3	A3	B3	R4	A4	B4	R8	—
B	R1	R2	A2	B2	R3	A3	B3	R4	A4	B4	R8	—
C	R1	R2	A2	B2	R3	A3	B3	R4	A4	B4	R8	—
D	R1	R2	A2	B2	R3	A3	B3	R4	A4	B4	R8	—
E	R5	A5	B5	R6	A6	B6	R7	A7	B7	A8	B8	C9
F	R5	A5	B5	R6	A6	B6	R7	A7	B7	A8	B8	C6
G	R5	A5	B5	R6	A6	B6	R7	A7	B7	A8	B8	C4
H	R5	A5	B5	R6	A6	B6	R7	A7	B7	A8	B8	C1

LEGEND:

A2–8 = Dilution series of *Sample solution A* (same solution is used on 3 plates, with 4 ng/mL as starting concentration).
 R1–8 = Dilution series of *Standard solution* (an independent *Standard solution* is prepared for each test plate, with 4 ng/mL as starting concentration; as a result, each *Sample solution* is only compared to the *Standard solution* on that plate).
 B2–8 = Dilution series of *Sample solution B* (same solution is used on 3 plates, with 4 ng/mL as starting concentration).
 — = No glucagon, but cells are present.
 C = *cAMP standard controls*, no glucagon. C1 contains 25 μM cAMP, C4 contains 0.75 μM cAMP, C6 contains 75 nM cAMP, and C9 contains no cAMP.

Table 3. Schematic Representation of the cAMP Standards Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	—	—	—	—	—	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—	—	—	—	—	—
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	—	—	—
D	C1	C2	C3	C4	C5	C6	C7	C8	C9	—	—	—
E	C1	C2	C3	C4	C5	C6	C7	C8	C9	—	—	—
F	—	—	—	—	—	—	—	—	—	—	—	—
G	—	—	—	—	—	—	—	—	—	—	—	—
H	—	—	—	—	—	—	—	—	—	—	—	—

LEGEND:

C1–9 = Dilution series of *cAMP standard controls* starting with C1 which contains 25 μM cAMP through C9 which does not contain cAMP.

System suitability criteria: *Sample solutions* A2–A8 and B2–B8 results and *Standard solutions* R1–R8 must be within the linear range of the *cAMP standard solutions* results (defined as C3–C8). NLT 3-fold difference between the signals obtained in the wells of cells treated without glucagon (R1) versus those with the maximal amount of glucagon (R8). The EC₅₀ for the *Standard solution* curves must be between 4.9 and 32.9 pg/mL. NMT 4 technical outliers may be omitted per standard curve. Any plate that fails one or more of these criteria is rejected and must be repeated.

Calculations: A minimum of two independent *Sample solution* preparations must be used for each test sample across three plates (assays). Outliers identified by Grubb's test (see chapter 111); but NMT 4/curve and NMT one data point from a replicate set) are omitted, and then the same number of *Standard solution* and *Sample solution* dose responses, including the 50% response (EC₅₀) of the standard/test sample within this range, are used to calculate the relative potency of the glucagon sample using statistical

methods for parallel-line analysis. For each individual *Sample solution* compared to the *Standard solution*, the statistical tests for linearity, slope, and parallelism must pass at the 95% level. Calculate the relative potency of the Glucagon samples by comparing the Reference Standard curve (from the *Standard solutions*) to the Glucagon sample curve (from the *Sample solutions*). Because a minimum of three valid assays are required, the estimated potencies are combined using the procedures in chapter $\langle 111 \rangle$, *Combination of Independent Assays*, and the confidence interval is calculated using suitable statistical methods. If the confidence limits are between 64% and 156%, the results are valid. If not, up to 2 more assay plates may be performed and combined until a valid confidence interval results, and the relative potency is then calculated from all valid independent runs. Calculate the potency of the Glucagon samples in USP rGlucagon Units/mg by multiplying the relative potency result by the potency of USP rGlucagon RS.

Acceptance criteria: NLT 0.80 USP rGlucagon Units/mg_{1S} (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **USP Reference Standards** $\langle 11 \rangle$

USP Dextrose RS

USP rGlucagon RS

■ USP Glucagon Receptor Cell Line RS_{1S} (USP39)

¹ Invitrogen catalog #31966-021 or suitable equivalent.

² Gibco catalog #10082-147 or suitable equivalent.

³ Calbiochem catalog #345812 or suitable equivalent.

⁴ Invitrogen catalog #14175 or suitable equivalent.

⁵ Corning catalog #3151 or suitable equivalent.

⁶ Gibco catalog #12563-011 or suitable equivalent.

⁷ PerkinElmer catalog #6005680 or suitable equivalent.

⁸ PerkinElmer catalog #6760625D or suitable equivalent.

⁹ PerkinElmer catalog #6760625D or suitable equivalent.

¹⁰ PerkinElmer catalog #6050195 or suitable equivalent.

11 PerkinElmer catalog #6760625D or suitable equivalent.

12 PerkinElmer catalog #6005185 or suitable equivalent.

13 PerkinElmer catalog #2300-001 or suitable equivalent.

BRIEFING

《 191 》 **Identification Tests—General**, *USP 38* page 216. It is proposed to modernize this chapter by providing an option of using instrumental identification methods in lieu of traditional wet-chemistry procedures. A new section, *Instrumental Identification Tests*, was added to the chapter. This section includes a *Procedure* (sample and standard solutions) and *Analysis* for each instrumental technique listed, as well as general guidance for selecting and implementing instrumental options for identification.

The *Introduction* was revised to indicate that unless otherwise specified in the monograph, if *Chemical Identification Tests* is selected for an ion, then all chemical test procedures for the ion shall be met. If *Instrumental Identification Tests* is selected for an ion, then only one instrumental technique is required.

The existing wet-chemistry procedures are now listed in the *Chemical Identification Tests* section. The specific changes are as follows:

- The flame tests under *Sodium*, *Calcium*, *Barium*, *Potassium*, *Lithium*, and *Borate* were removed to address safety concerns. Complementary wet-chemistry identification tests currently listed under *Chemical Identification Tests* for these ions are sufficient to verify the identity.
- Under *Hypophosphite*, the test evolving phosphine was also deleted.
- The section for *Phosphate* was divided into subsections for *Pyrophosphates* and *Orthophosphates* for clarity, to address comments received.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style, including the incorporation of letters (e.g., A, B, C) in the *Chemical Identification Tests* section for clarity and ease of navigation.

The monographs affected by the deletion of the flame tests are being revised in this issue and upcoming issues of *PF*. However, *USP* will coordinate the chapter and the monographs so that they become official at the same time.

For the drug substance and excipient monographs specifically referring to the flame test(s) under chapter 《 191 》, the following revisions are proposed:

- Where available, a replacement wet-chemistry test, adopted from another compendial source, is being proposed.
- For the articles where no replacement wet-chemistry procedures were found, an interim solution to retain the flame test is proposed. A complete description of the test is included in the monograph, and the manufacturers are encouraged to submit a replacement wet-chemistry or an instrumental procedure.

For the drug product monographs specifically referring to the flame test(s) under chapter 《

191 } , the following revisions are proposed:

- For the drug products where the identification test for *Sodium, Calcium, Barium, Potassium, Lithium, and/or Borate* is necessary to verify the identity of one of the active components (such as a monograph family of electrolytes), an interim solution to retain the flame test is proposed. A complete description of the test is included in the monograph, and the manufacturers are encouraged to submit a replacement wet-chemistry or an instrumental procedure.
- For all other drug product monographs, it is proposed to delete the flame tests found in the identification section of the monograph. These tests are often affected by interference from the excipient matrix and typically do not add value to dosage-form monographs.

The following Briefing list includes monographs and/or chapters that both reference the General Chapter under revision and require revision to keep references to the General Chapter accurate. Other monographs and/or chapters may also be listed, even where the reference to the General Chapter remains unchanged, as additional notice to stakeholders where there is believed to be potential for the change in the general chapter itself to affect pass-fail determinations for particular monograph articles. <https://www.usp.org/usp-nf/notices/general-chapter-191-identification-tests>

A companion [Stimuli](#) article is being published in this issue of *PF* to describe the background and rationale for this revision.

(GCCA: A. Hernandez-Cardoso.)
Correspondence Number—C88297

Comment deadline: May 31, 2015

⟨ 191 ⟩ IDENTIFICATION TESTS—GENERAL

INTRODUCTION

Change to read:

~~Under this heading are placed tests that are frequently referred to in the Pharmacopeia for the identification of official articles. Before using any acid or base to modify the pH of the sample solution, make sure that the added substance will not interfere with the results of the test. [Note—The tests are not intended to be applicable to mixtures of substances unless so specified.]~~

■ Procedures in this chapter are referenced in monographs for the identification of official articles and their components. Any acids, bases, or other reagents used in these procedures should not interfere with the results. Volumes may be scaled proportionally unless otherwise indicated. All of the tests include approximate amounts, except where specified.

Where “water,” without qualification, is mentioned in the tests or in directions for preparing solutions, *Purified Water, USP* is to be used.

Instrumental techniques described in this chapter may be used in lieu of chemical identification tests. In addition, other suitable, validated instrumental techniques may be used.

Unless otherwise specified in the monograph, if a chemical identification test is selected for an

ion, then all chemical test procedures for the ion shall be met. If an instrumental identification test is selected for an ion, then only one instrumental technique is required.

■ 1S (USP39)

CHEMICAL IDENTIFICATION TESTS

Change to read:

Acetate

■ ● **A.** ■ 1S (USP39)

Dissolve about 30 mg of the substance to be examined in 3 mL of water, or use 3 mL of the prescribed solution. Adjust the pH of the solution with sodium hydroxide to be slightly alkaline. Add 0.25 mL of lanthanum nitrate test solution (TS). If a white precipitate is formed, filter the solution. Add successively 0.1 mL of iodine, and potassium iodide TS 3, and 0.1 mL of ammonia TS 2 to the solution. If no blue color is observed, heat carefully to boiling. In the presence of acetates, a dark color develops or a blue precipitate is formed.

■ ● **B.** ■ 1S (USP39)

With neutral solutions of acetates, ferric chloride TS produces a red color that is destroyed by the addition of mineral acids.

Change to read:

Aluminum

■ ● **A.** ■ 1S (USP39)

By using 6 N ammonium hydroxide, solutions of aluminum salts yield a gelatinous, white precipitate that is insoluble in an excess of 6 N ammonium hydroxide.

■ ● **B.** ■ 1S (USP39)

1 N sodium hydroxide or sodium sulfide TS produces the same precipitate, which dissolves in an excess of either of these reagents.

Change to read:

Ammonium

■ ● **A.** ■ 1S (USP39)

Add 0.2 g of magnesium oxide to the solution under test. Prepare an indicator solution by mixing 1 mL of 0.1 M hydrochloric acid and 0.05 mL of methyl red TS 2. Pass a current of air through the

■ magnesium oxide ■ 1S (USP39)

mixture, and direct the gas that escapes to just beneath the surface of the indicator solution prepared previously. In the presence of ammonium, the color of the indicator solution is changed to yellow. After directing the gas into the indicator solution for a sufficient period of time, add 1 mL of freshly prepared sodium cobaltinitrite TS to the indicator solution. Upon the addition of the sodium cobaltinitrite TS, a yellow precipitate is formed when ammonium is present.

Change to read:

Antimony

■ ● **A.** ■ 1S (USP39)

With hydrogen sulfide, solutions of antimony(III) compounds, strongly acidified with hydrochloric acid, yield an orange precipitate of antimony sulfide that is insoluble in 6 N ammonium hydroxide but is soluble in ammonium sulfide TS.

Change to read:

Barium

- **A.** 1S (USP39)

Solutions of barium salts yield a white precipitate with the addition of 2 N sulfuric acid. This precipitate is insoluble in hydrochloric acid and in nitric acid.

- ~~**B.** Barium salts impart a yellowish-green color to a nonluminous flame that appears blue when viewed through green glass.~~

- 1S (USP39)

Change to read:

Benzoate

- **A.** 1S (USP39)

In neutral solutions, benzoates yield a salmon-colored precipitate with ferric chloride TS.

- **B.** 1S (USP39)

In moderately concentrated solutions, benzoates yield a precipitate of benzoic acid upon acidification when 2 N sulfuric acid is added. This precipitate is readily soluble in ethyl ether.

Bicarbonate

See *Carbonate*.

Change to read:

Bismuth

- **A.** 1S (USP39)

When dissolved in a slight excess of nitric acid or hydrochloric acid, bismuth salts yield a white precipitate upon dilution with water. This precipitate is colored brown by hydrogen sulfide, and the resulting compound dissolves in a warm mixture of equal parts of nitric acid and water.

Bisulfite

See *Sulfite*.

Change to read:

Borate

- **A.** 1S (USP39)

To 1 mL of a borate solution, acidified with hydrochloric acid to litmus, add 3 or 4 drops of iodine TS and 3 or 4 drops of polyvinyl alcohol solution (1:50): an intense blue color is produced.

- ~~**B.** When a borate is treated with sulfuric acid, methanol is added, and the mixture is ignited, it burns with a green-bordered flame.~~

- 1S (USP39)

Change to read:

Bromide

- **A.** ■1S (USP39)

Solutions of bromides, upon the addition of chlorine TS dropwise, liberate bromine, which is dissolved by shaking with chloroform, coloring the chloroform red-to-reddish brown.

- **B.** Silver nitrate TS produces, in solutions of bromides, a yellowish-white precipitate that is insoluble in nitric acid and is slightly soluble when 6 N ammonium hydroxide is added.

- 1S (USP39)

Change to read:

Calcium

- **A.** ■1S (USP39)

Solutions of calcium salts form insoluble oxalates when treated as follows. To a solution of the calcium salt (1:20) add 2 drops of methyl red TS and neutralize with 6 N ammonium hydroxide. Add 3 N hydrochloric acid dropwise, until the solution is acid to the indicator. Upon the addition of ammonium oxalate TS, a white precipitate is formed. This precipitate is insoluble when 6 N acetic acid is added but dissolves in hydrochloric acid.

- ~~**B.** Calcium salts moistened with hydrochloric acid impart a transient yellowish-red color to a nonluminous flame.~~

- 1S (USP39)

Change to read:

Carbonate

- **A.** ■1S (USP39)

Carbonates and bicarbonates effervesce with acids, evolving a colorless gas that, when passed into calcium hydroxide TS, produces a white precipitate immediately.

- **B.** A cold solution (1:20) of a soluble carbonate is colored red by phenolphthalein TS, whereas a similar solution of a bicarbonate remains unchanged or is only slightly colored.

- 1S (USP39)

Change to read:

Chlorate

- **A.** ■1S (USP39)

Solutions of chlorates yield no precipitate with silver nitrate TS. The addition of sulfurous acid to this mixture produces a white precipitate that is insoluble in nitric acid but is soluble in 6 N ammonium hydroxide.

- **B.** ■1S (USP39)

Upon ignition, chlorates yield chlorides, recognizable by appropriate tests.

- **C.** ■1S (USP39)

When sulfuric acid is added to a dry chlorate, decrepitation occurs and a greenish-yellow gas is evolved. [**Caution**—Use only a small amount of chlorate for this test, and exercise extreme caution in performing it.]

Change to read:

Chloride

■● A. 1S (USP39)

With silver nitrate TS, solutions of chlorides yield a white, curdy precipitate that is insoluble in nitric acid but is soluble in a slight excess of 6 N ammonium hydroxide.

■● B. 1S (USP39)

When testing amine (including alkaloidal) hydrochlorides that do not respond to the above test, add 1 drop of diluted nitric acid and 0.5 mL of silver nitrate TS to a solution of the substance being examined containing, unless otherwise directed in the monograph, about 2 mg of chloride ion in 2 mL: a white, curdy precipitate is formed. Centrifuge the mixture without delay, and decant the supernatant layer. Wash the precipitate with three 1-mL portions of nitric acid solution (1:100), and discard the washings. Add ammonia TS dropwise to this precipitate. It dissolves readily.

■● C. 1S (USP39)

When a monograph specifies that an article responds to the test for dry chlorides, mix the solid to be tested with an equal weight of manganese dioxide, moisten with sulfuric acid, and gently heat the mixture: chlorine, which is recognizable by the production of a blue color with moistened starch iodide paper, is evolved.

Change to read:**Citrate****■● A. 1S (USP39)**

To 15 mL of pyridine add a few mg of a citrate salt, dissolved or suspended in 1 mL of water, and shake. To this mixture add 5 mL of acetic anhydride, and shake: a light red color is produced.

Change to read:**Cobalt****■● A. 1S (USP39)**

Solutions of cobalt salts (1:20) in 3 N hydrochloric acid yield a red precipitate when heated on a steam bath with an equal volume of a hot, freshly prepared solution of 1-nitroso-2-naphthol (1:10) in 9 N acetic acid.

■● B. 1S (USP39)

Solutions of cobalt salts, when saturated with potassium chloride and treated with potassium nitrite and acetic acid, yield a yellow precipitate.

Change to read:**Copper****■● A. 1S (USP39)**

Cupric compounds, acidified with hydrochloric acid, deposit a red film of metallic copper upon a bright, untarnished surface of metallic iron.

■● B. 1S (USP39)

An excess of 6 N ammonium hydroxide, when added to a solution of a cupric salt, produces first a bluish precipitate and then a deep blue-colored solution.

■● C. 1S (USP39)

With potassium ferrocyanide TS, solutions of cupric salts yield a reddish-brown precipitate, insoluble in diluted acids.

Change to read:

Hypophosphite

- ~~A. When strongly heated, hypophosphites evolve spontaneously flammable phosphine.~~

■ **1S** (USP39)

- **A.** ■ **1S** (USP39)

Hypophosphites in solution yield a white precipitate with mercuric chloride TS. This precipitate becomes gray when an excess of hypophosphite is present.

- **B.** Solutions of hypophosphites, acidified with sulfuric acid and warmed with cupric sulfate TS, yield a red precipitate. ■ **1S** (USP39)

Change to read:

Iodide

- **A.** ■ **1S** (USP39)

Solutions of iodides, upon the addition of chlorine TS dropwise, liberate iodine, which colors the solution from yellow to red. When the solution is shaken with chloroform, the solution is colored violet. The iodine thus liberated gives a blue color with starch TS.

- **B.** ■ **1S** (USP39)

Silver nitrate TS produces, in solutions of iodides, a yellow, curdy precipitate that is insoluble in nitric acid and insoluble in 6 N ammonium hydroxide.

Change to read:

Iron

- **A.** ■ **1S** (USP39)

Ferrous and ferric compounds in solution yield a black precipitate with ammonium sulfide TS. This precipitate dissolves in cold, 3 N hydrochloric acid and evolves hydrogen sulfide.

Ferric Salts

- **A.** ■ **1S** (USP39)

Acid solutions of ferric salts yield a dark blue precipitate with potassium ferrocyanide TS.

- **B.** ■ **1S** (USP39)

With an excess of 1 N sodium hydroxide, a reddish-brown precipitate is formed.

- **C.** ■ **1S** (USP39)

With ammonium thiocyanate TS, solutions of ferric salts produce a deep red color that is not destroyed by dilute mineral acids.

Ferrous Salts

- **A.** ■ **1S** (USP39)

Solutions of ferrous salts yield a dark blue precipitate with potassium ferricyanide TS. This precipitate is insoluble in 3 N hydrochloric acid but is decomposed by 1 N sodium hydroxide.

- **B.** With 1 N sodium hydroxide, solutions of ferrous salts yield a greenish-white precipitate;

when shaken, the color changes rapidly to green and then to brown. ■1S (USP39)

Change to read:

Lactate

- A. ■1S (USP39)

When solutions of lactates are acidified with sulfuric acid, potassium permanganate TS is added; the mixture is heated, and acetaldehyde evolves. Acetaldehyde can be detected by allowing the vapor to come into contact with a filter paper that has been moistened with a freshly prepared mixture of equal volumes of 20% aqueous morpholine and sodium nitroferricyanide TS: a blue color is produced.

Change to read:

Lead

- A. ■1S (USP39)

With 2 N sulfuric acid, solutions of lead salts yield a white precipitate that is insoluble in 3 N hydrochloric or 2 N nitric acid but is soluble in warm 1 N sodium hydroxide and in ammonium acetate TS.

- B. ■1S (USP39)

With potassium chromate TS, solutions of lead salts, free or nearly free from mineral acids, yield a yellow precipitate that is insoluble in 6 N acetic acid but is soluble in 1 N sodium hydroxide.

Change to read:

Lithium

- A. ■1S (USP39)

With sodium carbonate TS, moderately concentrated solutions of lithium salts, made alkaline with sodium hydroxide, yield a white precipitate on boiling. The precipitate is soluble in ammonium chloride TS.

● ~~B. Lithium salts moistened with hydrochloric acid impart an intense crimson color to a nonluminous flame.~~

- B. Solutions of lithium salts are not precipitated by 2 N sulfuric acid or soluble sulfates (distinction from strontium). ■1S (USP39)

Change to read:

Magnesium

- A. ■1S (USP39)

Solutions of magnesium salts in the presence of ammonium chloride yield NMT a slightly hazy precipitate when neutralized with ammonium carbonate TS, but on the subsequent addition of dibasic sodium phosphate TS, a white, crystalline precipitate, which is insoluble in 6 N ammonium hydroxide, is formed.

Change to read:

Manganese

- A. ■1S (USP39)

With ammonium sulfide TS, solutions of manganous salts yield a salmon-colored precipitate that dissolves in acetic acid.

Change to read:

Mercury

- **A.** 1S (USP39)

When applied to bright copper foil, solutions of mercury salts, free from an excess of nitric acid, yield a deposit that, upon rubbing, becomes bright and silvery in appearance.

- **B.** 1S (USP39)

With hydrogen sulfide, solutions of mercury compounds yield a black precipitate that is insoluble in ammonium sulfide TS and in boiling 2 N nitric acid.

Mercuric Salts

- **A.** 1S (USP39)

Solutions of mercuric salts yield a yellow precipitate with 1 N sodium hydroxide.

- **B.** 1S (USP39)

Solutions of mercuric salts yield, in neutral solutions with potassium iodide TS, a scarlet precipitate that is very soluble in an excess of the reagent.

Mercurous Salts

- **A.** 1S (USP39)

Mercurous compounds are decomposed by 1 N sodium hydroxide, producing a black color.

- **B.** 1S (USP39)

With hydrochloric acid, solutions of mercurous salts yield a white precipitate that is blackened by the addition of 6 N ammonium hydroxide.

- **C.** With potassium iodide TS, a yellow precipitate, which may become green upon standing, is formed.

Change to read:

Nitrate

- **A.** 1S (USP39)

When a solution of a nitrate is mixed with an equal volume of sulfuric acid, the mixture is cooled, and a solution of ferrous sulfate is superimposed; a brown color is produced at the junction of the two liquids.

- **B.** When a nitrate is heated with sulfuric acid and metallic copper, brownish-red fumes evolve.

- **C.** Nitrates do not decolorize acidified potassium permanganate TS (distinction from nitrites). 1S (USP39)

Change to read:

Nitrite

- **A.** 1S (USP39)

When treated with dilute mineral acids or with 6 N acetic acid, nitrites evolve brownish-red fumes. The solution colors starch-iodide paper blue.

Change to read:**Oxalate**

- **A.** ■1S (USP39)

Neutral and alkaline solutions of oxalates yield a white precipitate with calcium chloride TS. This precipitate is insoluble in 6 N acetic acid but is dissolved by hydrochloric acid.

- **B.** Hot acidified solutions of oxalates decolorize potassium permanganate TS. ■1S (USP39)

Change to read:**Permanganate**

- **A.** ■1S (USP39)

Solutions of permanganates acidified with sulfuric acid are decolorized by hydrogen peroxide TS and by sodium bisulfite TS, in the cold, and by oxalic acid TS in a hot solution.

Change to read:**Peroxide**

- **A.** ■1S (USP39)

Solutions of peroxides slightly acidified with sulfuric acid yield a deep blue color upon the addition of potassium dichromate TS. On shaking the mixture with an equal volume of ethyl ether and allowing the liquids to separate, the blue color is found in the ethyl ether layer.

Change to read:

Phosphate [Note—Where the monograph specifies the identification test for *Phosphate*, use the tests for orthophosphates, unless the instructions specify the use of the pyrophosphate tests or indicate that the product is to be ignited before performing the test.]

■Orthophosphates

- **A.** ■1S (USP39)

With silver nitrate TS, neutral solutions of orthophosphates yield a yellow precipitate that is soluble in 2 N nitric acid and in 6 N ammonium hydroxide.

- **B.** ■1S (USP39)

With ammonium molybdate TS, acidified solutions of orthophosphates yield a yellow precipitate that is soluble in 6 N ammonium hydroxide. This precipitate may be slow to form.

■Pyrophosphates

- **C.** ■1S (USP39)

With silver nitrate TS, pyrophosphates obtained by ignition yield a white precipitate that is soluble in 2 N nitric acid and soluble in 6 N ammonium hydroxide.

- **D.** ■1S (USP39)

With ammonium molybdate TS, pyrophosphates obtained by ignition yield a yellow precipitate that is soluble in 6 N ammonium hydroxide.

Change to read:**Potassium**

- ~~**A.** Potassium compounds impart a violet color to a nonluminous flame, but the presence of small quantities of sodium masks the color unless the yellow color produced by sodium is~~

~~screened out by viewing through a blue filter that blocks emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). Traditionally, cobalt glass has been used, but other suitable filters are commercially available.~~

■ ■ 1S (USP39)

■ ● A. ■ 1S (USP39)

In neutral, concentrated, or moderately concentrated solutions of potassium salts (depending upon the solubility and the potassium content), sodium bitartrate TS produces a white crystalline precipitate that is soluble in 6 N ammonium hydroxide and in solutions of alkali hydroxides and carbonates. The formation of the precipitate, which is usually slow, is accelerated by stirring or rubbing the inside of the test tube with a glass rod. The addition of a small amount of glacial acetic acid or alcohol also promotes the precipitation.

Change to read:

Salicylate

■ ● A. ■ 1S (USP39)

In moderately dilute solutions of salicylates, ferric chloride TS produces a violet color.

■ ● B. ■ 1S (USP39)

Addition of acids to moderately concentrated solutions of salicylates produces a white, crystalline precipitate of salicylic acid that melts between 158° and 161°.

Change to read:

Silver

■ ● A. ■ 1S (USP39)

With hydrochloric acid, solutions of silver salts yield a white, curdy precipitate that is insoluble in nitric acid but is readily soluble in 6 N ammonium hydroxide.

■ ● B. A solution of a silver salt, to which 6 N ammonium hydroxide and a small quantity of formaldehyde TS are added, deposits, upon warming, a mirror of metallic silver upon the sides of the container. ■ 1S (USP39)

Change to read:

Sodium

■ ● A. ■ 1S (USP39)

Unless otherwise specified in an individual monograph, prepare a solution to contain 0.1 g of the sodium compound in 2 mL of water. Add 2 mL of 15% potassium carbonate, and heat to boiling. No precipitate is formed. Add 4 mL of potassium pyroantimonate TS, and heat to boiling. Allow to cool in ice water and, if necessary, rub the inside of the test tube with a glass rod. A dense precipitate is formed.

~~● B. Sodium compounds impart an intense yellow color to a nonluminous flame.~~

■ ■ 1S (USP39)

Change to read:

Sulfate

■ ● A. ■ 1S (USP39)

With barium chloride TS, solutions of sulfates yield a white precipitate that is insoluble in hydrochloric acid and in nitric acid.

■● **B.** 1S (USP39)

With lead acetate TS, neutral solutions of sulfates yield a white precipitate that is soluble in ammonium acetate TS.

■● **C.** 1S (USP39)

Hydrochloric acid produces no precipitate when added to solutions of sulfates (distinction from thiosulfates).

Change to read:

Sulfite

■● **A.** 1S (USP39)

When treated with 3 N hydrochloric acid, sulfites and bisulfites yield sulfur dioxide, which blackens filter paper moistened with mercurous nitrate TS.

Change to read:

Tartrate

■● **A.** 1S (USP39)

Dissolve a few mg of a tartrate salt in 2 drops of sodium metaperiodate solution (1:20). Add 1 drop of 1 N sulfuric acid and after 5 min add a few drops of sulfurous acid, followed by a few drops of fuchsin-sulfurous acid TS: a reddish-pink color is produced within 15 min.

Change to read:

Thiocyanate

■● **A.** 1S (USP39)

With ferric chloride TS, solutions of thiocyanates yield a red color that is not destroyed by moderately concentrated mineral acids.

Change to read:

Thiosulfate

■● **A.** 1S (USP39)

With hydrochloric acid, solutions of thiosulfates yield a white precipitate that soon turns yellow; with the addition of sulfur dioxide, filter paper moistened with mercurous nitrate TS blackens.

■● **B.** 1S (USP39)

The addition of ferric chloride TS to solutions of thiosulfates produces a dark violet color that disappears quickly.

Change to read:

Zinc

■● **A.** 1S (USP39)

In the presence of sodium acetate, solutions of zinc salts yield a white precipitate with hydrogen sulfide. This precipitate is insoluble in acetic acid but is dissolved by 3 N hydrochloric acid.

- **B.** ■1S (USP39)

Ammonium sulfide TS produces a similar precipitate in neutral and in alkaline solutions.

- **C.** ■1S (USP39)

With potassium ferrocyanide TS, zinc salts in solution yield a white precipitate that is insoluble in 3 N hydrochloric acid.

INSTRUMENTAL IDENTIFICATION TESTS

Add the following:

■ Instrumental techniques described in this section may be used in lieu of procedures described in the *Chemical Identification Tests* section. Instrumental techniques provide flexibility in the choice of identification tests. All instrumental techniques described below shall follow method validation procedures for identification tests (for additional information, see *Category IV* in general chapter *Validation of Compendial Procedures* 〈 1225 〉). *Instrumental Identification Tests* must demonstrate specificity.

The selection of the appropriate sample preparation depends on the material under test, must be appropriate for the technique being used, and is the responsibility of the analyst. The analyst may use any of the following preparation procedures, with the appropriate verification. When using solvents, the solvent must be free of interfering species. ■1S (USP39)

Add the following:

- **Identification Using X-Ray Fluorescence Spectrometry**

X-ray fluorescence spectrometry (XRF) can typically be used for identification of elements with atomic numbers from magnesium through uranium. The actual range of elements for which the instrument is capable is dependent on the instrument design. Guidance regarding the use of XRF may be found in general chapter *X-Ray Fluorescence Spectrometry* 〈 735 〉.

Sample Preparation

Powders/solids: Powders and solids can be used without further manipulation, or material can be made into pellets as described in chapter 〈 735 〉.

Neat liquids: Liquids can be used without further manipulation, provided the liquid is a single phase, is compatible with the sample holder, and has sufficiently low volatility.

Samples in solution: Samples may be dissolved in an appropriate solvent.

Procedure

Blank: For *Powders/solids* and *Neat liquids*, prepare a blank sample holder. For *Samples in solution*, use the solvent as a blank.

Reference Standard and Sample: Prepare each *Reference Standard* and *Sample* by using sufficient material as required for the specific manufacturer's instrumentation.

Analysis: Analyze the *Blank*, *Reference Standard*, and *Sample* according to the manufacturer's suggestions for the specific instrument. If there is any interference, use the *Blank* to make the correction. The spectrum of the *Reference Standard* exhibits radiation characteristic of the element(s) under investigation. The energy bands of the *Sample* exhibit radiation characteristic of the element(s) under investigation and compare

qualitatively with that of the *Reference Standard*. ■1S (USP39)

Add the following:

■● **Identification Using Atomic Spectroscopy Techniques: Atomic Absorption Spectroscopy, Inductively Coupled Plasma–Optical Emission Spectroscopy, Inductively Coupled Plasma–Mass Spectrometry**

Atomic absorption (AA) spectroscopy techniques can be used for the identification of many elements. Guidance regarding the use of AA spectroscopy may be found in general chapter *Atomic Absorption Spectroscopy* 〈 852 〉. Guidance regarding the use of inductively coupled plasma–optical emission spectroscopy (ICP–OES) or inductively coupled plasma–mass spectrometry (ICP–MS) may be found in general chapter *Plasma Spectrochemistry* 〈 730 〉.

Sample Preparation: Samples should be dissolved in an appropriate solvent. If dissolving the sample is not possible, digestion may be necessary.

Procedure

Blank: Prepare an appropriate solution that does not contain the analyte(s) of interest and is compatible with the technique being used.

Standard solution: Standards must contain the analyte of interest. All solutions should be matrix matched where possible.

Sample solution: Prepare the sample in the same solvent as the standard.

Analysis: Analyze the *Blank*, *Standard solution*, and *Sample solution* according to the manufacturer's suggestions for the specific instrument. When a wavelength-based technique is used, wherever possible select at least two wavelengths characteristic of the analyte(s) of interest. When a mass-based technique is used, wherever possible select at least two isotopes characteristic of the analyte(s) of interest. If two isotopes are unavailable, or if an element is monoisotopic, it may be possible to monitor the oxide of the analyte(s) ($m + 16$), if one is formed. If no oxide is formed, or if multiple isotopes cannot be evaluated because of interferences or because of a limited number of isotopes, then a different technique may be required. If interfering elements are present in the sample solution, wavelengths or masses examined must be selected to unequivocally identify the analyte(s) of interest. ■1S (USP39)

Add the following:

■● **Identification Using Ion Chromatography**

Ion chromatography (IC) can be used to identify a number of anions and cations found in drug substances (for additional information, see general chapter *Ion Chromatography* 〈 1065 〉).

Apparatus: Analyte detection will typically utilize suppressed conductivity, although other detection methods are possible, depending on the analyte (see 〈 1065 〉). The ion-exchange column must be capable of separating the analyte from any other ions of the same charge known to be in the sample at a concentration $\geq 5\%$ of the analyte.

Procedure

Blank: Use the sample solvent as a blank.

Standard and Sample solutions: Dissolve or dilute the sample in water. Other solvents can be used if compatible with the IC column.

Analysis: Analyze an equal volume of the *Blank*, *Reference Standard*, and sample (according to the manufacturer's suggestions for the specific instrument and column dimensions). The counterion is identified if a peak in the sample has the same retention time as the peak in the *Reference Standard*, and there is not a peak of $\geq 5\%$ of its size at the same retention time in the *Blank*. ■1S (USP39)

Add the following:

■● **Identification by Other Liquid Chromatography Techniques in Chromatography** (621)

Some of the ions suitable for IC identification can also be identified by other forms of liquid chromatography. *Sample* and *Reference Standard* concentrations, as well as injection volumes, need to be adjusted, depending on the detection technique used. All high-concentration components in the test substance should be analyzed to judge whether there is interference with the analyte of interest. ■1S (USP39)

Add the following:

■● **Identification Using Raman Spectroscopy**

Raman spectroscopy can be used for identification of counterions. Guidance regarding the use of Raman spectroscopy may be found in general chapter *Spectrophotometry and Light-Scattering* (851) .

Apparatus: Prepare the spectrometer for operation according to the instrument instruction manual and the instrument manufacturer's recommendations. Instrument performance and the quality of spectra collected should be verified daily whenever the instrument is in use. Wavelength accuracy should be verified as per applicable instrument operating procedures.

Procedure

Reference Standard and Sample: All reference material and sample spectra should be collected using identical instrumental parameters. These collection parameters may be determined by the analyst based on the nature of the sample and the type of analysis and should be selected based on the quality of spectra needed. Use the appropriate sample container and/or sample holding apparatus, depending on the type of reference material and sample (powder, liquid, paste, film, or other) being analyzed. Transfer the sample or reference material into the appropriate sample container and/or holder, and acquire the spectrum for each.

Analysis: Qualitatively compare the Raman spectra obtained from the reference material and the *Sample*. The *Sample* complies with the identity test if the spectrum exhibits maxima only at the same wavelengths as that of a similar preparation of the corresponding USP Reference Standard. ■1S (USP39)

Add the following:

■● **Identification Using Mid-Infrared Spectroscopy**

Mid-infrared spectroscopy techniques can be used for identification of counterions. Guidance regarding the use of mid-infrared spectroscopy may be found in general chapters *Mid-Infrared Spectroscopy* 〈 854 〉 and *Spectrophotometric Identification Tests* 〈 197 〉. If the monograph contains an identification test by IR (e.g., chapter 〈 197 〉) and a reference to general chapter 〈 191 〉, then mid-infrared cannot be used as an instrumental replacement for the chemical identification tests prescribed in chapter 〈 191 〉.

Procedure

Standard and Sample solutions: Sample preparation may be conducted using any of the procedures described in chapter 〈 854 〉 that are appropriate for the sample of concern. The standard preparation should be conducted in the same way but using the USP Reference Standard of the substance under test.

Analysis: Record the spectra of the test specimen and the corresponding USP Reference Standard over the range from about 4000 to 400 cm^{-1} (2.5–25 μm). The IR absorption spectrum of the preparation of the test specimen, previously dried under conditions specified for the corresponding *Reference Standard*, unless the *Reference Standard* is to be used without drying, exhibits maxima only at the same wavelengths as that of a similar preparation of the corresponding USP Reference Standard. ■1S (USP39)

BRIEFING

〈 341 〉 **Antimicrobial Agents—Content**, *USP 38* page 264 and *PF 39(3)* [May–June 2013]. The previously published proposal in *PF 39(3)* is canceled and replaced with the following proposal based on the comments received.

In an effort to modernize this general test chapter, it is proposed to replace the existing *General Gas Chromatographic Method* section that uses packed-column GC procedures with a combination of agent-specific capillary column GC and HPLC procedures. Furthermore, the *Polarographic Method for Thimerosal* is replaced with an HPLC procedure. The revisions are as follows:

1. Content of *Benzyl Alcohol*: A gas chromatographic procedure based on analyses performed with the J&W Scientific DBWax brand of G16 column with 0.5- μm film thickness, using phenol as the internal standard. The typical retention times reported for benzyl alcohol and phenol are 8 and 10 min, respectively.
2. Content of *Chlorobutanol*: A gas chromatographic procedure based on analyses performed with the J&W Scientific DBWax brand of G16 column with 0.25- μm film thickness. The typical retention times for chlorobutanol and 2,2,2-trichloroethanol are 6.0 and 8.1 min, respectively.
3. Content of *Phenol*: A gas chromatographic procedure based on analyses performed with the J&W Scientific DBWax brand of G16 column with 0.5- μm film thickness, using benzyl alcohol as the internal standard. The typical retention times for benzyl alcohol and phenol are 8 and 10 min, respectively.
4. Content of Parabens, *Methylparaben* and *Polyparaben*: A liquid chromatographic procedure based on analyses performed with the Phenomenex Inertsil 5- μm brand of L1 column, using a Phenomenex C18 Security Guard cartridge with an L1 guard column. The typical retention times for *p*-hydroxybenzoic acid, methylparaben,

ethylparaben, and propylparaben are 1.6, 2.7, 3.8, and 5.7 min, respectively.

5. Content of *Thimerosal*: The polarographic procedure is replaced with a liquid chromatographic procedure based on analyses performed with the ACE Excel C18-AR brand of L1 column. The HPLC procedure is adopted from the recent proposal published in *PF* 40(5) [Sept.–Oct. 2014] for the *Thimerosal* monograph. The typical retention time for thimerosal is about 5 min. Stakeholders are requested to provide their input on the proposed procedure.

Stakeholders who can provide a validated test procedure to replace the polarographic method for the content of *Phenylmercuric Nitrate* are encouraged to submit information to the Expert Committee. Also, USP seeks industry feedback on inclusion of other types of antimicrobial agents in this general chapter. Stakeholders are encouraged to submit comments for Expert Committee consideration.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCCA: S. Shivaprasad.)

Correspondence Number—C136371; 148392

Comment deadline: May 31, 2015

〈 341 〉 ANTIMICROBIAL AGENTS—CONTENT

Change to read:

An essential component of Injections preserved in multiple-dose containers is the agent or agents present to reduce the hazard of having introduced, in the course of removing some of the contents, accidental microbial contamination of the contents remaining. It is a Pharmacopeial requirement that the presence and amount added of such agent(s) be declared on the label of the container. ~~The methods provided herein for the most commonly used agents~~

■ This general chapter provides methods for the most commonly used antimicrobial agents.

These methods or other suitably validated methods. ■ 1S (*USP39*)

are to be used to demonstrate that the declared agent is present but does not exceed the labeled amount by more than 20%.

The concentration of an antimicrobial preservative added to a multiple-dose or single-dose parenteral, otic, nasal, and ophthalmic preparation may diminish during the shelf life of the product. ~~Because it is recognized that the antimicrobial preservative concentration in a given preparation may decrease during the product's shelf life,~~

■ Therefore, ■ 1S (*USP39*)

the manufacturer shall determine the lowest level at which the preservative is effective, and the product should be so formulated as to assure that this level is exceeded throughout the product's shelf life. At the time of its manufacture, the product should contain the declared amount of antimicrobial preservative (within $\pm 20\%$ to allow for manufacturing and analytical variations). The quantitative label statement of the preservative content is not intended to mean that the labeled quantity is retained during the shelf life of the product; rather, it is a statement of the amount added, within process limits, and which is not exceeded by more than 20%. An example of such a label statement is "____(unit) added as preservative". [Note —"____(unit)" would be a number followed by the unit of measurement, e.g., 0.015 mg/mL or

0.1%.]

The most commonly used agents include benzyl alcohol; chlorobutanol; phenol; the four homologous esters of *p*-hydroxybenzoic acid; and the two mercurials, phenylmercuric nitrate and thimerosal.

~~The methods for the first two named are polarographic, while quantitative gas chromatography is employed in the determination of the other agents.~~

■ The method used for phenylmercuric nitrate is polarographic, whereas quantitative liquid chromatography was used for thimerosal and the four homologous esters of *p*-hydroxybenzoic acid. Gas chromatography is used in the determination of phenol, benzyl alcohol, and chlorobutanol. ■ 1S (USP39)

Delete the following:

■ GENERAL GAS CHROMATOGRAPHIC METHOD

~~The general procedures set forth in the following paragraphs are applicable to the quantitative determination of benzyl alcohol, chlorobutanol, phenol, and the methyl, ethyl, propyl, and butyl esters of *p*-hydroxybenzoic acid, the latter being treated as a group, the individual members of which, if present, are capable of separate determination. Prepare the *Internal Standard Solution* and the *Standard Preparation* for each agent as directed individually below. Unless otherwise directed below, prepare the *Test Preparation* from accurately measured portions of the *Internal Standard Solution* and the sample under test, of such size that the concentration of the agent and the composition of the solvent correspond closely to the concentration and composition of the *Standard Preparation*. Suggested operating parameters of the gas chromatograph apparatus are given in the accompanying table, the carrier gas being helium or nitrogen, and the detector being the flame ionization type.~~

Suggested Operating Parameters of Gas Chromatograph Apparatus

Agent	Column Size		Column Packing Phases and Support	Flow Rate, mL per minute	Column Temperature
	Length	ID			
Benzyl Alcohol	1.8 m	3 mm	5% G16/S1A	50	140 [°]
Chlorobutanol	1.8 m	2 mm	5% G16/S1A	20	110 [°]
Phenol	1.2 m	3 mm	5% G16/S1A	50	145 [°]
Parabens	1.8 m	2 mm	5% G2/S1A	20	150 [°]

● **Benzyl Alcohol**

Internal Standard Solution— Dissolve about 380 mg of phenol in 10 mL of methanol contained in a 200-mL volumetric flask. Add water to volume, and mix.

Standard Preparation— Dissolve about 180 mg of USP Benzyl Alcohol RS, accurately weighed, in 20.0 mL of methanol contained in a 100-mL volumetric flask. Add *Internal Standard Solution* to volume, and mix.

Procedure— Separately inject equal volumes (about 5 µL) of the *Standard Preparation* and the *Test Preparation* into the chromatograph, record the chromatograms with the apparatus adjusted to the parameters set forth in the accompanying table, and measure

the areas under the peaks for benzyl alcohol and phenol. Calculate the content, in mg per mL, of benzyl alcohol (C_7H_8O) in the specimen taken by the formula:

$$100(C/V)(p_1/p_2)(P_2/P_1)$$

in which C is the concentration, in mg per mL, of benzyl alcohol in the *Standard Preparation*; V is the volume, in mL, of the specimen under test used in preparing each 100 mL of the *Test Preparation*; p_1 and p_2 are the peak areas for benzyl alcohol and phenol, respectively, obtained from the *Test Preparation*; and P_1 and P_2 are the peak areas of benzyl alcohol and phenol, respectively, obtained from the *Standard Preparation*.

- **Chlorobutanol**

Internal Standard Solution— Transfer about 140 mg of benzaldehyde to a 100-mL volumetric flask, add 10 mL of methanol, and swirl to dissolve. Dilute with water to volume, and mix.

Standard Preparation— Transfer about 125 mg of USP Chlorobutanol RS, accurately weighed, to a 25-mL volumetric flask. Add 2 mL of methanol, swirl to dissolve, dilute with water to volume, and mix. Transfer 5.0 mL of this solution and 5.0 mL of *Internal Standard Solution* to a 25-mL flask, and mix to obtain a solution having a known concentration of about 2.5 mg of chlorobutanol per mL.

Test Preparation— Quantitatively dilute, if necessary, an accurately measured volume of the specimen under test with methanol to obtain a solution containing not more than about 5.0 mg of chlorobutanol per mL. Combine 3.0 mL of this solution with 3.0 mL of *Internal Standard Solution*, and mix.

Chromatographic System (see *Chromatography* (621))— [Note—See accompanying table for column dimensions, column packing phase and support, flow rate, and column temperature.] The injection port temperature is maintained at 180° , and the detector temperature is maintained at 220° . Chromatograph the *Standard Preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for benzaldehyde and 1.0 for chlorobutanol; the resolution, R , between benzaldehyde and the chlorobutanol is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— Separately inject equal volumes (about 1 μ L) of the *Standard Preparation* and the *Test Preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of chlorobutanol ($C_4H_7Cl_3O$) in each mL of the specimen under test by the formula:

$$C(L/D)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of chlorobutanol, calculated on the anhydrous basis, in the *Standard Preparation*; L is the labeled quantity, in mg, of chlorobutanol in each mL of the specimen under test; D is the concentration, in mg per mL, of chlorobutanol in the *Test Preparation*, based on the volume of specimen under test taken and the extent of dilution; and R_U and R_S are the ratios of the chlorobutanol peak to the benzaldehyde peak obtained from the *Test Preparation* and the *Standard Preparation*, respectively.

- **Phenol**

Internal Standard Solution— Pipet 1 mL of USP Benzyl Alcohol RS into a 500-mL volumetric flask, add methanol to volume, and mix.

Standard Preparation— Dissolve about 75 mg of USP Phenol RS, accurately weighed, in

7.5 mL of methanol contained in a 100 mL volumetric flask. Add 20.0 mL of *Internal Standard Solution*, then add water to volume, and mix.

Procedure— Separately inject equal volumes (about 3 μ L) of the *Standard Preparation* and the *Test Preparation* into the chromatograph, record the chromatograms with the apparatus adjusted to the parameters set forth in the accompanying table, and measure the areas under the peaks for phenol and benzyl alcohol. Calculate the content, in mg per mL, of phenol (C_6H_6O) in each mL of the specimen taken by the formula:

$$100(C/V)(p_1/p_2)(P_2/P_1)$$

in which C is the concentration, in mg per mL, of phenol in the *Standard Preparation*; V is the volume, in mL, of the specimen under test used in preparing each 100 mL of the *Test Preparation*; p_1 and p_2 are the peak areas for phenol and benzyl alcohol, respectively, obtained from the *Test Preparation*; and P_1 and P_2 are the peak areas of phenol and benzyl alcohol, respectively, obtained from the *Standard Preparation*.

- **Methylparaben and Propylparaben**

Internal Standard Solution— Place about 200 mg of benzophenone in a 250 mL volumetric flask, dilute with ether to volume, and mix.

Standard Preparation— Place 100 mg of USP Methylparaben RS and 10 mg of USP Propylparaben RS, each accurately weighed, in a 200 mL volumetric flask, dilute with *Internal Standard Solution* to volume, and mix. Place 10 mL of this solution in a 25 mL conical flask, and proceed as directed for *Test Preparation*, beginning with "Add 3 mL of pyridine."

Test Preparation— Pipet 10 mL of the specimen under test and 10 mL of the *Internal Standard Solution* into a small separator. Shake vigorously, allow the layers to separate, draw off the aqueous layer into a second separator, and transfer the ether layer into a small flask through a funnel containing anhydrous sodium sulfate. Extract the aqueous layer with two 10 mL portions of ether, also filtering the extracts through the anhydrous sodium sulfate. Evaporate the combined extracts under a current of dry air until the volume is reduced to about 10 mL, then transfer the residue to a 25 mL conical flask. Add 3 mL of pyridine, complete the evaporation of the ether, and boil on a hot plate until the volume is reduced to about 1 mL. Cool, and add 1 mL of a suitable silylation agent, such as bis(trimethylsilyl)trifluoroacetamide, bis(trimethylsilyl)acetamide, or a mixture of hexamethyldisilazane and trimethylchlorosilane [2:1 or 3:1 (v/v)]. Mix, and allow to stand for not less than 15 minutes.

Procedure— Separately inject equal volumes (2 μ L) of the silanized solution from the *Standard Preparation* and the *Test Preparation* into the chromatograph, record the chromatograms with the apparatus adjusted to the parameters set forth in the accompanying table, and measure the areas under the peaks for methylparaben, propylparaben, and benzophenone. Calculate the content, in μ g per mL, of methylparaben ($C_8H_8O_3$) in the sample under test by the formula:

$$10(C_M/V)(p_1/p_3)(P_3/P_1)$$

in which C_M is the concentration, in μ g per mL, of methylparaben in the *Standard Preparation*; V is the volume, in mL, of the specimen taken; p_1 and p_3 are the peak areas for methylparaben and benzophenone, respectively, obtained from the *Test Preparation*; and P_1 and P_3 are the peak areas of methylparaben and benzophenone, respectively, obtained from the *Standard Preparation*. Similarly, calculate the content, in μ g per mL, of propylparaben ($C_{10}H_{12}O_3$) in the specimen under test by the formula:

$$10(C_p/V)(p_2/p_3)(P_3/P_2)$$

in which C_p is the concentration, in μg per mL, of propylparaben in the *Standard Preparation*; V is the volume, in mL, of the specimen taken; p_2 and p_3 are the peak areas for propylparaben and benzophenone, respectively, obtained from the *Test Preparation*; and P_2 and P_3 are the peak areas of propylparaben and benzophenone, respectively, obtained from the *Standard Preparation*.

Ethylparaben and butylparaben may be determined in a similar manner.

■ 1S (USP39)

Add the following:

■ **GENERAL GAS AND LIQUID CHROMATOGRAPHIC METHODS**

The general gas chromatography procedures set forth in the following paragraphs are applicable to the quantitative determination of benzyl alcohol, chlorobutanol, and phenol. Prepare the *Internal standard solution* and the *Standard solution* for each antimicrobial agent as directed below. Unless otherwise directed, prepare the *Sample solution* from accurately measured portions of the sample under test and the *Internal standard solution* such that the concentration of the agent and the composition of the solvent correspond closely to the concentration and composition of the *Standard solution*. Suggested operating parameters of the gas chromatograph are provided in this section.

The general HPLC procedures set forth in the following paragraphs are applicable to the quantitative determination of parabens and *Thimerosal*. Prepare the *Internal standard solution* and the *Standard solution* for each agent as directed below. Unless otherwise directed, prepare the *Sample solution* from accurately measured portions of the sample under test and the *Internal standard solution*, if applicable, such that the concentration of the agent and the composition of the solvent correspond closely to the concentration and composition of the *Standard solution*. Suggested operating parameters of the liquid chromatograph are provided in this section.

● **Benzyl Alcohol**

Diluent: Methanol and water (20:80)

Internal standard solution: 3.8 mg/mL of phenol prepared as follows. Dissolve a suitable amount of phenol in 10% of the flask volume of methanol, and dilute with water to volume.

Standard solution: 1.8 mg/mL of USP Benzyl Alcohol RS and 1.5 mg/mL of phenol prepared as follows. Dissolve 180 mg of USP Benzyl Alcohol RS in 20 mL of methanol contained in a 100-mL volumetric flask. Add 40.0 mL of *Internal standard solution*, and dilute with water to volume.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 30-m \times 0.32-mm fused-silica; bonded with a 0.5- μm film of phase G16

Temperatures

Injection port: 200 $^{\circ}$

Detector: 310 $^{\circ}$

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
150	0	150	5
150	10	230	7

Carrier gas: Helium

Flow rate (constant): 2 mL/min

Injection volume: 1 µL

Split ratio: 10:1

Run time: 20 min

System suitability

Sample: *Standard solution*

[Note—The relative retention times for benzyl alcohol and phenol are about 1.0 and 1.25, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the benzyl alcohol and phenol peaks

Tailing factor: NMT 2.0 for the benzyl alcohol peak

Relative standard deviation: NMT 2.0% for the peak response ratio of benzyl alcohol to phenol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzyl alcohol (C₇H₈O) in the portion of the sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{U}}$ peak response ratio of benzyl alcohol to phenol from the *Sample solution*

$R_{\bar{S}}$ peak response ratio of benzyl alcohol to phenol from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Benzyl Alcohol RS in the *Standard solution*

$C_{\bar{U}}$ nominal concentration of benzyl alcohol in the *Sample solution*

● Chlorobutanol

Diluent: Methanol and water (50:50)

Internal standard solution: 10 mg/mL of 2,2,2-trichloroethanol in *Diluent*

Standard stock solution: 5 mg/mL of USP Chlorobutanol RS in methanol

Standard solution: 1.25 mg/mL of USP Chlorobutanol RS and 2 mg/mL of 2,2,2-trichloroethanol prepared as follows. Transfer 2.5 mL of *Standard stock solution*, 2.0 mL of *Internal standard solution*, and 0.5 mL of methanol to a 10-mL volumetric flask. Dilute with water to volume.

Sample stock solution: Quantitatively dilute, if necessary, an accurately measured volume corresponding to 2.5 mg/mL of chlorobutanol in water.

Sample solution: Combine 5.0 mL of *Sample stock solution* with 2.0 mL of *Internal standard solution* in a 10-mL volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 30-m × 0.32-mm fused-silica; bonded with a 0.25-μm film of phase G16

Temperatures

Injection port: 260°

Detector: 280°

Column: 135°

Carrier gas: Helium

Flow rate: 1 mL/min

Injection volume: 0.5 μL

Split ratio: 10:1

Run time: 12 min

System suitability

Sample: *Standard solution*

[Note—The relative retention times for chlorobutanol and 2,2,2-trichloroethanol are about 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 2.0 between chlorobutanol and 2,2,2-trichloroethanol

Relative standard deviation: NMT 1.0% for the peak response ratio of chlorobutanol to 2,2,2-trichloroethanol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlorobutanol (C₄H₇Cl₃O), on the anhydrous basis, in the portion of the sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{U}}$ peak response ratio of chlorobutanol to 2,2,2-trichloroethanol from the *Sample solution*

$R_{\bar{S}}$ peak response ratio of chlorobutanol to 2,2,2-trichloroethanol from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Chlorobutanol RS in the *Standard solution*

$C_{\bar{U}}$ nominal concentration of chlorobutanol in the *Sample solution*

● **Phenol**

Internal standard solution: 2 mg/mL of USP Benzyl Alcohol RS in methanol

Standard stock solution: 4 mg/mL of USP Phenol RS in water

Standard solution: 0.4 mg/mL each of USP Phenol RS and USP Benzyl Alcohol RS prepared as follows. Combine 5.0 mL of *Standard stock solution* with 10.0 mL of *Internal standard solution* in a 50-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨ 621 ⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 30-m × 0.32-mm fused-silica; bonded with a 0.5-μm film of phase G16

Temperatures

Injection port: 200°

Detector: 310°

Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
150	0	150	5
150	10	230	7

Carrier gas: Helium

Flow rate (constant flow): 2 mL/min

Injection volume: 1 µL

Split ratio: 10:1

Run time: 20 min

System suitability

Sample: *Standard solution*

[Note—The relative retention times for benzyl alcohol and phenol are about 0.85 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between benzyl alcohol and phenol

Tailing factor: NMT 2.0 for the phenol peak

Relative standard deviation: NMT 1.0% for the peak response ratio of phenol to benzyl alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenol (C₆H₆O) in the portion of the sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{U}}$ peak response ratio of phenol to benzyl alcohol from the *Sample solution*

$R_{\bar{S}}$ peak response ratio of phenol to benzyl alcohol from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Phenol RS in the *Standard solution*

$C_{\bar{U}}$ nominal concentration of phenol in the *Sample solution*

● **Methylparaben and Propylparaben**

Buffer: 7 g/L of monobasic potassium phosphate in water

Mobile phase: Methanol and *Buffer* (65:35)

Internal standard solution: 0.013 mg/mL of USP Ethylparaben RS in *Mobile phase*

System suitability solution: 0.01 mg/mL each of USP Butylparaben RS, USP Propylparaben RS, USP Ethylparaben RS, USP Methylparaben RS, and *p*-hydroxybenzoic acid in *Mobile phase*

Standard stock solution: 0.2 mg/mL of USP Methylparaben RS and 0.03 mg/mL of USP Propylparaben RS in *Mobile phase*

Standard solution: Combine 5 mL of *Standard stock solution* with 5 mL of *Internal standard solution*, and extract three times with 10-mL aliquots of diethyl ether. Filter the combined ether layers through anhydrous sodium sulfate. Evaporate the ether extract to dryness, and dissolve the residue in 50 mL of *Mobile phase*.

Sample solution: Combine 5 mL of the specimen under test with 5 mL of *Internal standard*

solution, and extract three times with 10-mL aliquots of diethyl ether. Filter the combined ether layers through anhydrous sodium sulfate. Evaporate the ether extract to dryness, and dissolve the residue in 50 mL of *Mobile phase*.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 272 nm

Columns

Guard: 4.0-mm × 3-mm; packing L1

Analytical: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1.3 mL/min

Injection volume: 10 μL

Run time: 10 min

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for *p*-hydroxybenzoic acid, methylparaben, ethylparaben, and propylparaben are about 0.58, 1.0, 1.4, and 2.1, respectively.]

Suitability requirements

Resolution: NLT 2.0 between *p*-hydroxybenzoic acid and methylparaben, NLT 2.0 between methylparaben and ethylparaben; *System suitability solution*

Tailing factor: NMT 2.0 for the methylparaben and propylparaben peaks, *Standard solution*

Relative standard deviation: NMT 2.0% for the peak response ratio of methylparaben to ethylparaben, NMT 2.0% for the peak response ratio of propylparaben to ethylparaben; *System suitability solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylparaben (C₈H₈O₃) in the portion of the sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{U}}$ peak response ratio of methylparaben to ethylparaben from the *Sample solution*

$R_{\bar{S}}$ peak response ratio of methylparaben to ethylparaben from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Methylparaben RS in the *Standard solution*

$C_{\bar{U}}$ nominal concentration of methylparaben in the *Sample solution*

Calculate the percentage of the labeled amount of propylparaben (C₁₀H₁₂O₃) in the portion of the sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{U}}$ peak response ratio of propylparaben to ethylparaben from the *Sample solution*

$R_{\bar{S}}$ peak response ratio of propylparaben to ethylparaben from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Propylparaben RS in the *Standard solution*

$C_{\bar{U}}$ nominal concentration of propylparaben in the *Sample solution*

Ethylparaben and butylparaben may be determined in a similar manner using appropriate

internal standard solutions. However, because the extraction recovery is matrix dependent, the user should verify the suitability of the procedure for their drug product and for different product formulations.

- **Thimerosal**

Solution A: Trifluoroacetic acid and water (0.5: 1000)

Mobile phase: Methanol and *Solution A* (60:40)

Standard solution: 25 µg/mL of USP Thimerosal RS in water

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 222 nm

Column: 2.1-mm × 10-cm; 2-µm packing L1

Autosampler temperature: 4°

Flow rate: 0.35 mL/min

Injection volume: 2.5 µL

System suitability

Sample: *Standard solution*

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of thimerosal (C₉H₉HgNaO₂S) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of thimerosal from the *Sample solution*

r_S = peak response of thimerosal from the *Standard solution*

C_S = concentration of USP Thimerosal RS in the *Standard solution*

C_U = nominal concentration of thimerosal in the *Sample solution*

■ 1S (USP39)

Change to read:

POLAROGRAPHIC METHOD

- **Phenylmercuric Nitrate**

Standard stock solution: 0.1 mg/mL of phenylmercuric nitrate in sodium hydroxide solution (1 in 250). Warm, if necessary, to dissolve.

Standard solution: Pipet 10 mL of *Standard stock solution* into a 25-mL volumetric flask, and proceed as directed under *Sample solution* beginning with "add 2 mL of potassium nitrate solution (1 in 100)".

Sample solution: Pipet 10 mL of the specimen under test into a 25-mL volumetric flask, add 2 mL of potassium nitrate solution (1 in 100) and 10 mL of pH 9.2 alkaline borate buffer (see in *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), and adjust to a pH of 9.2, if necessary, by the addition of 2 N nitric acid. Add 1.5 mL of freshly prepared gelatin solution (1 in 1000), then add the pH 9.2 alkaline borate buffer to volume.

Analysis: Pipet a portion of the *Sample solution* into the polarographic cell, and deaerate

by bubbling nitrogen through the solution for 15 min. Insert the dropping mercury electrode of a suitable polarograph (see *Polarography* 〈 801 〉), and record the polarogram from -0.6 to -1.5 volts versus the saturated calomel electrode. Calculate the quantity, in $\mu\text{g/mL}$, of phenylmercuric nitrate ($\text{C}_6\text{H}_5\text{HgNO}_3$) in the portion of the sample taken:

$$\text{Result} = 2.5C[(i_d)_U/(i_d)_S]$$

C = concentration of phenylmercuric nitrate in the *Standard solution* ($\mu\text{g/mL}$)

$(i_d)_U$ diffusion current of the *Sample solution*, as the difference between the residual current and the limiting current

$(i_d)_S$ diffusion current of the *Standard solution*, as the difference between the residual current and the limiting current

◆ **Thimerosal**

Standard solution: On the day of use, place about 25 mg of USP Thimerosal RS, accurately weighed, in a 250 mL volumetric flask, add water to volume, and mix. Protect from light. Pipet 15 mL of this solution into a 25 mL volumetric flask, add 1.5 mL of gelatin solution (1 in 1000), then add potassium nitrate solution (1 in 100) to volume, and mix.

Sample solution: Pipet 15 mL of the test specimen into a 25 mL volumetric flask, add 1.5 mL of gelatin solution (1 in 1000), add potassium nitrate solution (1 in 100) to volume.

Analysis: Transfer a portion of the *Sample solution* to a polarographic cell, and deaerate by bubbling nitrogen through the solution for 15 minutes. Insert the dropping mercury electrode of a suitable polarograph (see *Polarography* 〈 801 〉), and record the polarogram from -0.2 to -1.4 volts versus the saturated calomel electrode. Determine the diffusion current, $(i_d)_U$, as the difference between the residual current and the limiting current. Similarly and concomitantly determine the diffusion current, $(i_d)_S$, of the *Standard solution*.

Calculate the quantity, in μg , of thimerosal ($\text{C}_6\text{H}_9\text{HgNaO}_2\text{S}$) in each mL of the test specimen taken by the formula:

$$\text{Result} = 1.667C[(i_d)_U/(i_d)_S]$$

in which C is the concentration, in $\mu\text{g per mL}$, of thimerosal in the *Standard solution*; and the other terms are as defined therein.

■ **1S (USP39)**

Add the following:

•

■ **USP Reference Standards** 〈 11 〉

USP Benzyl Alcohol RS
 USP Butylparaben RS
 USP Chlorobutanol RS
 USP Ethylparaben RS
 USP Methylparaben RS
 USP Phenol RS

USP Propylparaben RS
USP Thimerosal RS ■ 1S (USP39)

BRIEFING

〈 381 〉 **Elastomeric Closures for Injections**, *USP 38* page 270 and *PF 40(5)* [Sept.–Oct. 2014]. Due to the impending omission of *Heavy Metals* 〈 231 〉, the USP Packaging, Storage, and Distribution Expert Committee is proposing the following revision while the Expert Committee works to modernize the chapter. The modernized chapter will include specific testing requirements for elastomeric materials of construction and elastomeric materials used in packaging systems. As part of this modernization effort, the elemental impurities tests for elastomeric materials and materials used in final packaging systems will also be updated.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCPS: D. Hunt.)
Correspondence Number—C154446

Comment deadline: May 31, 2015

〈 381 〉 ELASTOMERIC CLOSURES FOR INJECTIONS

INTRODUCTION

Elastomeric closures for containers used in the types of preparations defined in the general test chapter *Injections and Implanted Drug Products* 〈 1 〉 are made of materials obtained by vulcanization (cross-linking) polymerization, polyaddition, or polycondensation of macromolecular organic substances (elastomers). Closure formulations contain natural or synthetic elastomers and inorganic and organic additives to aid or control vulcanization, impart physical and chemical properties or color, or stabilize the closure formulation.

This chapter applies to closures used for long-term storage of preparations defined in 〈 1 〉. Such closures are typically used as part of a vial, bottle, or pre-fill syringe package system. This chapter applies to closures formulated with natural or synthetic elastomeric substances. This chapter does not apply to closures made from silicone elastomer; however, it does apply to closures treated with silicone (e.g., *Dimethicone, NF*). When performing the tests in this chapter, it is not required that closures be treated with silicone, although there is no restriction prohibiting the use of siliconized closures.

This chapter also applies to closures coated with other lubricious materials (e.g., materials chemically or mechanically bonded to the closure) that are not intended to, and in fact do not provide, a barrier to the base elastomer. When performing the tests, closures with lubricious nonbarrier coatings are to be tested in their coated state.

The following comments relate solely to closures laminated or coated with materials intended to provide, or in fact function as, a barrier to the base elastomer [e.g., polytetrafluoroethylene (PTFE) or lacquer coatings]. It is not permissible to use a barrier material in an attempt to change a closure that does not meet compendial requirements to one that does conform. Therefore, all *Physicochemical Tests* apply to the base formula of such closures, as well as to the coated or laminated closure. To obtain *Physicochemical Tests* results, the tests are to be

performed on uncoated or nonlaminated closures of the same elastomeric compound, as well as to the laminated or coated closure. The *Functionality Tests* apply to and are to be performed using the laminated or coated elastomeric closure. *Biological Tests* apply to the lamination or coating material, as well as to the base formula. *Biological Tests* may be performed on the laminated or coated closure, or they may be performed on the laminate/coating material and the uncoated or nonlaminated closures of the same elastomeric compound. In the latter case, the results are to be reported separately. The base formula used for physicochemical or biological tests intended to support the compendial compliance of a barrier-coated closure should be similar to the corresponding coated closure in configuration and size.

For all *Elastomeric Closures for Injections* { 381 } tests performed on any closure type, it is important to document the closure being tested, including a full description of the elastomer, and any lubrication, coating, laminations, or treatments applied.

This chapter states test limits for Type I and Type II elastomeric closures. Type I closures are typically used for aqueous preparations. Type II closures are typically intended for nonaqueous preparations and are those which, having properties optimized for special uses, may not meet all requirements listed for Type I closures because of physical configuration, material of construction, or both. If a closure fails to meet one or more of the Type I test requirements but still meets the Type II requirements for the test(s), the closure is assigned a final classification of Type II. All elastomeric closures suitable for use with injectable preparations must comply with either Type I or Type II test limits. However, this specification is not intended to serve as the sole evaluation criteria for the selection of such closures.

It is appropriate to use this chapter when identifying elastomeric closures that might be acceptable for use with injectable preparations on the basis of their biological reactivity, their aqueous extract physicochemical properties, and their functionality.

The following closure evaluation requirements are beyond the scope of this chapter:

- The establishment of closure identification tests and specifications
- The verification of closure–product physicochemical compatibility
- The identification and safety determination of closure leachables found in the packaged product
- The verification of packaged product closure functionality under actual storage and use conditions

The manufacturer of the injectable product (the end user) must obtain from the closure supplier an assurance that the composition of the closure does not vary and that it is the same as that of the closure used during compatibility testing. When the supplier informs the end user of changes in the composition, compatibility testing must be repeated, totally or partly, depending on the nature of the changes. Closures must be properly stored, cleaned for removal of environmental contaminants and endotoxins, and, for aseptic processes, sterilized before use in packaging injectable products.

CHARACTERISTICS

Elastomeric closures are translucent or opaque and have no characteristic color, the latter depending on the additives used. They are homogeneous and practically free from flash and adventitious materials (e.g., fibers, foreign particles, and waste rubber).

IDENTIFICATION

Closures are made of a wide variety of elastomeric materials and optional polymeric coatings. For this reason, it is beyond the scope of this chapter to specify identification tests that encompass all possible closure presentations. However, it is the responsibility of the closure supplier and the injectable product manufacturer (the end user) to verify the closure elastomeric formulation and any coating or laminate materials used according to suitable identification tests. Examples of some of the analytical test methodologies that may be used include specific gravity, percentage of ash analysis, sulfur content determination, Fourier transform infrared spectroscopy/attenuated total reflectance (FTIR-ATR) test, thin-layer chromatography (TLC) of an extract, UV absorption spectrophotometry of an extract, or infrared (IR) absorption spectrophotometry of a pyrolysate.

TEST PROCEDURES

Elastomeric closures shall conform to biological, physicochemical, and functionality requirements both as they are shipped by the closure supplier to the injectable product manufacturer (the end user) and in their final ready-to-use state by the end user.

For those elastomeric closures processed by the supplier before distribution to the end user, the supplier shall demonstrate compendial conformance of closures exposed to such processing and/or sterilization steps. Similarly, if elastomeric closures received by the end user are subsequently processed or sterilized, the end user is responsible for demonstrating the continued conformance of closures to compendial requirements subsequent to such processing and/or sterilization conditions (i.e., in their ready-to-use state). This is especially important if closures shall be exposed to processes or conditions that may significantly affect the biological, physicochemical, or functionality characteristics of the closure (e.g., gamma irradiation). For closures that are normally lubricated with silicone before use, it is permissible to perform physicochemical testing on nonlubricated closures to avoid potential method interference and/or difficulties in interpreting test results. For closures supplied with other lubricious nonbarrier coatings, all tests are to be performed using the coated closure.

For closures coated or laminated with coatings intended to provide a barrier function (e.g., PTFE or lacquer coatings), physicochemical compendial tests apply to the uncoated base elastomer, as well as to the coated closure. In this case, suppliers are responsible for demonstrating physicochemical compendial compliance of the coated closure, as well as of the uncoated closure, processed or treated in a manner simulating conditions typically followed by the supplier for such coated closures before shipment to the end user. The uncoated closure subject to physicochemical tests should be similar to the corresponding coated closure in size and configuration. End users of coated closures are also responsible for demonstrating the continued physicochemical compendial conformance of the coated closure, processed or treated in a manner simulating conditions typically used by the end user before use.

In all cases, it is appropriate to document all conditions of closure processing, pretreatment, sterilization, or lubrication when reporting test results.

Table 1 summarizes the testing requirements of closures, and the responsibilities of the supplier and the end user.

Table 1

Closure Types (as supplied or used)	Test Requirements		
	Physicochemical Tests	Functionality Tests	Biological Tests
Closure with or without silicone coating	• Tests are to be performed.	• Tests are to be performed.	• Tests are to be performed.
	• Silicone use is optional.	• Silicone use is optional.	• Silicone use is optional.
	• Responsibility: supplier and end user	• Responsibility: supplier and end user	• Responsibility: supplier and end user
Closures with lubricious coating (nonbarrier material; not silicone)	• Tests are to be performed on coated closures.	• Tests are to be performed on coated closures.	• Tests are to be performed on coated closures.
	• Responsibility: supplier and end user	• Responsibility: supplier and end user	• Responsibility: supplier and end user
Closures with barrier coating	• Tests are to be performed on coated closures.	• Tests are to be performed on coated closures.	• Tests are to be performed on coated closures.
	• Responsibility: supplier and end user		OR:
	AND: • Tests are to be performed on uncoated closures (base formula).	• Responsibility: supplier and end user	• Tests are to be performed on uncoated closures (base formula) and the laminate/coating material (report results separately).
	• Responsibility: supplier		• Responsibility: supplier and end user

BIOLOGICAL TESTS

Two stages of testing are indicated. The first stage is the performance of an in vitro test procedure as described in general test chapter *Biological Reactivity Tests, In Vitro* (87). Materials that do not meet the requirements of the in vitro test are subjected to the second stage of testing, which is the performance of the in vivo tests, *Systemic Injection Test* and *Intracutaneous Test*, according to the procedures set forth in the general test chapter *Biological Reactivity Tests, In Vivo* (88). Materials that meet the requirements of the in vitro test are not required to undergo in vivo testing. Type I and Type II closures must both conform to the requirements of either the in vitro or the in vivo biological reactivity tests. [Note—Also see the general information chapter *The Biocompatibility of Materials Used in Drug Containers, Medical Devices, and Implants* (1031).]

Change to read:

PHYSICOCHEMICAL TESTS

Preparation of *Solution S*

Place whole, uncut closures corresponding to a surface area of $100 \pm 10 \text{ cm}^2$ into a suitable glass container. Cover the closures with 200 mL of Purified Water or Water for Injection. If it is not possible to achieve the prescribed closure surface area ($100 \pm 10 \text{ cm}^2$) using uncut closures, select the number of closures that will most closely approximate 100 cm^2 , and adjust the volume of water used to the equivalent of 2 mL per each 1 cm^2 of actual closure surface area used. Boil for 5 min, and rinse five times with cold Purified Water or Water for Injection. Place the washed closures into a Type I glass, wide-necked flask (see *Containers—Glass* $\langle 660 \rangle$), add the same quantity of Purified Water or Water for Injection initially added to the closures, and weigh. Cover the mouth of the flask with a Type I glass beaker. Heat in an autoclave so that a temperature of $121 \pm 2^\circ$ is reached within 20–30 min, and maintain this temperature for 30 min. Cool to room temperature over a period of about 30 min. Add Purified Water or Water for Injection to bring it up to the original mass. Shake, and immediately decant and collect the solution. [Note—This solution must be shaken before being used in each of the tests.]

Preparation of Blank

Prepare a blank solution similarly, using 200 mL of Purified Water or Water for Injection omitting the closures.

Appearance of Solution (Turbidity/Opaescence and Color)

DETERMINATION OF TURBIDITY (OPAESCENCE)

Note—The determination of turbidity may be performed by visual comparison (*Procedure A*), or instrumentally using a suitable ratio turbidimeter (*Procedure B*). For a discussion of turbidimetry, see *Spectrophotometry and Light-Scattering* $\langle 851 \rangle$. Instrumental assessment of clarity provides a more discriminatory test that does not depend on the visual acuity of the analyst.

Hydrazine sulfate solution: Dissolve 1.0 g of hydrazine sulfate in water, and dilute with water to 100.0 mL. Allow to stand for 4–6 h.

Hexamethylenetetramine solution: Dissolve 2.5 g of hexamethylenetetramine in 25.0 mL of water in a 100-mL glass-stoppered flask.

Opaescence stock suspension: Add 25.0 mL of *Hydrazine sulfate solution* to the *Hexamethylenetetramine solution* in the flask. Mix, and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Opaescence standard suspension: Prepare a suspension by diluting 15.0 mL of the *Opaescence stock suspension* with water to 1000.0 mL. *Opaescence standard suspension* is stable for about 24 h after preparation.

Reference suspensions: Prepare according to *Table 2*. Mix and shake before use. [Note—Stabilized formazin suspensions that can be used to prepare stable, diluted turbidity standards are available commercially and may be used after comparison with the standards prepared as described.]

Table 2

	Reference suspension A	Reference suspension B	Reference suspension C	Reference suspension D
Standard of opalescence	5.0 mL	10.0 mL	30.0 mL	50.0 mL
Water	95.0 mL	90.0 mL	70.0 mL	50.0 mL
Nephelometric turbidity units (NTU)	3 NTU	6 NTU	18 NTU	30 NTU

Procedure A, visual comparison: Use identical test tubes made of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm. Fill one tube to a depth of 40 mm with *Solution S*, one tube to the same depth with water, and four others to the same depth with *Reference suspensions A, B, C, and D*. Compare the solutions in diffuse daylight 5 min after preparation of the *Reference suspensions*, viewing vertically against a black background. The light conditions shall be such that *Reference suspension A* can be readily distinguished from water and that *Reference suspension B* can be readily distinguished from *Reference suspension A*.

Requirement—*Solution S* is not more opalescent than *Reference suspension B* for Type I closures and not more opalescent than *Reference suspension C* for Type II closures. *Solution S* is considered clear if its clarity is the same as that of water when examined as described above, or if its opalescence is not more pronounced than that of *Reference suspension A* (refer to *Table 3*).

Procedure B, instrumental comparison: Measure the turbidity of the *Reference suspensions* in a suitable calibrated turbidimeter (see $\langle 851 \rangle$). The blank should be run, and the results should be corrected for the blank. *Reference suspensions A, B, C, and D* represent 3, 6, 18, and 30 NTU, respectively. Measure the turbidity of *Solution S* using the calibrated turbidimeter.

Requirement—The turbidity of *Solution S* is NMT that for *Reference suspension B* (6 NTU FTU) for Type I closures and is NMT that for *Reference suspension C* (18 NTU FTU) for Type II closures (refer to *Table 3*).

Table 3

Comparison Method		
Opalescence Requirements	Procedure A (visual)	Procedure B (instrumental)
Type I closures	No more opalescent than <i>Reference suspension B</i>	NMT 6 NTU
Type II closures	No more opalescent than <i>Reference suspension C</i>	NMT 18 NTU

DETERMINATION OF COLOR

Color standard: Prepare a solution by diluting 3.0 mL of *Matching Fluid O* (see *Color and Achromicity* $\langle 631 \rangle$) with 97.0 mL of diluted hydrochloric acid.

Procedure: Use identical tubes made of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm. Fill one tube to a depth of 40 mm with *Solution S*, and the second tube with the *Color standard*. Compare the liquids in diffuse daylight, viewing vertically against a white background.

Requirement: *Solution S* is not more intensely colored than the *Color standard*.

Acidity or Alkalinity

Bromothymol blue solution: Dissolve 50 mg of bromothymol blue in a mixture of 4 mL of 0.02 M sodium hydroxide and 20 mL of alcohol. Dilute with water to 100 mL.

Procedure: To 20 mL of *Solution S* add 0.1 mL of *Bromothymol blue solution*. If the solution is yellow, titrate with 0.01 N sodium hydroxide until a blue endpoint is reached. If the solution is blue, titrate with 0.01 N hydrochloric acid until a yellow endpoint is reached. If the solution is green, it is neutral and no titration is required.

Blank correction: Test 20 mL of blank similarly. Correct the results obtained for *Solution S* by subtracting or adding the volume of titrant required for the blank, as appropriate. (See *Titrimetry* 〈 541 〉.)

Requirement: NMT 0.3 mL of 0.01 N sodium hydroxide produces a blue color, or NMT 0.8 mL of 0.01 N hydrochloric acid produces a yellow color, or no titration is required.

Absorbance

Procedure: [Note—Perform this test within 5 h of preparing *Solution S*.] Pass *Solution S* through a filter of 0.45- μ m pore size, discarding the first few mL of filtrate. Measure the absorbance of the filtrate at wavelengths between 220 and 360 nm in a 1-cm cell using the blank in a matched cell in the reference beam. If dilution of the filtrate is required before measurement of the absorbance, correct the test results for the dilution.

Requirement: The absorbances at these wavelengths do not exceed 0.2 for Type I closures or 4.0 for Type II closures.

Reducing Substances

Procedure: [Note—Perform this test within 4 h of preparing *Solution S*.] To 20.0 mL of *Solution S* add 1 mL of diluted sulfuric acid and 20.0 mL of 0.002 M potassium permanganate. Boil for 3 min. Cool, add 1 g of potassium iodide, and titrate immediately with 0.01 M sodium thiosulfate, using 0.25 mL of starch solution TS as the indicator. Perform a titration using 20.0 mL of blank, and note the difference in volume of 0.01 M sodium thiosulfate required.

Requirement: The difference between the titration volumes is NMT 3.0 mL for Type I closures and NMT 7.0 mL for Type II closures.

Heavy Metals

~~*Procedure*—Proceed as directed for *Method I* under *Heavy Metals* 〈 231 〉. Prepare the *Test Preparation* using 10.0 mL of *Solution S*.~~

~~*Requirement*—*Solution S* contains not more than 2 ppm of heavy metals as lead.~~

■ ■ 2S (USP38)

■

Elemental Impurities

Limits for elemental impurities in marketed pharmaceutical drug products can be found in *Elemental Impurities—Limits* 〈 232 〉. These limits are not specific to packaging components; a strategy to limit elemental impurities in a final drug product should consider all potential sources

that include a packaging component. ■ 1S (USP39)

Extractable Zinc

Test solution: Prepare a *Test solution* by diluting 10.0 mL of *Solution S* with 0.1 N hydrochloric acid to 100 mL. Prepare a test blank similarly, using the blank for *Solution S*.

Zinc standard solution: Prepare a solution (10 ppm of zinc) by dissolving zinc sulfate in 0.1 N hydrochloric acid.

Reference solutions: Prepare NLT 3 *Reference solutions* by diluting the *Zinc standard solution* with 0.1 N hydrochloric acid. The concentrations of zinc in these *Reference solutions* are to span the expected limit of the *Test solution*.

Procedure: Use a suitable atomic absorption spectrophotometer (see { 851 }) equipped with a zinc hollow-cathode lamp and an air-acetylene flame. An alternative procedure, such as an appropriately validated inductively coupled plasma analysis (ICP), may be used. Test each of the *Reference solutions* at the zinc emission line of 213.9 nm at least three times. Record the steady readings. Rinse the apparatus with the test blank solution each time to ensure that the reading returns to the initial blank value. Prepare a calibration curve from the mean of the readings obtained for each *Reference solution*. Record the absorbance of the *Test solution*. Determine the ppm of zinc concentration of the *Test solution* using the calibration curve.

Requirement: *Solution S* contains NMT 5 ppm of extractable zinc.

Ammonium

Alkaline potassium tetraiodomercurate solution: Prepare a 100-mL solution containing 11 g of potassium iodide and 15 g of mercuric iodide in water. Immediately before use, mix 1 volume of this solution with an equal volume of a 250-g/L solution of sodium hydroxide.

Test solution: Dilute 5 mL of *Solution S* with water to 14 mL. Make alkaline if necessary by adding 1 N sodium hydroxide, and dilute with water to 15 mL. Add 0.3 mL of *Alkaline potassium tetraiodomercurate solution*, and close the container.

Ammonium standard solution: Prepare a solution of ammonium chloride in water (1 ppm of ammonium). Mix 10 mL of the 1 ppm of ammonium chloride solution with 5 mL of water and 0.3 mL of *Alkaline potassium tetraiodomercurate solution*. Close the container.

Requirement: After 5 min, any yellow color in the *Test solution* is no darker than the *Ammonium standard solution* (NMT 2 ppm of ammonium in *Solution S*).

Volatile Sulfides

Procedure: Place closures, cut if necessary, with a total surface area of $20 \pm 2 \text{ cm}^2$ in a 100-mL flask, and add 50 mL of a 20-g/L citric acid solution. In the same manner and at the same time, prepare a control solution in a separate 100-mL flask by dissolving 0.154 mg of sodium sulfide in 50 mL of a 20-g/L citric acid solution. Place a piece of lead acetate paper over the mouth of each flask, and hold the paper in position by placing over it an inverted weighing bottle. Heat the flasks in an autoclave at $121 \pm 2^\circ$ for 30 min.

Requirement: Any black stain on the paper produced by the test solution is not more intense than that produced by the control substance.

FUNCTIONALITY TESTS

Note—Samples treated as described for preparation of *Solution S* and air dried should be used for *Functionality Tests of Penetrability, Fragmentation, and Self-Sealing Capacity*. *Functionality Tests* are performed on closures intended to be pierced by a hypodermic needle. The *Self-Sealing Capacity* test is required only for closures intended for multiple-dose containers. The needle specified for each test is a lubricated, long-bevel (bevel angle, $12 \pm 2^\circ$) hypodermic needle.¹

Penetrability

Procedure: Fill 10 suitable vials to the nominal volume with water, fit the closures to be examined, and secure with a cap. Using a new hypodermic needle as described above for each closure, pierce the closure with the needle perpendicular to the surface.

Requirement: The force for piercing is NMT 10 N (1 kgf) for each closure, determined with an accuracy of ± 0.25 N (25 gf).

Fragmentation

Closures for liquid preparations: Fill 12 clean vials with water to 4 mL less than the nominal capacity. Fit the closures to be examined, secure with a cap, and allow to stand for 16 h.

Closures for dry preparations: Fit closures to be examined into 12 clean vials, and secure each with a cap.

Procedure: Using a hypodermic needle as described above fitted to a clean syringe, inject into each vial 1 mL of water while removing 1 mL of air. Repeat this procedure four times for each closure, piercing each time at a different site. Use a new needle for each closure, checking that the needle is not blunted during the test. Pass the total volume of liquid in all of the vials through a single filter with a nominal pore size NMT 0.5 μm . Count the rubber fragments on the surface of the filter visible to the naked eye.

Requirement: There are NMT five fragments visible. This limit is based on the assumption that fragments with a diameter >50 μm are visible to the naked eye. In case of doubt or dispute, the particles are examined microscopically to verify their nature and size.

Self-Sealing Capacity

Procedure: Fill 10 suitable vials with water to the nominal volume. Fit the closures that are to be examined, and cap. Using a new hypodermic needle as described above for each closure, pierce each closure 10 times, piercing each time at a different site. Immerse the 10 vials in a solution of 0.1% (1 g/L) methylene blue, and reduce the external pressure by 27 kPa for 10 min. Restore to atmospheric pressure, and leave the vials immersed for 30 min. Rinse the outside of the vials.

Requirement: None of the vials contain any trace of blue solution.

¹ Refer to ISO 7864:1993. Sterile hypodermic needles for single use.

http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=14793

BRIEFING

《 507 》 **Protein Determination Procedures.** The USP Total Protein Measurement Expert Panel and the General Chapters—Biological Analysis Expert Committee propose the following new general test chapter. The chapter contains multiple validated methods for the measurement of total protein, providing users with options depending on their sample matrix and measurement purpose. Because it is impossible to test every potential protein and sample matrix for this general purpose, any selected method must be verified before use as suitable for its intended purpose. The liquid chromatographic procedure in *Amino Acid Analysis* is based on analyses performed with the Biochrom Sodium Prewash 4.6-mm × 10-cm, 20-µm Ultropac 4 packing brand of L58 column and Biochrom Sodium High Performance Protein Hydrolysate 4.6-mm × 20-cm, 8-µm Ultropac 8 packing brand of L58 column. An associated USP BSA for Protein Quantitation Reference Standard is also proposed for assuring system suitability or for approximate protein measurements when a specific protein Reference Standard is unavailable for that purpose. Readers with alternative, validated total protein determination procedures are encouraged to submit their methods to USP for possible inclusion in a future chapter revision.

(GCBA: M. Kibbey.)

Correspondence Number—C118014

Comment deadline: May 31, 2015

Add the following:

■ 《 507 》 PROTEIN DETERMINATION PROCEDURES

INTRODUCTION

Several procedures exist to determine the total protein in pharmaceutical drug substances and products. Procedures may differ based on the physical characteristic of the protein, which serves as the basis of the measurement principle (e.g., absorbance of ultraviolet light by aromatic amino acid residues or by dye binding). Understanding the protein structure and sample matrix is important when selecting a total protein quantitation procedure. Users must verify that the chosen validated method below is suitable for their specific purpose (see *Verification of Compendial Procedures* 《 1226 》).

PROCEDURE

Two options for Method I are presented: *Method IA*, using denaturing conditions; and *Method IB*, using native conditions. Depending on the protein sample (e.g., a strong coiled structure with most aromatic amino acids in the interior of the protein), one method may prove superior to the other, and users must verify which is more suitable for their particular protein sample. At a minimum, both methods are dependent on the following conditions being met: 1) the amino acid sequence and molecular weight of the protein must be known; and 2) the protein must contain tyrosines or tryptophans, and no other chromophores (other than cystines) that absorb near 280 nm (including nucleic acids and diluent components) should be present.

- **Method IA. Ultraviolet Light Absorbance Under Denaturing Conditions**

Sample buffer A: Prepare 20 mM sodium phosphate, pH 6.5 by dissolving 0.24 g of sodium

dihydrogen phosphate in about 80 mL of water. Adjust the pH with sodium hydroxide solution (10% w/w), and fill with water to 100 mL. Filter.

Sample buffer B: Prepare 7 M guanidine hydrochloride and 20 mM sodium phosphate, pH 6.5 by dissolving 0.24 g of sodium dihydrogen phosphate and 66.87 guanidine hydrochloride in about 50 mL of water. Adjust with sodium hydroxide solution (10% w/w), and fill with water to 100 mL. Filter.

Reference solution: Prepare 6 M guanidine hydrochloride and 20 mM sodium phosphate, pH 6.5 by mixing 330 μL of *Sample buffer A* and 2000 μL of *Sample buffer B*.

Sample preparation A: Dilute the test sample by adding *Sample buffer A* and *Sample buffer B* to achieve a final concentration of 6 M guanidine hydrochloride and about 0.4 mg/mL of protein, achieving an absorbance of about 0.6. [Note—Gravimetric dilutions can be used and sometimes improve accuracy.] Prepare at least three replicates per test sample.

Analysis

Samples: *Sample buffer A*, *Sample buffer B*, *Reference solution*, and *Sample preparation A*

Determination of the protein concentration in denaturing buffer: Using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* (857)), measure the absorbance of *Sample preparation A* in 1-cm cuvettes at an optical density of 280 nm against the *Reference solution*, and subtract the absorbance value at 330 nm to obtain $A_{\text{corrected}}$.

Calculate the protein concentration c in the test sample:

$$c = A_{\text{corrected}} \times DF \times MW / (\epsilon_{280 \text{ nm}} \times 1 \text{ cm})$$

$A_{\text{corrected}}$ = absorbance at 280 nm minus the absorbance at 330 nm

DF = dilution factor

MW = calculated molecular weight of the protein

$\epsilon_{280 \text{ nm}}$ = extinction coefficient of the test sample at 280 nm

$$\epsilon_{280 \text{ nm}} = (n_{\text{Trp}} \times 5690 + n_{\text{Tyr}} \times 1280 + n_{\text{Cys}} \times 120) \text{ M}^{-1} \times \text{cm}^{-1}$$

n_{Trp} = number of tryptophans

n_{Tyr} = number of tyrosines

n_{Cys} = number of cystines

System suitability

The test sample UV spectra must show a maximum between 270 and 285 nm and must show a minimum between 243 and 257 nm. The measured absorbance at 280 nm must be NLT 0.4 and NMT 0.6. The relative standard deviation (RSD) of the calculated concentration of all replicates must be NMT 3.0%.

• Method IB. Ultraviolet Light Absorbance Under Native Conditions

Sample buffer A: Prepare 20 mM sodium phosphate, pH 6.5 by dissolving 0.24 g of sodium dihydrogen phosphate in about 80 mL of water. Adjust the pH with sodium hydroxide solution (10% w/w), and fill with water to 100 mL. Filter.

Sample preparation A: Dilute the test sample by adding *Sample buffer A* to achieve a final concentration of about 0.4 mg/mL of protein, achieving an absorbance of about 0.6. [Note—Gravimetric dilutions can be used and sometimes improve accuracy.] Prepare at least

three replicates per test sample.

Analysis

Samples: *Sample buffer A* and *Sample preparation A*

Determination of the protein concentration in native buffer: Using a suitable

spectrophotometer (see chapter (857)), measure the absorbance of *Sample preparation A* in 1-cm cuvettes at 280 nm against *Sample buffer A* and subtract the absorbance value at 330 nm to obtain $A_{corrected}$.

Calculate the protein concentration c of the test sample:

$$c = A_{corrected} \times DF \times MW / (\epsilon_{280\text{ nm-native2}} \times 1\text{ cm})$$

$A_{corrected}$ = absorbance at 280 nm minus the absorbance at 330 nm

DF = dilution factor

MW = calculated molecular weight of the protein

$\epsilon_{280\text{ nm-native2}}$ = extinction coefficient of the test sample at 280 nm

where the extinction coefficient $\epsilon_{280\text{ nm-native2}}$ is calculated as:

$$\epsilon_{280\text{ nm-native2}} = (n_{Trp} \times 5500 + n_{Tyr} \times 1490 + n_{Cys} \times 125) \text{ M}^{-1} \times \text{cm}^{-1}$$

n_{Trp} = number of tryptophans

n_{Tyr} = number of tyrosines

n_{Cys} = number of cystines

System suitability

The test sample UV spectra must show a maximum between 270 and 285 nm and must show a minimum between 243 and 257 nm. The measured absorbance at 280 nm must be NLT 0.4 and NMT 0.6. The RSD of the calculated concentration of all replicates must be NMT 3.0%.

• Method II. Bicinchoninic Acid Method

BCA reagent solution:¹ Prepare a solution of 10 g/L of bicinchoninic acid (BCA), 20 g/L of sodium carbonate monohydrate, 1.6 g/L of sodium tartrate, 4 g/L of sodium hydroxide, and 9.5 g/L of sodium bicarbonate in water to a pH of 11.25.

Copper sulfate reagent solution: Prepare a solution of 40 g/L of cupric sulfate pentahydrate in water.

BCA working reagent solution: Mix 50 volumes of *BCA reagent solution* with 1 volume of *Copper sulfate reagent solution*. Mix until the green solution is clear.

Sample dilution buffer: Water or another suitable, non-interfering buffer

Standard solutions: Ideally, use the USP Reference Standard for the protein under test. If either the protein of interest is unknown, is a mixture, or is a specific USP Reference Standard that is unavailable, use USP BSA for Protein Quantitation RS. Dilute portions of this solution with the *Sample dilution buffer* to obtain NLT five evenly spaced *Standard solutions* in triplicate. The selected *Standard solution* concentrations should result in a linear curve, typically in the range of 25–1000 µg/mL of protein.

Suitability standard solution: Prepare an independent dilution of the same protein used to make the *Standard solutions* above using *Sample dilution buffer* to obtain a *Suitability standard solution* having a concentration near the midpoint of the standard curve. Prepare the *Suitability standard solution* in triplicate.

Sample solution: Prepare a *Sample solution* with an expected concentration within the standard curve by diluting with the *Sample dilution buffer*. Prepare triplicate samples.

Analysis

Samples: *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solution*

Accurately transfer 100 μL of *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solution* to individual test tubes. [Note—96-well, plate-based, and automated methods can also be used with suitable volume adjustments to the procedure below.] Add 2.0 mL of *BCA working reagent solution* to each tube, and mix well.

Cover and incubate the solutions at $37 \pm 2^\circ$ for 30 ± 1 min, then allow the samples to cool to room temperature for NLT 5 min and NMT 60 min. Determine the absorbances of the solutions at 562 nm with a suitable spectrophotometer (see chapter [857](#)). Autozero the instrument with the *Sample dilution buffer* sample.

Measure the absorbance of all *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solution* samples within 10 min.

Calculations: Plot the absorbances of the *Standard solutions* versus the protein concentrations of the *Standard solutions*, and prepare a standard curve using the linear regression method. Use this standard curve and the absorbance of the *Sample solution* to determine the concentration of protein in each *Suitability standard solution* and *Sample solution*.

System suitability

Samples: *Standard solutions* and *Suitability standard solution*

Suitability requirements

Linearity: The coefficient of determination (r^2) for a linear fit of all *Standard solutions* is NLT 0.99.

Relative standard deviation: NMT 5% for triplicate results of the *Suitability standard solution*

Accuracy: The calculated concentration of the *Suitability standard solution* must be within 90%–110% of the theoretical concentration.

Acceptance criteria

Relative standard deviation: NMT 5% for triplicate *Sample solution* results

● Method III. Bradford Method

Sample dilution buffer: Water or another suitable, non-interfering buffer

Standard solutions: Use the USP Reference Standard for the protein under test or USP BSA for Protein Quantitation RS if either the protein of interest is unknown, a mixture, or a specific USP Reference Standard is unavailable. Dilute portions of this solution with the *Sample dilution buffer* to obtain NLT five evenly spaced *Standard solutions* in triplicate. Two standard curve ranges are provided for use: either 0.1–1.0 mg/mL of protein (see *Analysis procedure A*, below) or 5–25 $\mu\text{g/mL}$ of protein (see *Analysis procedure B*, below).

Suitability standard solution: Dilute the USP Reference Standard for the protein under test or USP BSA for Protein Quantitation RS with *Sample dilution buffer* to obtain a *Suitability standard solution* having a concentration near the midpoint of the standard curve. Prepare the *Suitability standard solution* in triplicate.

Sample solution: Prepare a *Sample solution* with an expected concentration within one of the two concentration ranges in options *A* or *B* below by diluting with the *Sample dilution buffer*. Prepare triplicate samples.

Coomassie reagent solution: Dissolve 100 mg of brilliant blue G² in 50 mL of alcohol. Add 100 mL of phosphoric acid, dilute with water to 1 L, and mix. Pass the solution through filter paper (Whatman No. 1 or equivalent), and store the filtered reagent in an amber bottle at room temperature. [Note—Slow precipitation of the dye will occur during storage of the reagent. Filter the reagent before use.]

Analysis: Select one of the two concentration ranges listed below that is suitable for the expected concentrations of the *Sample solutions*.

A. Protein concentration range: 0.1–1.0 mg/mL

Samples: *Sample dilution buffer*, *Standard solutions*, and *Sample solution*

Add 1 mL of the *Coomassie reagent solution* to 20 μ L of each *Sample dilution buffer*, *Standard solutions*, and *Sample solution* samples. Mix by inversion, avoiding foaming.

Incubate 10–30 min. By using a suitable spectrophotometer (see chapter { 857 }) [Note—Do not use quartz (silica) cuvettes.], use the *Sample dilution buffer* to set the instrument to zero at 595 nm. Autozero the instrument with the *Sample dilution buffer* sample.

Measure the absorbance of all *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solution* samples within 60 min.

B. Protein concentration range: 5–25 μ g/mL

Samples: *Sample dilution buffer*, *Standard solutions*, and *Sample solution*

Add 0.5 mL of the *Coomassie reagent solution* to 0.5 mL each of *Sample dilution buffer*, *Standard solutions*, and *Sample solution* samples. Mix by inversion, avoiding foaming.

Incubate 10–30 min. By using a suitable spectrophotometer (see chapter { 857 }) [Note—Do not use quartz (silica) cuvettes.], use the *Sample dilution buffer* to set the instrument to zero at 595 nm. Autozero the instrument with the *Sample dilution buffer* sample. Measure the absorbance of all *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solution* samples within 60 min.

Calculations: [Note—The relationship of absorbance to protein concentration is nonlinear; however, if the standard curve concentration range is sufficiently small, it will approach linearity.] Plot the absorbances of the *Standard solutions* versus the protein concentrations, and determine the standard curve best fitting the plotted points. From this standard curve and the absorbance of the *Sample solution*, determine the concentration of protein in the *Sample solution*.

System suitability

Samples: *Standard solutions* and *Suitability standard solutions*

Suitability requirements

Linearity: r^2 for a fit of all *Standard solutions* is NLT 0.99.

Relative standard deviation: NMT 15% for triplicate results of the *Suitability standard solution*

Recovery: The calculated concentration of the *Suitability standard solution* must be within 90%–110% of the theoretical concentration.

Acceptance criteria: %CV of triplicates of each *Sample solution* is NMT 15%.

● Method IV. Lowry Method

SDS solution: If required, prepare a solution of 50 g/L of sodium dodecyl sulfate (SDS).

Lowry reagent A: Prepare a solution of 10 g/L of sodium hydroxide in water, and prepare a solution of 50 g/L of sodium carbonate anhydrous in water. Mix equal volumes (2 volumes and 2 volumes) of each solution, and dilute with water or *SDS solution*, if required, to 5

volumes. [Note—SDS may be added to *Lowry reagent A* when the test protein contains detergents or lipids or is poorly soluble after treatment for interfering substances.]

Lowry reagent B: Prepare a fresh solution of 29.8 g/L of disodium tartrate dihydrate in water. Prepare a solution of 12.5 g/L of cupric sulfate pentahydrate in water. Mix equal volumes (2 volumes and 2 volumes) of each solution, and dilute with water to 5 volumes.

Lowry reagent C: Mix 50 volumes of *Lowry reagent A* with 1 volume of *Lowry reagent B*. [Note—Prepare fresh and protect from light.]

Sample dilution buffer: Purified water or other non-interfering buffer

Standard solutions: Use the USP Reference Standard for the protein under test or USP BSA for Protein Quantitation RS if the protein of interest is unknown, a mixture, or a specific USP Reference Standard is unavailable. Dilute portions of this solution with the *Sample dilution buffer* to obtain NLT five evenly spaced *Standard solutions*. The standard concentrations should be selected to result in a linear curve, typically in the range of 10–200 µg/mL of protein.

Suitability standard solution: Dilute the USP Reference Standard for the protein under test or USP BSA for Protein Quantitation RS with *Sample dilution buffer* to obtain a *Suitability standard solution* having a concentration near the midpoint of the standard curve. Prepare and analyze the *Suitability standard solution* in triplicate.

Sample solutions: Prepare a sample solution with an expected concentration within the standard curve by diluting with the *Sample dilution buffer*. Prepare and analyze samples in triplicate.

Diluted Folin–Ciocalteu's phenol reagent: Dilute Folin–Ciocalteu's phenol reagent 1:1 with water. Alternative dilutions may be used provided that the pH of the samples (i.e., *Standard solutions* and *Sample solutions* after addition of *Lowry reagent C* and the *Diluted Folin–Ciocalteu's phenol reagent*) is 10.0–10.6.

Analysis

Samples: *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solutions*

Accurately transfer 1.0 mL of *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solutions* to individual test tubes. To each test tube add 5.0 mL of *Lowry reagent C*. Allow to stand at room temperature for 10 min. Add 0.5 mL of *Diluted Folin–Ciocalteu's phenol reagent* to each test tube, mix immediately, and incubate at room temperature for 30 min. Determine the absorbance of each solution at 750 nm with a suitable spectrophotometer (see chapter { 857 }). Autozero the instrument with the *Sample dilution buffer*.

Calculations: Plot the absorbances of the *Standard solutions* versus the protein concentrations of the *Standard solutions*, and prepare a standard curve using the linear regression method. Use this standard curve and the absorbances of the *Sample solutions* to determine the concentration of protein in each of the *Sample solutions* and *Suitability standard solution*.

System suitability

Sample: *Suitability standard solution*

Suitability requirements

Linearity: r^2 for a linear fit of all standards is NLT 0.995.

Relative standard deviation: NMT 5% for triplicate results of the *Suitability standard solution*

Recovery: The calculated concentration of the *Suitability standard solution* must be

within 90%–110% of the theoretical concentration.

Acceptance criteria

Relative standard deviation: NMT 5% for triplicate sample results

• Method V. Amino Acid Analysis

Separation and determination of hydrolyzed amino acids by ion-exchange chromatography, followed by postcolumn ninhydrin derivatization, is described. [Note—For additional information, see *Biotechnology-Derived Articles—Amino Acid Analysis* 〈 1052 〉, which may be a helpful but not mandatory resource.]

Hydrolysis solution: 6 N hydrochloric acid

Sample hydrolysate preparations: Prepare a protein sample such that the content of amino acids is within the established linear working range of the procedure. [Note—Glassware used for hydrolysis must be clean.] Place suitable volumes of the sample and *Hydrolysis solution* in a hydrolysis tube. [Note—Common volumes range from 100 to 500 μL for the sample and from 1 to 2 mL for the *Hydrolysis solution* such that complete protein digestion is demonstrated within the hydrolysis incubation time.] Cool the mixture until solidification, and evacuate until the pressure is <0.2 bar. Flame seal the hydrolysis tube in vacuum by melting the neck of the tube. Hydrolyze at 110^o for 22 h. Allow the tube to cool, and remove the solvent by centrifugal vacuum concentrator.

Chromatography

Solution A: Sodium citrate buffer (0.2 N in sodium), containing 2.0% trisodium citrate dihydrate, 0.1% phenol, 0.2% thiodiglycol, and 2.0% propan-2-ol. Adjust with concentrated hydrochloric acid to a pH of 3.2.

Solution B: Sodium citrate buffer (0.2 N in sodium), containing 2.0% trisodium citrate dihydrate and 0.1% phenol. Adjust with concentrated hydrochloric acid to a pH of 4.25.

Solution C: Sodium citrate buffer (1.2 N in sodium), containing 2.0% trisodium citrate dihydrate, 0.1% phenol, and 5.8% sodium chloride. Adjust with concentrated hydrochloric acid to a pH of 6.45.

Solution D: 0.4 M sodium hydroxide containing 0.1% ethylenediaminetetraacetic acid.

Mobile phase: See *Table 1*. Equilibrate the system before each run.

Table 1

Time (min)	Solution (100%)	Column Temperature	Flow, Mobile Phase (mL/h)	Flow, Postcolumn Reagent (mL/h)
0	A	49 ^o	35	25
8.5	A	49 ^o	35	25
8.51	B	51 ^o	35	25
23.0	B	51 ^o	35	25
23.01	C	51 ^o	35	25
24.0	C	51 ^o	35	25
24.01	C	95 ^o	35	25
41.5	C	95 ^o	35	25
41.51	D	95 ^o	35	25

Time (min)	Solution (100%)	Column Temperature	Flow, Mobile Phase (mL/h)	Flow, Postcolumn Reagent (mL/h)
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45.5	D	95°	35	25
45.51	A	49°	35	25
49.0	A	49°	35	25
49.01	50% Isopropanol	49°	0	0
51.0	50% Isopropanol	49°	0	0
51.01	A	49°	44	0
62.0	A	49°	44	0
62.01	A	49°	35	25
64.0	A	49°	35	25

Loading buffer: Sodium citrate buffer (0.2 N in sodium), containing 2.0% trisodium citrate dihydrate, 0.1% phenol, and 2.0% thiodiglycol. Adjust with concentrated hydrochloric acid to a pH of 2.2.

Postcolumn reagent

Solution 1: Solution containing 7.6% acetic acid, 47.7% ethylene glycol, 28.7% water, and 16.0% potassium acetate

Solution 2: Solution containing 9% methanol, 8% ninhydrin, 46.4% diethylene glycolmethyl ether, 36% ethylene glycol, and 0.6% hydrindantin. Prepare *Postcolumn reagent* by mixing 1.75 L of *Solution 1* and 0.25 L of *Solution 2*. Use nitrogen sparging during preparation and mixing. Keep the solution under nitrogen pressure.

Standard solution: Prepare a solution having known equimolar amounts of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine with half the equimolar amount of L-cystine. [Note—Suitable concentrations are 250 and 125 nmol/mL, respectively.]

Sample solution: Reconstitute the *Sample hydrolysate preparations* in a suitable volume of *Loading buffer*.

Blank: *Hydrolysis solution* that has been taken through analytical steps including hydrolysis

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: 440 and 570 nm

Columns

Prewash: 4.6-mm × 10-cm; 20-µm sodium form, packing L58

Analytical: 4.6-mm × 20-cm; 8-µm sodium form, packing L58

Injection volume: 40 µL, at 1 min with baseline reset

Postcolumn reaction: As the amino acids are eluted from the column, *Postcolumn reagent* is added at a rate of 25 mL/h. After mixing, the column effluent and the *Postcolumn reagent* are passed through a reaction coil of approximately 10-m × 0.3-mm

PTFE tubing at a temperature of approximately 135^o, where a purple color is developed.

Reaction coil temperature: 135^o

System suitability

Sample: *Standard solution*

Suitability requirements: All 17 amino acid peaks must be visible in the *Standard solution*.

Resolution: NLT 1.2 between the pairs of: l-threonine and l-serine; l-cystine and l-valine; and l-isoleucine and l-leucine

Tailing factor: 0.8–1.5 for the peak of l-aspartic acid

Relative standard deviation: NMT 2.0% determined from the l-aspartic acid peak

Analysis

Samples: *Blank, Standard solution, and Sample solution*

Record and measure the responses for each amino acid peak in the *Standard solution* and *Sample solution*. Use the responses obtained from the *Standard solution* to calculate the content of each amino acid in the sample. Analyze the *Blank* to confirm purity of the reagent and diluent.

Calculations: For unknown protein samples, calculate the mass, in μg , of each recovered amino acid by the formula $(m \times Mw)/1000$, in which m is the recovered quantity in nmol of the amino acid under test, and Mw is the molecular weight for that amino acid corrected for the weight of the water molecule that was eliminated during peptide bond formation (see *Table 2*). The sum of the masses of the recovered amino acids will give an estimate of the total mass of the protein or peptide analyzed after appropriate correction for partially and completely destroyed amino acids.

Protein samples with known weight and amino acid composition: Use well-recovered amino acids to quantify the protein. Divide the quantity, in nmol, of each of the well-recovered amino acids by the expected number of residues for that amino acid to obtain the protein content based on each well-recovered amino acid. Average the protein content results calculated. Discard protein content values that deviate >5% from the mean. Recalculate the mean protein content from the remaining values to obtain the protein content of the sample.

Table 2

Amino Acid	Abbreviation	Protein/Peptide Residue (Mw)
Alanine	Ala	71.08
Arginine	Arg	156.19
Aspartic acid	Asp	115.09
Cysteine	Cys	103.14
Glutamic acid	Glu	129.12
Glycine	Gly	57.05
Histidine	His	137.14
Isoleucine	Ile	113.16
Leucine	Leu	113.16
Lysine	Lys	128.17
Methionine	Met	131.20
Phenylalanine	Phe	147.18

Amino Acid	Abbreviation	Protein/Peptide Residue (Mw)
Proline	Pro	97.12
Serine	Ser	87.08
Threonine	Thr	101.11
Tyrosine	Tyr	163.18
Valine	Val	99.13

- **USP Reference Standards** 〈 11 〉

USP BSA for Protein Quantitation RS

- 1S (USP39)

¹ Suitable BCA reagents are available from Thermo Scientific catalog #23225 or equivalent.

² Suitable brilliant blue G dye is available from Sigma-Aldrich, catalog #B0770.

BRIEFING

〈 800 〉 **Hazardous Drugs—Handling in Healthcare Settings**, PF 40(3) [May–Jun. 2013].

Based on the public comments received for the proposed 〈 800 〉 in PF 40(3), the USP Compounding Expert Committee has developed a revised chapter. This chapter has been created to identify the requirements for receipt, storage, compounding, dispensing, and administration of hazardous drugs (HDs) to protect the patient, healthcare personnel, and environment. Facility requirements that differ from *Pharmaceutical Compounding—Sterile Preparations* 〈 797 〉 and this chapter will be harmonized through an upcoming revision of 〈 797 〉, which will include the following:

- Elimination of the current allowance in 〈 797 〉 for facilities that prepare a low volume of HDs that permits placement of a Biological Safety Cabinet (BSC) or Caseptic containment isolator (ACI) in a non-negative pressure room. All HD compounding must be done in a separate area designated for HD compounding.
- Addition of an allowance in 〈 800 〉 for a Containment Segregated Compounding Area (C-SCA), a separate, negative pressure room with at least 12 air changes per hour (ACPH) for use when compounding HDs. Low- and medium-risk HD compounded sterile preparation (CSP) may be prepared in a BSC or compounding aseptic containment isolator (CACI) located in a C-SCA, provided the beyond-use date of the CSP does not exceed 12 hours.

Major changes from the proposal of 〈 800 〉 in PF 40(3) include:

- Clarified wording in many sections.
- Removed statement concerning no acceptable level of HDs.
- Revised section on list of HDs, to allow entities to perform an assessment of risk for non-antineoplastic drugs and final dosage forms to determine alternative containment strategies and/or work practices.
- Clarified that HDs may be unpacked in either a neutral/normal or negative pressure area.
- Allowance for either external venting or redundant high-efficiency particulate air

(HEPA) filtration of containment primary engineering controls (C-PECs) used for nonsterile compounding.

The proposed chapter is posted online at www.usp.org/usp-nf/notices/general-chapter-hazardous-drugs-handling-healthcare-settings with line numbers. Please provide the line numbers corresponding to your comments when submitting comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C151881

Comment deadline: May 31, 2015

Add the following:

■ 〈 800 〉 HAZARDOUS DRUGS—HANDLING IN HEALTHCARE SETTINGS

1. INTRODUCTION AND SCOPE

This chapter describes practice and quality standards for handling hazardous drugs (HDs) to promote patient safety, worker safety, and environmental protection. Handling HDs includes, but is not limited to, the receipt, storage, compounding, dispensing, administration, and disposal of sterile and nonsterile products and preparations.

This chapter applies to all healthcare personnel who handle HD preparations and all entities which store, prepare, transport, or administer HDs (e.g., pharmacies, hospitals and other healthcare institutions, patient treatment clinics, physicians' practice facilities, or veterinarians' offices). Personnel who may potentially be exposed to HDs include, but are not limited to: pharmacists, pharmacy technicians, nurses, physicians, physician assistants, home healthcare workers, veterinarians, and veterinary technicians.

Entities that handle HDs must incorporate the standards in this chapter into their occupational safety plan. The entity's health and safety management system must, at a minimum, include:

- Engineering controls
- Competent personnel
- Safe work practices
- Proper use of appropriate Personal Protective Equipment (PPE)
- Policies for HD waste segregation and disposal

The chapter is organized into the following main sections:

1. Introduction and Scope
2. List of Hazardous Drugs
3. Types of Exposure
4. Responsibilities of Personnel Handling Hazardous Drugs
5. Facilities
6. Environmental Quality and Control
7. Personal Protective Equipment
8. Hazard Communication Program
9. Personnel Training
10. Receiving

11. Labeling, Packaging, and Transport
12. Dispensing Final Dosage Forms
13. Compounding
14. Administering
15. Deactivation/Decontamination, Cleaning, and Disinfection
16. Spill Control
17. Disposal
18. Documentation and Standard Operating Procedures
19. Medical Surveillance

Appendix A: Acronyms and Definitions

Appendix B: Examples of Design for Hazardous Drugs Compounding Areas

Appendix C: Types of Biological Safety Cabinets

Appendix D: Bibliography

2. LIST OF HAZARDOUS DRUGS

The National Institute for Occupational Safety and Health (NIOSH) maintains a list of antineoplastic and other HDs used in healthcare. An entity must maintain a list of HDs, which may include items on the current NIOSH list in addition to other agents not on the NIOSH list. The entity's list must be reviewed at least annually and whenever a new agent or dosage form is used.

The NIOSH list of antineoplastic and other HDs provides the criteria used to identify HDs. These criteria must be used to identify HDs that enter the market after the most recent version of the NIOSH list, or that enter the entity as an investigational drug. If the information available on this drug is deemed insufficient to make an informed decision, consider the drug hazardous until more information is available.

Box 1: Containment Requirements

- Any antineoplastic HD requiring manipulation and HD Active Pharmaceutical Ingredients (API) on the NIOSH list must follow the requirements in this chapter.
 - Final antineoplastic dosage forms that do not require any further manipulation other than counting final dosage forms may be dispensed without any further requirements for containment unless required by the manufacturer.
- For dosage forms of other HDs on the NIOSH list, the entity may perform an assessment of risk to determine alternative containment strategies and/work practices.

Some dosage forms of drugs defined as hazardous may not pose a significant risk of direct occupational exposure because of their dosage formulation (e.g., tablets or capsules—solid, intact medications that are administered to patients without modifying the formulation). However, dust from tablets and capsules may present a risk of exposure by skin contact and/or inhalation. An assessment of risk may be performed for these dosage forms to determine alternative containment strategies and/or work practices.

The assessment of risk must, at a minimum, consider the following:

- Type of HD (e.g., antineoplastic, non-antineoplastic, reproductive risk)
- Risk of exposure

- Packaging
- Manipulation

If an assessment of risk approach is taken, the entity must document what alternative containment strategies and/or work practices are being employed for specific dosage forms to minimize occupational exposure. If used, the assessment of risk must be reviewed at least annually and the review documented.

3. TYPES OF EXPOSURE

Routes of unintentional entry of HDs into the body include dermal and mucosal absorption, inhalation, injection, and ingestion (e.g., contaminated foodstuffs, spills, or mouth contact with contaminated hands). Both clinical and nonclinical personnel may be exposed to HDs when they handle HDs or touch contaminated surfaces. *Table 1* lists examples of potential routes of exposure based on activity.

Table 1. Examples of Potential Routes of Exposure Based on Activity

Activity	Potential Route of Exposure
Dispensing	<ul style="list-style-type: none"> • Counting tablets and capsules from bulk containers
Compounding	<ul style="list-style-type: none"> • Crushing tablets or opening capsules • Pouring oral or topical liquids from one container to another • Weighing or mixing components • Constituting or reconstituting powdered or lyophilized HDs • Withdrawing or diluting injectable HDs from parenteral containers • Expelling air or HDs from syringes • Contacting HD residue present on PPE or other garments • Deactivating, decontaminating, cleaning, and disinfecting areas contaminated with or suspected to be contaminated with HDs • Maintenance activities for potentially contaminated equipment and devices
Administration	<ul style="list-style-type: none"> • Generating aerosols during administration of HDs by various routes (e.g. injection, irrigation, oral, inhalation, or topical application) • Performing certain specialized procedures (e.g., intraoperative intraperitoneal injection or bladder instillation) • Priming an IV administration set
Patient-care activities	<ul style="list-style-type: none"> • Handling body fluids (e.g., urine, feces, sweat, or vomit) or body-fluid-contaminated clothing, dressings, linens, and other materials
Spills	<ul style="list-style-type: none"> • Spill generation, management, and disposal
Receipt	<ul style="list-style-type: none"> • Contacting with HD residues present on drug container, individual dosage units, outer containers, work surfaces, or floors
Transport	<ul style="list-style-type: none"> • Moving HDs within a healthcare setting

4. RESPONSIBILITIES OF PERSONNEL HANDLING HAZARDOUS DRUGS

Each entity must have a designated person who is qualified and trained to be responsible for developing and implementing appropriate procedures; overseeing entity compliance with this chapter and other applicable laws, regulations, and standards; ensuring competency of personnel; and ensuring environmental control of the storage and compounding areas. The designated individual must thoroughly understand the rationale for risk-prevention policies, risks to themselves and others, risks of non-compliance that may compromise safety, and the responsibility to report potentially hazardous situations to the management team. The designated individual must also be responsible for the continuous monitoring of the facility and maintaining reports of testing/sampling performed in facilities.

All personnel who handle HDs are responsible for understanding the fundamental practices and precautions and for continually evaluating these procedures and the quality of final HDs to prevent harm to patients, minimize exposure to personnel, and minimize contamination of the work and care environment.

5. FACILITIES

HDs must be handled under conditions that promote patient safety, worker safety, environmental protection, and infection prevention. Access to areas where HDs are handled must be restricted to authorized personnel to protect persons not involved in HD handling. HD handling areas must be located away from break rooms and refreshment areas for personnel, patients, or visitors to reduce risk of exposure. Signs designating the hazard must be prominently displayed before the entrance to the HD handling areas.

Designated areas must be available for:

- Receipt and unpacking of antineoplastic HDs or API
- Storage of HDs
- Nonsterile HD compounding (if performed by the entity)
- Sterile HD compounding (if performed by the entity)

5.1 Receipt

Antineoplastic HDs and APIs must be unpacked (i.e., removal from external shipping containers) in an area that is neutral/normal or negative pressure relative to the surrounding areas. HDs must not be unpacked from their shipping containers in sterile compounding areas or in positive pressure areas.

5.2 Storage

HDs must be stored in a manner that prevents spillage or breakage if the container falls. Do not store HDs on the floor. In areas prone to specific types of natural disasters (e.g., earthquakes) the manner of storage must meet applicable safety precautions, such as secure shelves with raised front lips.

Non-antineoplastic, reproductive risk only, and final dosage forms of antineoplastic HDs may be stored with other inventory. Antineoplastic HDs requiring manipulation other than counting final dosage forms and any HD API must be stored separately from non-HDs in a manner that prevents contamination and personnel exposure. These HDs must be stored in a negative-pressure room with at least 12 air changes per hour (ACPH).

Sterile and nonsterile HDs may be stored together. Depending upon facility design, HDs may be stored within a negative pressure buffer room with at least 12 ACPH. However, only HDs used for sterile compounding may be stored in the negative pressure buffer room.

Refrigerated antineoplastic HDs must be stored in a dedicated refrigerator in a negative pressure area with at least 12 ACPH [e.g., storage room, buffer room, or containment segregated compounding area (C-SCA)]. If a refrigerator is placed in a negative pressure buffer room, an exhaust located adjacent to the refrigerator's compressor and behind the refrigerator should be considered.

5.3 Compounding

Engineering controls are required to protect the preparation from cross-contamination and microbial contamination (if preparation is intended to be sterile) during all phases of the compounding process. Engineering controls for containment are divided into three categories representing primary, secondary, and supplementary levels of control. A containment primary engineering control (C-PEC) is a ventilated device designed to minimize worker and environmental HD exposure while directly handling HDs. Containment secondary engineering controls (C-SEC) is the room in which the C-PEC is placed. Supplemental engineering controls [e.g., closed-system drug-transfer device (CSTD)] are adjunct controls to offer additional levels of protection. *Appendix B* provides examples for designs of HD compounding areas. Sterile and nonsterile HDs must be compounded within a C-PEC located in a C-SEC. The C-SEC used for sterile and nonsterile compounding must:

- Be externally vented through high-efficiency particulate air (HEPA) filtration
- Be physically separated (i.e., a different room from other preparation areas)
- Have a negative pressure between 0.01 and 0.03 inches of water column

The C-PEC must operate continuously if used for sterile compounding or if the C-PEC supplies the negative pressure. If there is any loss of power to the unit, or if repair or moving occurs, all activities occurring in the C-PEC must be suspended immediately. If necessary, protect the unit by covering it appropriately per the manufacturer's recommendations. Once the C-PEC can be powered on, decontaminate, clean, and disinfect (if used for sterile compounding) all interior surfaces and wait the manufacturer-specified recovery time before resuming compounding. A sink must be available for hand washing as well as emergency access to water for removal of hazardous substances from eyes and skin. An eyewash station and/or other emergency or safety precautions that meet applicable laws and regulations must be readily available. However, care must be taken to locate them in areas where their presence will not interfere with required ISO classifications.

For entities that compound both nonsterile and sterile HDs, the respective C-PECs must be placed in segregated rooms separate from each other, unless those C-PECs used for nonsterile compounding are sufficiently effective that the room can continuously maintain ISO 7 classification throughout the nonsterile compounding activity. If the C-PECs used for sterile and nonsterile compounding are placed in the same room, they must be placed at least 1 meter apart and particle-generating activity must not be performed when sterile compounding is in process.

5.3.1 NONSTERILE COMPOUNDING

In addition to this chapter, nonsterile compounding must follow standards in *Pharmaceutical*

Compounding—Nonsterile Preparations 〈 795 〉. A C-PEC is not required if manipulations are limited to handling of final dosage forms (e.g., tablets and capsules) that do not produce particles, aerosols, or gasses.

The C-PECs used for manipulation of nonsterile HDs must be either externally vented (preferred) or redundant-HEPA filtered in series. Nonsterile HD compounding must be performed in a C-PEC that provides personnel and environmental protection, such as a Class I Biological Safety Cabinet (BSC) or Containment Ventilated Enclosure (CVE). A Class II BSC or a compounding aseptic containment isolator (CACI) may be also be used. For occasional nonsterile HD compounding, a C-PEC used for sterile compounding (e.g., Class II BSC or CACI) may be used but must be decontaminated, cleaned, and disinfected before resuming sterile compounding in that C-PEC. The C-PEC used for nonsterile compounding does not need to have unidirectional airflow because the critical environment does not need to be ISO classified.

The C-PEC must be placed in a C-SEC that has at least 12 ACPH. *Table 2* summarizes the engineering controls required for nonsterile HD compounding.

Due to the difficulty of cleaning HD contamination from surfaces, the architectural finish requirements (e.g., smooth, seamless, or impervious surfaces) described in *Pharmaceutical Compounding—Sterile Preparations* 〈 797 〉 also apply to nonsterile compounding areas.

Table 2. Engineering Controls for Nonsterile HD Compounding

C-PEC	C-SEC Requirements
<ul style="list-style-type: none"> • Externally vented (preferred) or redundant-HEPA filtered in series • Examples: CVE, Class I or II BSC, CACI 	<ul style="list-style-type: none"> • 12 ACPH • Externally vented • Negative pressure between 0.01 and 0.03 inches of water column

5.3.2 STERILE COMPOUNDING

In addition to this chapter, applicable sterile compounding standards in 〈 797 〉 must be followed.

All C-PECs used for manipulation of sterile HDs must be externally vented. Sterile HD compounding must be performed in a C-PEC that provides a Class 5 or better air quality, such as a Class II or III BSC or CACI. Class II BSC types A2, B1, or B2 are all acceptable. For most known HDs, type A2 cabinets offer a simple and reliable integration with the ventilation and pressurization requirements of the secondary engineering control. Class II type B2 BSCs are typically reserved for use with volatile components. *Appendix C* describes the different types of BSCs.

A laminar airflow workbench (LAFW) or compounding aseptic isolator (CAI) must not be used for the compounding of an antineoplastic HD. A BSC or CACI used for the preparation of HDs must not be used for the preparation of a non-HD unless the non-HD preparation is placed into a protective outer wrapper during removal from the C-PEC and is labeled to require PPE handling precautions.

The C-PEC must be located in a C-SEC, which may either be an ISO Class 7 buffer room (preferred) or an unclassified containment segregated compounding area (C-SCA). If the C-PEC is placed in a C-SCA, the beyond-use date (BUD) of all compounded sterile preparation (CSP) prepared must be limited as defined in 〈 797 〉 for CSPs prepared in a segregated compounding area. *Table 3* summarizes the engineering controls required for sterile HD compounding.

Table 3. Engineering Controls for Sterile HD Compounding

Configuration	C-PEC	C-SEC	Maximum BUD
ISO Class 7 Buffer Room	<ul style="list-style-type: none"> Externally Vented Examples: Class II BSC or CACI 	<ul style="list-style-type: none"> 30 ACPH Externally vented Negative pressure between 0.01 and 0.03 inches of water column 	As described in { 797 }
C-SCA	<ul style="list-style-type: none"> Externally Vented Examples: Class II BSC or CACI 	<ul style="list-style-type: none"> 12 ACPH Externally vented Negative pressure between 0.01 and 0.03 inches of water column 	As described in { 797 } for segregated compounding area

ISO class 7 buffer room: The C-PEC may be placed in an ISO Class 7 buffer room that has a negative pressure between 0.01 and 0.03 inches of water column and has a minimum of 30 ACPH of HEPA-filtered supply air.

Because the room through which entry into the HD buffer room (e.g., ante-area or non-HD buffer room) plays an important role in terms of total contamination control, the following is required:

- Minimum of 30 ACPH of HEPA-filtered supply air
- Maintain a positive pressure of 0.02 inches of water column relative to all adjacent unclassified spaces
- Maintain an air quality of ISO Class 7 or better

This provides for inward air migration of equal cleanliness classified air into the negative pressure buffer room to contain any airborne HD. A hand-washing sink must be placed at least 1 meter from the entrance of the buffer room to avoid contamination migration into the negative pressure HD buffer room.

Although not a recommended facility design, if the negative-pressure HD buffer room is entered through the positive-pressure non-HD buffer room, the following is required:

- A line of demarcation must be defined within the negative-pressure buffer area for garbing and degarbing
- A method to transport HDs, CSPs, and waste into and out of the negative pressure buffer room to minimize the spread of HD contamination. This may be accomplished by use of a pass-through between the negative-pressure buffer area and adjacent space. The pass-through must be included in the facility's certification to ensure that particles are not compromising the air quality of the negative-pressure buffer room. Do not use a refrigerator pass-through. Other methods of containment (such as sealed containers) may be used if the entity can demonstrate HD containment and appropriate environmental control.

HD CSPs prepared in an ISO Class 7 buffer room may use the BUDs described in { 797 }, based on the categories of CSP, sterility testing, and storage temperature.

Containment segregated compounding area (C-SCA): The C-PEC may be placed in an unclassified C-SCA that has a negative pressure between 0.01 and 0.03 inches of water column

relative to all adjacent spaces and has a minimum of 12 ACPH of HEPA-filtered supply air. A hand-washing sink must be placed at least 1 meter from C-PEC.

Only low- and medium-risk HD CSPs may be prepared in a C-SCA. HD CSPs prepared in the C-SCA must not exceed the BUDs described in § 797 for CSPs prepared in a segregated compounding area.

5.4 Containment Supplemental Engineering Controls

Containment supplemental engineering controls, such as CSTDs, provide adjunct controls to offer additional levels of protection during compounding or administration. Some CSTDs have been shown to limit the potential of generating aerosols during compounding. However, there is no certainty that all CSTDs will perform adequately. Since there is no published universal performance standard for evaluation of CSTD containment, users should carefully evaluate the performance claims associated with available CSTDs based on independent studies and demonstrated containment reduction.

A CSTD must not be used as a substitute for a C-PEC when compounding. CSTDs should be used when compounding HDs when the dosage form allows. CSTDs must be used when administering HDs when the dosage form allows.

6. ENVIRONMENTAL QUALITY AND CONTROL

Environmental wipe sampling should be performed routinely (e.g., initially as a benchmark and at least every 6 months, or more often as needed, to verify containment). Surface wipe sampling should include:

- Interior of the C-PEC and equipment contained in it
- Staging or work areas near the C-PEC
- Areas adjacent to C-PECs (e.g., floors directly under staging and dispensing area)
- Patient administration areas

There are currently no studies demonstrating the effectiveness of a specific number or size of wipe samples in determining levels of HD contamination. Wipe sampling kits should be verified before use to ensure the method and reagent used have been tested to recover a specific percentage of known marker drugs from various surface types found in the sampled area. There are currently no certifying agencies for vendors of wipe sample kits.

There is currently no standard for acceptable limits for HD surface contamination. Common marker HDs that can be assayed include cyclophosphamide, ifosfamide, methotrexate, fluorouracil, and platinum-containing drugs. An example of contamination would be cyclophosphamide levels $>1.00 \text{ ng/cm}^2$, which were shown in some studies to result in uptake of the drug in exposed workers. If any measurable contamination is found, the compounding supervisor must identify, document, and contain the cause of contamination. Such action may include reevaluating work practices, re-training personnel, performing thorough deactivation/decontamination and cleaning, and improving engineering controls. Repeat the wipe sampling to validate that the deactivation/decontamination and cleaning steps have been effective.

7. PERSONAL PROTECTIVE EQUIPMENT

Personal Protective Equipment (PPE) provides worker protection to reduce exposure to HD aerosols and residues. When performing a task in situations where C-PECs are not generally available, such as treating a patient or cleaning a spill, additional PPE may be required. The NIOSH list of antineoplastic and other HDs provides some general guidance on PPE for possible scenarios that may be encountered in healthcare settings.

Gloves, gowns, head, hair, and shoe covers are required for compounding sterile and nonsterile HDs. Gloves are required for administering antineoplastic HDs. Gowns are required when administering injectable antineoplastic HDs. For all other activities, the entity's SOP must describe the appropriate PPE to be worn based on its occupational safety plan and assessment of risk (if used). The entity must develop SOPs for PPE based on the risk of exposure (see *Types of Exposure*) and activities performed.

Appropriate PPE must be worn when handling HDs including during:

- Receipt
- Storage
- Transport
- Compounding (sterile and nonsterile)
- Administration
- Deactivation/Decontamination, Cleaning, and Disinfecting
- Spill Control

7.1 Gloves

When required, chemotherapy gloves must be tested to American Society for Testing and Materials (ASTM) standard D6978 (or its successor). Chemotherapy gloves must be powder-free because powder can contaminate the work area and can adsorb and retain HDs. Gloves must be inspected for physical defects before use. Do not use gloves with pin holes or weak spots.

Chemotherapy gloves must be changed every 30 min or when torn, punctured, or contaminated.

7.2 Gowns

When required, disposable gowns must be tested and shown to resist permeability by HDs. Gowns must be selected based on the HDs handled. Disposable gowns made of polyethylene-coated polypropylene or other laminate materials offer better protection than those made of uncoated materials. Gowns must close in the back (i.e., no open front), be long sleeved, and have closed cuffs that are elastic or knit. Gowns must not have seams or closures that could allow drugs to pass through.

Cloth laboratory coats, surgical scrubs, isolation gowns, or other absorbent materials are not appropriate outerwear when handling HDs because they permit the permeation of HDs and can hold spilled drugs against the skin, thereby increasing exposure. Clothing may also retain HD residue from contact, and may transfer to other healthcare workers or various surfaces.

Washing of non-disposable clothing contaminated with HD residue may transfer drug residue to other clothing.

Gowns must be changed per the manufacturer's information for permeation of the gown. If no permeation information is available for the gowns used, change them every 2–3 h or immediately after a spill or splash. Gowns worn in HD handling areas must not be worn to other

areas in order avoid spreading HD contamination and exposing other healthcare workers.

7.3 Head, Hair, Shoe, and Sleeve Covers

Head and hair covers (including beard and moustache, if applicable) and shoe covers provide protection from contact with HD residue on surfaces and floors. When compounding sterile HDs, a second pair of shoe covers must be donned before entering the buffer room and removed when exiting the buffer room. Shoe covers worn in HD handling areas must not be worn to other areas to avoid spreading HD contamination and exposing other healthcare workers. Disposable sleeve covers constructed of coated materials may be used to protect areas of the arm that may come in contact with HDs. If used, sleeve covers must be carefully removed and properly disposed of after the task is completed.

7.4 Eye and Face Protection

Many HDs are irritating to the eyes and mucous membranes. Appropriate eye and face protection must be worn when there is a risk for spills or splashes of HDs or HD waste materials when working outside of a C-PEC (e.g., administration in the surgical suite, working at or above eye level, or cleaning a spill). A full-facepiece respirator provides eye and face protection. Goggles must be used when eye protection is needed. Eye glasses alone or safety glasses with side shields do not protect the eyes adequately from splashes. Face shields in combination with goggles provide a full range of protection against splashes to the face and eyes. Face shields alone do not provide full eye and face protection.

7.5 Respiratory Protection

For most activities requiring respiratory protection, a fit-tested NIOSH-certified N95 or more protective respirator is sufficient to protect against airborne particles. However, N95 respirators offer no protection against gases and vapors and little protection against direct liquid splashes (see the Centers for Disease Control and Prevention's (CDC's) Respirator Trusted-Source Information).

Surgical masks do not provide respiratory protection from drug exposure and must not be used when respiratory protection is required. A surgical N95 respirator provides the respiratory protection of an N95 respirator, and like a surgical mask, provides a barrier to splashes, droplets, and sprays around the nose and mouth.

Personnel who are unpacking HDs that are not contained in plastic should wear an elastomeric half-mask with a multi-gas cartridge and P100-filter. If the type of drug can be better defined, then a more targeted cartridge should be used.

Fit test the respirator and train workers to use respiratory protection. Follow all requirements in the Occupational Safety and Health Administration (OSHA) respiratory protection standard (29 CFR 1910.134). An appropriate full-facepiece, chemical cartridge-type respirator must be worn when attending to HD spills larger than what can be contained with a spill kit, or when there is a known or suspected airborne exposure to powders or vapors.

7.6 Disposal of Used Personal Protective Equipment

Consider all PPE worn when handling HDs to be contaminated with, at minimum, trace quantities of HDs. PPE must be placed in an appropriate waste container and further disposed of per local,

state, and federal regulations. PPE used during compounding should be disposed of in the proper waste container before leaving the C-SEC. Chemotherapy gloves worn during compounding must be carefully removed and discarded immediately in an approved HD waste container inside the C-PEC or contained in a sealable bag for discarding outside the C-PEC. Potentially contaminated clothing must not be taken home under any circumstances.

8. HAZARD COMMUNICATION PROGRAM

Entities are required to establish policies and procedures that ensure worker safety during all aspects of HD handling. The entity must develop SOPs to ensure effective training regarding proper labeling, transport, and storage of the HDs and use of Safety Data Sheets (SDS), based on the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

Elements of the plan must include:

- A written plan that describes how the standard will be implemented.
- All containers of hazardous chemicals must be labeled, tagged, or marked with the identity of the material and appropriate hazard warnings.
- Entities must have an SDS for each hazardous chemical they use.
- Entities must ensure that the SDSs for each hazardous chemical used are readily accessible to personnel during each work shift and when they are in their work areas.
- Personnel who may be exposed to hazardous chemicals when working must be provided information and training before the initial assignment to work with a hazardous chemical, and also whenever the hazard changes.

9. PERSONNEL TRAINING

All personnel who handle HDs must be fully trained based on their job functions (e.g., in the receipt, storage, handling, compounding, dispensing, and disposal of HDs). Training must occur before the employee independently handles HDs. The effectiveness of training for HD handling competencies must be demonstrated by each employee. Personnel competency must be reassessed at least every 12 months and when a new HD or new equipment is used or a new or significant change in process or SOP occurs. All training and competency assessment must be documented.

The training must include at least the following:

- Overview of entity's list of HDs and their risks
- Review of the entity's SOPs related to handling of HDs
- Proper use of PPE
- Proper use of equipment and devices (e.g., engineering controls)
- Spill management
- Response to known or suspected HD exposure

10. RECEIVING

The entity must establish SOPs for receiving HDs. HDs should be received from the supplier sealed in impervious plastic to segregate them from other drugs and to improve safety in the receiving and internal transfer process. HDs must be delivered to the HD storage area

immediately upon arrival.

PPE, including ASTM-tested, powder-free chemotherapy gloves, must be worn when unpacking HDs (see *Personnel Protective Equipment*). A spill kit must be accessible in the receiving area. The entity must enforce policies that include a tiered approach, starting with visual examination of the shipping container for signs of damage or breakage (e.g., visible stains from leakage, sounds of broken glass containers, and others). *Table 4* summarizes the steps for receiving and handling of damaged shipping containers.

Table 4. Summary of Requirements for Receiving and Handling Damaged HD Shipping Containers

<p>If the shipping container appear damaged</p>	<ul style="list-style-type: none"> • Seal container without opening and contact the supplier for instructions • If the unopened package is to be returned to the supplier, enclose the package in an impervious container and label the outer container "Hazardous" • If the supplier declines return, dispose of properly
<p>If a damaged shipping container must be opened</p>	<ul style="list-style-type: none"> • Seal the container in plastic or an impervious container • Transport it to a C-PEC and place on a plastic-backed preparation mat • Open the package and remove usable items. • Wipe the outside of the usable items with a disposable wipe. • Enclose the damaged item(s) in an impervious container and label the outer container "Hazardous" • If the supplier declines return, dispose of properly • Decontaminate/deactivate and clean the C-PEC (see <i>Deactivation/Decontamination, Cleaning, and Disinfection</i>) and discard the mat and cleaning disposables as hazardous waste

When opening damaged shipping containers, they should preferably be transported to a C-PEC designated for nonsterile compounding. If a C-PEC designated for sterile compounding is the only one available, it must be thoroughly disinfected after the decontamination/deactivation and cleaning step before returning to any sterile compounding activity.

Damaged packages or shipping cartons must be considered spills that must be reported to the designated person and managed according to the entity's SOPs. Clean up must comply with established SOPs.

11. LABELING, PACKAGING, AND TRANSPORT

The entity must establish SOPs for the labeling, handling, packaging, and transport of HDs. The SOPs must address prevention of accidental exposures or spills, personnel training on response to exposure, and use of a spill kit. Examples of special exposure-reducing strategies include small-bore connectors (such as Luer Lock) and syringes, syringe caps, CSTDs, the capping of container ports, sealed impervious plastic bags, impact-resistant and/or water-tight containers, and cautionary labeling.

11.1 Labeling

HDs identified by the entity as requiring special HD handling precautions must be clearly labeled

at all times during their transport.

11.2 Packaging

Compounding personnel must select and use packing containers and materials that will maintain physical integrity, stability, and sterility (if needed) of the HDs during transport. Packaging materials must protect the HD from damage, leakage, contamination, and degradation, while protecting healthcare workers who transport HDs. The entity must have written SOPs to describe appropriate shipping containers and insulating materials, based on information from product specifications, vendors, mode of transport, and experience of the compounding personnel.

11.3 Transport

HDs that need to be transported must be labeled, stored, and handled in accordance with applicable federal, state, and local regulations. HDs must be transported in containers that minimize the risk of breakage or leakage. Pneumatic tubes must not be used to transport any liquid or antineoplastic HDs because of the potential for breakage and contamination. When shipping HDs to locations outside the entity, the entity must consult the Transport Information on the SDS. The entity must ensure that labels and accessory labeling for the HDs include storage instructions, disposal instructions, and HD category information in a format that is consistent with the courier's policies.

12. DISPENSING FINAL DOSAGE FORMS

HDs that do not require any further manipulation other than counting final dosage forms may be dispensed without any further requirements for containment unless required by the manufacturer or if visual indicators of HD exposure hazards (e.g., HD dust or leakage) are present.

Counting of HDs should be done carefully. Clean equipment should be dedicated for use with these drugs. Tablet and capsule forms of HDs must not be placed in automated counting or packaging machines, which subject them to stress and may introduce powdered contaminants into the work area.

13. COMPOUNDING

Entities and personnel involved in compounding HDs must be compliant with the appropriate USP standards for compounding including $\langle 795 \rangle$ and $\langle 797 \rangle$. Compounding must be done in proper engineering controls as described in *Compounding*. When compounding nonsterile and sterile HD preparations in a C-PEC, a plastic-backed preparation mat must be placed on the work surface of the C-PEC. The mat should be changed immediately if a spill occurs and regularly during use, and should be discarded at the end of the daily compounding activity. Disposable or clean equipment for compounding (such as mortars and pestles, spatulas, and others) must be dedicated for use with HDs. Compounding personnel must ensure that the labeling processes for compounded preparations do not introduce contamination into non-HD handling areas.

When compounding nonsterile HD preparations, use commercially available products as starting

ingredients whenever possible. Liquid formulations are preferred over crushing tablets or opening capsules. APIs should only be used when there are no other options. When compounding sterile HD preparations, APIs should be avoided if a suitable manufactured product is available and appropriate for use (e.g., use an injectable product rather than API). Bulk containers of liquid and API HD must be handled carefully to avoid spills. If used, APIs should be handled in a C-PEC to protect against occupational exposure, especially during particle generating activities (such as crushing tablets, opening capsules, and weighing powder).

14. ADMINISTERING

HDs must be administered safely using protective medical devices and techniques. Examples of protective medical devices include needleless and closed systems. Examples of protective techniques include spiking or priming of IV tubing in a C-PEC and crushing tablets in plastic sleeves.

Appropriate PPE must be worn when administering HDs. After use, PPE must be removed and disposed of in an approved HD waste container at the site of drug administration. Equipment (such as tubing and needles) and packaging materials must be disposed of properly, such as in HD waste containers after administration.

CSTDs must be used for administration when the dosage form allows. Techniques and ancillary devices that minimize the risk posed by open systems must be used when administering HDs through certain routes. Administration into certain organs or body cavities (e.g., the bladder, eye, peritoneal cavity, or chest cavity) often requires equipment for which locking connections may not be readily available or possible.

Healthcare personnel should avoid manipulating HDs such as crushing tablets or opening capsules if possible. Liquid formulations are preferred if solid oral dosage forms are not appropriate for the patient. If HD dosage forms do require manipulation such as crushing tablet(s) or opening capsule(s) for a single dose, personnel must don appropriate PPE and use a plastic sleeve to contain any dust or particles generated.

The Oncology Nursing Society (ONS) Safe Handling of Hazardous Drugs publication contains additional information on handling HDs for administration.

15. DEACTIVATION/DECONTAMINATION, CLEANING, AND DISINFECTION

All areas where HDs are handled (e.g., such as during receiving, compounding, transport, administering, and disposal) and all reusable equipment and devices (e.g., C-PEC, carts, and trays) must be routinely deactivated/decontaminated and cleaned. Additionally, sterile compounding areas and devices must be subsequently disinfected.

All healthcare personnel who perform deactivation/decontamination, cleaning, and disinfection activities in HD handling areas must be trained in appropriate procedures to protect themselves and the environment from contamination. All personnel performing these activities must wear appropriate PPE resistant to the cleaning agents used, including two pairs of ASTM-tested chemotherapy gloves and impermeable disposable gowns. Consult manufacturer or supplier information for compatibility with cleaning agents used. Additionally, eye protection and face shields must be used if splashing is possible. Respiratory protection must be used if warranted by the activity.

The entity must establish written procedures for decontamination, deactivation, cleaning, and disinfection (for sterile compounding areas). Cleaning of nonsterile and sterile compounding areas must also comply with $\langle 795 \rangle$ and $\langle 797 \rangle$. Written procedures for cleaning must include procedures, agents used, dilutions used, frequency, and documentation requirements. *Table 5* summarizes the purpose and example agents for each step.

The deactivating, decontaminating, cleaning, and disinfecting agents selected must be appropriate for the type of HD contaminant(s), location, and surface materials. The products used must not contaminate the surfaces with substances that are toxic, volatile, corrosive, or otherwise harmful to the surface material. Perform cleaning in areas that are sufficiently ventilated to prevent accumulation of hazardous airborne drug concentrations and decontamination agents.

Table 5. Summary of Cleaning Steps

Cleaning Step	Purpose	Agents
Deactivation	Render compound inert or inactive	As listed in the HD labeling or if no specific information available, sodium hypochlorite or other Environmental Protection Agency (EPA)-registered oxidizer
Decontamination	Remove inactivated residue	Sterile alcohol, sterile water, peroxide, or sodium hypochlorite
Cleaning	Remove organic and inorganic material	Germicidal detergent and sterile water
Disinfection	Destroy microorganisms	Sterile alcohol or other EPA-registered disinfectant appropriate for use

15.1 Deactivation/Decontamination

Deactivation renders a compound inert or inactive. Decontamination occurs by physically removing HD residue from non-disposable surfaces and transferring it to absorbent, disposable materials (e.g., wipes, pads, or towels) appropriate to the area being cleaned. All disposable materials must be discarded as contaminated HD waste.

Chemical deactivation of HD residue is preferred, but no single process has been found to deactivate all currently available HDs. Studies have examined oxidizing agents such as potassium permanganate, hydrogen peroxide, and sodium hypochlorite; vaporized hydrogen peroxide and detergents; and high- and low-pH solutions, all with varying results. Some potential deactivators have produced byproducts that are as hazardous as the original drug. Other deactivators have respiratory effects or result in caustic damage to surfaces. Note that sodium hypochlorite is corrosive to stainless steel surfaces if left untreated; therefore, sodium hypochlorite must be neutralized with sodium thiosulfate or followed by use of a germicidal detergent.

A multi-component deactivation system is theoretically more efficient than a single-agent system because of the diverse nature of HDs. One commercially available product provides a system for decontamination and deactivation using sodium hypochlorite, surfactant, and thiosulfate neutralizer. This combination product, followed by rinsing, has been shown to be effective for cleaning HD-contaminated surfaces. Other products use combinations of

deactivating agents and/or cleaning agents, followed by rinsing and disinfecting. Because of the growing number of assays available for HDs, additional surface wipe sampling is now possible and should be done to document the effectiveness of any agent used for decontamination of HD residue from work surfaces (see *Environmental Quality and Control*).

15.2 Cleaning and Disinfection

Cleaning is a process that results in the removal of contaminants (e.g., soil, microbial contamination, HD residue) from objects and surfaces using water, detergents, surfactants, solvents, and/or other chemicals. Disinfection is a process of destroying microorganisms. Disinfection must be done for areas intended to be sterile including the sterile compounding areas.

15.3 Cleaning the Compounding Area

The *Cleaning and Disinfecting the Compounding Area* section in *797* applies to both sterile and nonsterile HD compounding areas. Cleaning agents used on compounding equipment should not introduce microbial contamination.

All C-PEC used for either nonsterile or sterile compounding must be decontaminated between compounding of different HDs, any time a spill occurs, before and after certification, any time voluntary interruption occurs, and if the ventilation tool is moved. No cleaning step may be performed when compounding activities are occurring.

The amount of HD contamination introduced into the C-PEC may be reduced by surface decontamination (i.e., wiping down) of HD containers. Although no wipe-down procedures have been studied, the use of disposable material moistened with alcohol, sterile water, peroxide, or sodium hypochlorite solutions may be effective. To avoid spreading HD residue, spray the wiper, not the HD container. The solution used for wiping HD packaging must not alter the product label.

C-PECs may have areas under the work tray where contamination can build up. These areas must be cleaned at least monthly to reduce the contamination level in the C-PEC. Accessing this area may be difficult. Clean as much as possible of the C-PEC surfaces before accessing the area under the work tray. When cleaning the area under the work tray of a C-PEC, the containment airflows are compromised by opening the cabinets. To provide protection to the worker performing this task, respiratory protection may be required. An NIOSH-approved respirator worn by a worker who has been fit tested and cleared to use a respirator would be appropriate.

16. SPILL CONTROL

All personnel who may be required to clean up a spill of HDs must receive proper training in spill management and the use of PPE and NIOSH-certified respirators (see *Personal Protective Equipment*). Spills must be contained and cleaned immediately only by qualified personnel with appropriate PPE. Qualified personnel must be available at all times in entities handling HDs. Signs must be available for restricting access to the spill area. Spill kits containing all of the materials needed to clean HD spills must be readily available in all areas where HDs are routinely handled. If HDs are being prepared or administered in a non-routine healthcare area, a spill kit and respirator must be available. All spill materials must be disposed of as hazardous waste.

The circumstances and management of spills must be documented. Personnel who are potentially exposed during the spill or spill clean up or who have direct skin or eye contact with HDs require immediate evaluation. Non-employees exposed to an HD spill should report to the designated emergency service for initial evaluation and also complete an incident report or exposure form.

SOPs must be developed to prevent spills and to direct the clean up of HD spills. SOPs must address the size and scope of the spill and specify who is responsible for spill management and the type of PPE required. The management of the spill (e.g., decontamination, deactivation, and cleaning) may be dependent on the size and type of spill. The SOP must address the location of spill kits and clean up materials as well as the capacity of the spill kit. Written procedures should address use of appropriate full-facepiece, chemical cartridge-type respirators if the capacity of the spill kit is exceeded or if there is known or suspected airborne exposure to vapors or gases.

17. DISPOSAL

Disposal of all HD waste (including unused and unusable HDs) must comply with all applicable federal, state, and local regulations. All personnel who perform routine custodial waste removal and cleaning activities in HD handling areas must be trained in appropriate procedures to protect themselves and the environment to prevent HD contamination.

18. DOCUMENTATION AND STANDARD OPERATING PROCEDURES

Activities that must be documented include, but are not limited to, the acquisition, preparation, and dispensing of an HD; personnel training; and the use and maintenance of equipment and supplies. These records must be available for review. Personnel who transport, compound, or administer HDs must document their training according to OSHA standards (see OSHA Standard 1910.120 Hazardous Waste Operations and Emergency Response) and other applicable laws and regulations.

The entity must maintain SOPs for the safe handling of HDs for all situations in which these HDs are used throughout a facility. The SOPs must be reviewed at least annually by the designated responsible individual, and the review must be documented. Revisions in forms or records must be made as needed and communicated to all personnel handling HDs.

The SOPs for handling of HDs should include:

- Hazard communication program
- Occupational safety program
- Labeling of HDs
- Procurement of HDs
- Use of proper engineering controls (e.g., C-PECs, C-SECs)
- Use of PPE based on activity (e.g., receipt, transport, compounding, administration, spill, and disposal)
- Decontamination/deactivation, cleaning, and disinfection
- Transport
- Environmental monitoring
- Spill control
- Medical surveillance

19. MEDICAL SURVEILLANCE

Medical surveillance is part of a comprehensive exposure control program complementing engineering controls, safe work processes, and use of PPE. Entities should ensure that healthcare workers who handle HDs as a regular part of their job assignment are enrolled in a medical surveillance program. The general purpose of surveillance is to minimize adverse health effects in personnel potentially exposed to HDs. Medical surveillance programs involve assessment and documentation of symptom complaints, physical findings, and laboratory values (such as a blood count) to determine whether there is a deviation from the expected norms. Medical surveillance can also be viewed as a secondary prevention tool that may provide a means of early detection if a health problem develops. Tracking personnel through medical surveillance allows the comparison of health variables over time in individual workers, which may facilitate early detection of a change in a laboratory value or health condition. Medical surveillance programs also look for trends in populations of workers. Examining grouped data compared with data from unexposed workers may reveal a small alteration or increase in the frequency of a health effect that would be obscured if individual workers' results alone were considered.

Medical surveillance evaluates the protection afforded by engineering controls, other administrative controls, safe work processes, PPE, and worker education about the hazards of the materials they work with in the course of their duties. The data-gathering elements of a medical surveillance program are used to establish a baseline of workers' health and then to monitor their future health for any changes that may result from exposure to HDs.

Elements of a medical surveillance program should be consistent with the entity's Human Resource policies and should include:

- Development of an organized approach to identify workers who are potentially exposed to HDs on the basis of their job duties
- Use of an 'entity-based' or contracted employee health service to perform the medical surveillance while protecting the confidentiality of the employees' personal medical information
- Initial baseline assessment (pre-placement) of a worker's health status and medical history. Data elements collected include a medical (including reproductive) history and work history to assess exposure to HDs, physical examination, and laboratory testing. Methods used to assess exposure history include a review of:
 - Records of HDs handled, with quantities and dosage forms
 - Number of HD preparations/administrations per week
 - Estimates of hours spent handling HDs per week and/or per month
 - Performance of a physical assessment and laboratory studies linked to target organs of commonly used HDs, such as a baseline complete blood count. Note that biological monitoring to determine blood or urine levels of specific HDs is not currently recommended in surveillance protocols, but may have a role in the follow-up of acute spills with a specific agent.
- Medical records of surveillance should be maintained according to OSHA regulation concerning access to employee exposure and medical records
- Monitoring workers' health prospectively through periodic surveillance using the elements of data gathering described above (updated health and exposure history, physical assessment, and laboratory measures, if appropriate)

- Monitoring of the data to identify prevention failure leading to health effects; this monitoring may occur in collaboration with the employee health service
- Development of a follow-up plan for workers who have shown health changes suggesting toxicity or who have experienced an acute exposure. This follow-up should include evaluation of current engineering and administrative controls and equipment to ensure that all systems are appropriately and accurately implemented (see *Follow-Up Plan* below).
- Completion of an exit examination when a worker's employment at the entity ends, to document the information on the employee's medical, reproductive, and exposure histories. Examination and laboratory evaluation should be guided by the individual's history of exposures and follow the outline of the periodic evaluation.

19.1 Follow-Up Plan

The occurrence of exposure-related health changes should prompt immediate re-evaluation of primary preventive measures (e.g., administrative and engineering controls, PPE, and others). In this manner, medical surveillance acts as a check on the effectiveness of controls already in use.

The entity should take the following actions:

- Perform a post-exposure examination tailored to the type of exposure (e.g., spills or needle sticks from syringes containing HDs). An assessment of the extent of exposure should be conducted and included in a confidential database and in an incident report. The physical examination should focus on the involved area as well as other organ systems commonly affected (i.e., the skin and mucous membranes for direct contact or inhalation; the pulmonary system for aerosolized HDs). Treatment and laboratory studies will follow as indicated and be guided by emergency protocols.
- Compare performance of controls with recommended standards; conduct environmental sampling when analytical methods are available.
- Verify and document that all controls are in proper operating condition.
- Verify and document that the worker complied with existing policies. Review policies for the use of PPE and employee compliance with PPE use and policies. Review availability of appropriate PPE (see *Personal Protective Equipment*).
- Develop and document a plan of action that will prevent additional exposure of workers.
- Ensure confidential, two-way communication between the worker and the employee health unit(s) regarding notification, discussions about a change in health condition, or detection of an adverse health effect.
- Provide and document a follow-up medical survey to demonstrate that the plan implemented is effective.
- Ensure that any exposed worker receives confidential notification of any adverse health effect. Offer alternative duty or temporary reassignment.
- Provide ongoing medical surveillance of all workers at risk for exposure to HDs to determine whether the plan implemented is effective.

APPENDIX A: ACRONYMS AND DEFINITIONS

Acronyms

ACPH	Air changes per hour
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API	Active pharmaceutical ingredient
ASTM	American Society for Testing and Materials
BSC	Biological safety cabinet
BUD	Beyond-use date
CACI	Compounding aseptic containment isolator
CAI	Compounding aseptic isolator
CDC	Centers for Disease Control and Prevention
C-PEC	Containment primary engineering control
C-SCA	Containment segregated compounding area
C-SEC	Containment secondary engineering control
CSP	Compounded sterile preparation
CSTD	Closed-system drug-transfer device
CVE	Containment ventilated enclosure
EPA	Environmental Protection Agency
GHS	Globally Harmonized System of Classification and Labeling of Chemicals
HD	Hazardous drug
HEPA	High-efficiency particulate air
IV	Intravenous
LAFW	Laminar airflow workbench
NIOSH	National Institute for Occupational Safety and Health
ONS	Oncology Nursing Society
OSHA	Occupational Safety and Health Administration
PPE	Personal protective equipment
SDS	Safety Data Sheet
SOP	Standard operating procedure
ULPA	Ultra-low particulate air

Definitions

Active pharmaceutical ingredient (API): Any substance or mixture of substances intended to be used in the compounding of a drug preparation, thereby becoming the active ingredient in that preparation and furnishing pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease in humans and animals or affecting the structure and function of the body.

Alternative duty: Performance of other tasks that do not include the direct handling of HDs.

Assessment of risk: Evaluation of risk to determine alternative containment strategies and/or work practices.

Beyond-use date (BUD): The date or time after which a compounded preparation must not be used, stored, or transported (see $\langle 795 \rangle$ and $\langle 797 \rangle$).

Biological safety cabinet (BSC): A ventilated cabinet often used for preparation of hazardous drugs. These cabinets are divided into three general classes (Class I, Class II, and Class III). Class II BSCs are further divided into types (Type A1, Type A2, Type B1, and Type B2). See *Appendix C* for details.

Buffer room: Part of the HD compounding area under negative pressure where the C-PEC is physically located. Activities that occur in this area are limited to the preparation and staging

of components and supplies used when compounding HDs.

Chemotherapy glove: A medical glove that meets the ASTM Standard Practice for Assessment of Resistance of Medical Gloves to Permeation by Chemotherapy Drugs (D6978) or its successor.

Cleaning: The removal of soil (e.g., organic and inorganic material) from objects and surfaces, normally accomplished by manually or mechanically using water with detergents or enzymatic products.

Closed-system drug-transfer device (CSTD): A drug transfer device that mechanically prohibits the transfer of environmental contaminants into the system and the escape of HD or vapor concentrations outside the system.

Compounded preparation: A nonsterile or sterile drug or nutrient preparation that is compounded in a licensed pharmacy or other healthcare-related facility in response to or anticipation of a prescription or a medication order from a licensed prescriber.

Compounding aseptic containment isolator (CACI): A specific type of CAI that is designed for the compounding of sterile HDs. The CACI is designed to provide worker protection from exposure to undesirable levels of airborne drugs throughout the compounding and material transfer processes and to provide an aseptic environment with unidirectional airflow for compounding sterile preparations.

Compounding aseptic isolator (CAI): An isolator specifically designed for compounding sterile, non-hazardous pharmaceutical ingredients or preparations. The CAI is designed to maintain an aseptic compounding environment within the isolator throughout the compounding and material transfer processes.

Compounding personnel: Individuals who participate in the compounding process.

Compounding supervisor: Individual(s) responsible for developing and implementing appropriate procedures; overseeing facility compliance with this chapter and other applicable laws, regulations, and standards; ensuring the competency of personnel; and maintaining environmental control of the compounding areas.

Containment primary engineering control (C-PEC): A ventilated device designed and operated to minimize worker and environmental exposures to HDs by controlling emissions of airborne contaminants through the following:

- The full or partial enclosure of a potential contaminant source
- The use of airflow capture velocities to trap and remove airborne contaminants near their point of generation
- The use of air pressure relationships that define the direction of airflow into the cabinet
- The use of HEPA filtration on all potentially contaminated exhaust streams

Examples of C-PECs include Class I, II, or III BSCs, CACIs, and CVE (e.g., powder hood). C-PECs used for nonsterile compounding do not need to have ISO Class 5 air quality, whereas C-PECs used for sterile compounding must have ISO Class 5 air quality (see *Table 2* and *3*).

Containment secondary engineering control (C-SEC): The C-SEC is the room in which the C-PEC is placed. It incorporates specific design and operational parameters required to contain the potential hazard within the compounding room.

Containment segregated compounding area (C-SCA): A type of C-SEC with nominal requirements for airflow and room pressurization as they pertain to HD compounding.

Containment ventilated enclosure (CVE): A full or partial enclosure that uses ventilation principles to capture, contain, and remove airborne contaminants through HEPA filtration and prevent their release into the work environment.

Deactivation: Treatment of an HD contaminant on surfaces with a chemical, heat, ultraviolet

light, or another agent to transform the HD into a less hazardous agent.

Decontamination: Inactivation, neutralization, or removal of HD contaminants on surfaces, usually by chemical means.

Disinfectant: A chemical agent that destroys or inhibits the growth of microorganisms.

Engineering control: Primary, secondary, and supplemental devices designed to eliminate or reduce worker exposure to a chemical, biological, radiological, ergonomic, or physical hazard, and in the case of CSPs, to protect the compounded preparation from environmental contamination.

Entity: Pharmacy, hospital, physician's office, clinic, veterinary office, or other location where HDs are received, stored, prepared, dispensed, administered, and distributed.

EPA-registered disinfectant: Antimicrobial products registered with the Environmental Protection Agency (EPA) for healthcare use against pathogens specified in the product labeling.

Externally vented: Exhausted to the outside

Globally Harmonized System of Classification and Labeling of Chemicals (GHS): A system for standardizing and harmonizing the classification and labeling of chemicals.

Goggles: Tight-fitting eye protection that completely covers the eyes, eye sockets, and facial area that immediately surrounds the eyes. Goggles provide protection from impact, dust, and splashes. Some goggles fit over corrective lenses.

Hazardous drug (HD): Any drug identified as hazardous or potentially hazardous on the basis of at least one of the following six criteria:

- Carcinogenicity
- Teratogenicity or developmental toxicity
- Reproductive toxicity in humans
- Organ toxicity at low doses in humans or animals
- Genotoxicity
- New drugs that mimic existing HDs in structure or toxicity

High-efficiency particulate air (HEPA) filtration: An extended-medium, dry-type filter in a rigid frame, having a minimum particle collection efficiency of 99.97% for particles with a mass median diameter of 0.3 μm when tested at a rated airflow in accordance with MIL STD 282 using IEST Recommended Standard RP-CC001.5.

Negative-pressure room: A room that is maintained at a lower pressure than the adjacent spaces; therefore the net flow of air is into the room.

Pass-through: An enclosure with interlocking doors that is positioned between two spaces for the purpose of reducing particulate transfer while moving materials from one space to another. A pass-through serving negative-pressure rooms needs to be equipped with sealed doors.

Personal protective equipment (PPE): Items such as gloves, gowns, respirators, goggles, faceshields, and others that protect individual workers from hazardous physical or chemical exposures.

Positive-pressure room: A room that is maintained at a higher pressure than the adjacent spaces; therefore, the net flow of air is out of the room.

Safety data sheet (SDS): An informational document that provides written or printed material concerning a hazardous chemical. The SDS is prepared in accordance with the HCS [previously known as a Material Safety Data Sheet (MSDS)].

Spill kit: A container of supplies, warning signage, and related materials used to contain the spill of an HD.

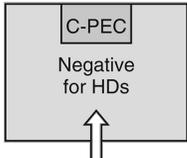
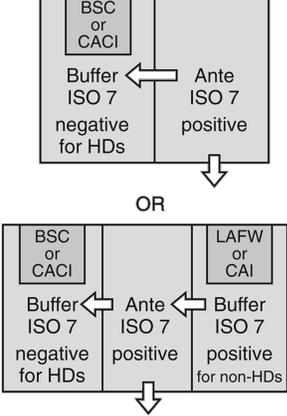
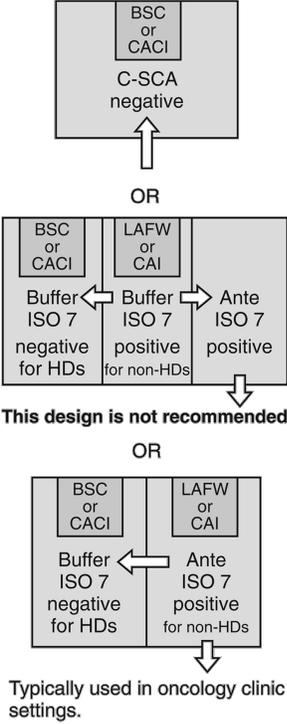
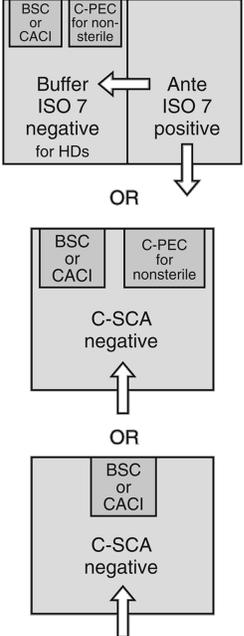
Standard operating procedure (SOP): Written procedures describing operations, testing, sampling, interpretation of results, and corrective actions that relate to the operations that are taking place.

Supplemental engineering control: An adjunct control (e.g., CSTD) used concurrently with primary and secondary engineering controls. Supplemental engineering controls offer additional levels of protection and may facilitate enhanced occupational protection, especially when handling HDs outside of primary and secondary engineering controls (e.g., during administering).

Trace chemotherapy waste: Components such as vials, ampules, IV bags, and others that once held antineoplastic agents.

Trace contaminated waste: Items used in the handling, compounding, dispensing, administration, or disposal of antineoplastic agents that are not overtly contaminated (e.g., gowns, gloves, goggles, wipes).

APPENDIX B: EXAMPLES OF DESIGNS FOR HAZARDOUS DRUGS COMPOUNDING AREAS^a

Use	Optimal Primary and Secondary Control	Minimum ACPH	Limitations Primary and Secondary Control	Minimum ACPH	Notes for limitations
Nonsterile HD compounding		12			
Sterile HD compounding		30		12 30 30	<p>Maximum BUD as described in <797> for segregated compounding area.</p> <p>If this design is in place, measures must be taken to avoid contamination of the positive-pressure buffer room.</p> <p>Maximum BUD as described in <797>.</p>
Both sterile HD and nonsterile HD compounding	A separate room for sterile and nonsterile compounding is recommended			30 12 12	<p>For rooms used for both sterile and nonsterile compounding, particle-generating activity must not be performed when sterile compounding is in process. C-PECs must be at least 1 meter apart.</p> <p>Maximum BUD as described in <797> for segregated compounding area.</p> <p>Maximum BUD as described in <797> for segregated compounding area.</p>

^a The arrows indicate direction of airflow.

APPENDIX C: TYPES OF BIOLOGICAL SAFETY CABINETS

Class I: A BSC that protects personnel and the environment but does not protect the product/preparation. A minimum velocity of 75 linear feet/min of unfiltered room air is drawn through the front opening and across the work surface, providing personnel protection. The air is then passed through a HEPA/ULPA (ultra-low particulate air) filter, either into the room or to the outside in the exhaust plenum, providing environmental protection.

Class II: Class II (Types A1, A2, B1, and B2) BSCs are partial barrier systems that rely on the movement of air to provide personnel, environmental, and product/preparation protection. Personnel and product/preparation protection are provided by the combination of inward and downward airflow captured by the front grille of the cabinet. Side-to-side cross-contamination of products/preparations is minimized by the internal downward flow of HEPA/ULPA filtered air moving toward the work surface and then drawn into the front and rear intake grilles. Environmental protection is provided when the cabinet exhaust air is passed through a HEPA/ULPA filter.

Type A1 (formerly, Type A): These Class II BSCs maintain a minimum inflow velocity of 75 feet/min; have HEPA-filtered, down-flow air that is a portion of the mixed down-flow and inflow air from a common plenum; may exhaust HEPA-filtered air back into the laboratory or to the environment through an exhaust canopy; and may have positive-pressure contaminated ducts and plenums that are not surrounded by negative-pressure plenums. Type A1 BSCs are not suitable for use with volatile toxic chemicals and volatile radionucleotides.

Type A2 (formerly, Type B3): These Class II BSCs maintain a minimum inflow velocity of 100 feet/min; have HEPA-filtered, down-flow air that is a portion of the mixed down-flow and inflow air from a common exhaust plenum; may exhaust HEPA-filtered air back into the laboratory or to the environment through an exhaust canopy; and have all contaminated ducts and plenums under negative pressure or surrounded by negative-pressure ducts and plenums. If these cabinets are used for minute quantities of volatile toxic chemicals and trace amounts of radionucleotides, they must be exhausted through properly functioning exhaust canopies.

Type B1: These Class II BSCs maintain a minimum inflow velocity of 100 feet/min; have HEPA-filtered, down-flow air composed largely of uncontaminated, recirculated inflow air; exhaust most of the contaminated down-flow air through a dedicated duct exhausted to the atmosphere after passing it through a HEPA filter; and have all contaminated ducts and plenums under negative pressure or surrounded by negative-pressure ducts and plenums. If these cabinets are used for work involving minute quantities of volatile toxic chemicals and trace amounts of radionucleotides, the work must be done in the directly exhausted portion of the cabinet.

Type B2 (total exhaust): These Class II BSCs maintain a minimum inflow velocity of 100 feet/min; have HEPA-filtered, down-flow air drawn from the laboratory or the outside; exhaust all inflow and down-flow air to the atmosphere after filtration through a HEPA filter without recirculation inside the cabinet or return to the laboratory; and have all contaminated ducts and plenums under negative pressure or surrounded by directly exhausted negative-pressure ducts and plenums. These cabinets may be used with volatile toxic chemicals and radionucleotides.

Class III: The Class III BSC is designed for work with highly infectious microbiological agents and other hazardous operations. It provides maximum protection for the environment and the

worker. It is a gas-tight enclosure with a viewing window that is secured with locks and/or requires the use of tools to open. Both supply and exhaust air are HEPA/ULPA filtered. Exhaust air must pass through two HEPA/ULPA filters in series before discharge to the outdoors.

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BRIEFING

〈 1050.1 〉 **Design, Evaluation, and Characterization of Viral Clearance Procedures.**

This proposed new general information chapter expands upon general information chapter *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*

〈 1050 〉. In 1999, 〈 1050 〉 was adopted, essentially unchanged, from the International Conference on Harmonization Q5A *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* (ICH Q5A). Since then, knowledge and technologies regarding viral clearance have improved. A revision of 〈 1050 〉 was therefore proposed in PF 36(3) [May-June 2010], but comments on the proposed revision, especially with regard to deviations from and additions to the original ICH language, prompted the USP General Chapters—Biological Analysis Expert Committee to decide that chapter 〈 1050 〉 should be left intact and the core content of the potential revision should be proposed as a separate companion chapter. This new chapter, *Design, Evaluation, and Characterization of Viral Clearance Procedures* 〈 1050.1 〉 provides current best practices for, and examples of, virus selection, critical process challenges, sampling, assay qualification, and additional aspects of viral clearance studies. Viral clearance studies are necessary for demonstrating product safety before human exposure, and thus should follow the principles of ICH Q5A. Although ICH Q5A suggests general approaches for evaluation of viral clearance in manufacturing processes, it lacks sufficient content on experimental methodology, and therefore this new chapter is

proposed to address this need. In addition, although the scope of this new chapter differs somewhat from that proposed in *PF 36(3)*, where possible this new chapter also addresses the public comments received for that revision proposal.

(GCBA: M. Kibbey.)

Correspondence Number—C149666

Comment deadline: May 31, 2015

Add the following:

■ 〈 1050.1 〉 DESIGN, EVALUATION, AND CHARACTERIZATION OF VIRAL CLEARANCE PROCEDURES

INTRODUCTION

Chapter 〈 1050.1 〉 is a companion document to general information chapter *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* 〈 1050 〉, which was adapted, essentially unchanged, from the International Conference on Harmonization Q5A. Chapter 〈 1050.1 〉 provides users with practical guidance regarding the design, evaluation, and characterization of viral clearance procedures. The chapter scope is the same as that described in chapter 〈 1050 〉 and covers biotechnology products for human use that are derived from cell lines of human or animal origin. Viral clearance studies performed according to the principles outlined in this chapter will provide meaningful data about the ability of the overall production and purification processes to remove or inactivate a broad spectrum of viral types that may affect the safety of biotechnology-derived products. [Note—The *Appendix* contains definitions of terms used in this chapter that are not already defined in chapter 〈 1050 〉.]

The regulations for licensing biotechnology products stipulate that cell banks, biologically derived raw materials, and bulk harvest must be controlled and tested for viral safety; however, many of the viral detection approaches that can be used have inherent limitations. In addition, certain detection methods may be so specific for a particular virus that they may fail to detect viral variants (e.g., noncytopathic strains, or non-laboratory-adapted wild-type strains, or mutated variants). Some newly emerging viruses may be missed. Finally, detection methods typically are limited by their sensitivities, and infectious viruses at a low titer may go undetected. Viral contamination of manufacturing processes can come from many sources, including cell substrates and raw materials of biological origin (such as cell culture supplements and other production raw materials), and contamination during production. In addition to the required viral safety testing of these materials, manufacturers must evaluate the effectiveness of downstream purification process steps, which together must remove or inactivate any viruses potentially present (see 〈 1050 〉).

As suggested in the U.S. Food and Drug Administration "Points to Consider" documents (*Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use* and *Draft Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals*), as well as chapter 〈 1050 〉, a multi-step approach is typically used to ensure viral safety. Clearance studies can generally be considered generic or modular. As described in the FDA's

Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use, a generic clearance study is one in which virus removal and inactivation is demonstrated for several steps in the purification process of a model monoclonal antibody (mAb) in which the data may be extrapolated to other mAbs following the same purification and virus removal/inactivation scheme as the model mAb. A modular clearance study demonstrates virus removal or inactivation of individual steps during the purification process (column chromatography, filtration, pasteurization, solvent/detergent, low pH, and others) so that each module in the purification scheme may be studied independently of the other modules. If necessary, an alternative model mAb can be used to demonstrate viral clearance in different modules. Identical clearance modules can be extrapolated to the product mAb. This chapter complements these documents by providing users strategies to perform and assess viral clearance.

GOALS AND PRINCIPLES OF VIRAL CLEARANCE STUDIES

A viral clearance study should evaluate the ability of the overall purification process to remove or inactivate a broad spectrum of virus types, including viruses that are known to contaminate or have the potential to contaminate the raw materials, and those that can be introduced during manufacturing. Viral clearance studies typically involve substudies performed on specific and suitable individual steps of the manufacturing and purification process. The studies should be performed in a manner that generates quantitative data, allowing for identification of effective clearance steps and estimation of viral reduction factors (VRFs, also known as log reduction factors, LRF). The VRFs of an individual clearance step represent the ratio of the viral load in the pretreatment material (used to challenge the clearance step) to the viral load in the post-treatment material. The VRFs derived from specific process steps are used to evaluate the overall capacity of the entire production process to remove or inactivate process-specific or nonspecific viruses.

One of the key goals of a manufacturing purification process is to achieve maximal viral clearance without compromising product quality. Critical attributes of strategic viral clearance steps in the manufacturing process must be characterized and validated. In the context of viral clearance, robustness has two main components: 1) the ability of process steps to remove or inactivate viruses under worst-case conditions or over a wide operational range for parameters (e.g., temperature, protein concentration, pressure, flow rate, conductivity, and pH), and 2) the ability of process steps to consistently remove or inactivate nonspecific viruses that possess a broad spectrum of physical and chemical characteristics (e.g., pH, heat, solvent/detergent treatment, or filtration). A validated process that provides robust viral clearance should establish the VRF achievable for a panel of "relevant" or "model" viruses (see next section for definitions). The validated process also should demonstrate robustness of dedicated inactivation or removal steps for which critical operational parameters that could affect viral clearance are well established. Therefore, a demonstration of viral clearance robustness provides confidence that the manufacturing process can remove or inactivate potential viral contaminants.

Viral clearance study protocols begin with an overall action plan and design of experiments.

Chapter **1050** (see *Section V* and *Table 4*) describes five major action plans (*Cases A–E*) and can serve as a guide. Cell substrates categorized in *Cases C, D, and E* represent special cases, and manufacturers who use these substrates should discuss study design with the applicable regulatory authorities. Unique issues for each production process must be considered

on a case-by-case basis. These issues may relate to the starting materials, production process, product, and intended use of the product. The overall action plan dictates the choice of viruses used in the viral clearance study, i.e., the study must include relevant, specific, and nonspecific model viruses. Further, the viral clearance study protocol may include the following elements: descriptions of the study facility and staff responsible for executing the study, the scaled-down purification models, justification of the appropriateness of the scaled-down model, and the study design. The study report may include these elements, as well as a stepwise analysis of calculated VRFs and the overall viral clearance capacity of the purification process. Viral clearance studies should be conducted in a well-documented, controlled manner that complies with current regulatory requirements.

CONSIDERATIONS FOR PERFORMANCE OF VIRAL CLEARANCE STUDIES

Selection of Viruses

The choice and number of viruses that may be used in a viral clearance study are dictated by the nature and origin of the production cell line, as well as the nature and origin of the animal-derived materials used in production and purification. In general, at least two viruses, one enveloped and one nonenveloped (preferably a parvovirus), are used in the early clinical phases of product development, but three or more viruses may be used to generate data for registration-enabling studies. At least two orthogonal virus removal/inactivation steps (steps with different mechanisms of clearance) should be evaluated. The reproducibility of an effective step should be assessed by performing at least two independent experiments, or reproducibility should be supported by relevant in-house data. Model viruses for process evaluation and process characterization studies should be similar to the virus that may contaminate the product; however, other viruses with a wide range of physical and chemical characteristics should also be examined. The latter is important for showing that the purification process is capable of inactivating or removing a wide variety of viruses, including newly emerging viruses (e.g., vesivirus 2117, circoviruses, or newly discovered animal and human parvoviruses) or unexpected contaminants (e.g., BVDV, MVM, epizootic hemorrhagic disease virus, and Cache valley virus). The manufacturer should justify the choice of viruses in accordance with the aims of the process evaluation and process characterization studies and the guidance provided in this document, as well as relevant regulatory guidelines.

Viruses used in clearance studies fall into three categories: relevant viruses, specific model viruses, and nonspecific model viruses. "Relevant viruses" are viruses used in process evaluation studies which are either the identified virus, or of the same species as viruses of the virus that is known, or likely to contaminate the cell substrate or any other reagents or materials used in the production process. When a relevant virus is not available or when it is not well adapted for this purpose (e.g., it cannot be grown in vitro to a sufficiently high titer or cannot be detected using cell-based viral titration endpoints required for assessing inactivation), a "specific model virus" can be used as a substitute. An appropriate specific model virus may be a virus that is closely related to the known or suspected virus (same genus or family), having similar physical and chemical properties to those of the observed or suspected virus. For example, cell lines derived from rodents usually contain endogenous retroviral particles or retroviral-like particles that can be infectious or noninfectious. The capacity of the manufacturing process to remove and/or inactivate rodent retroviruses present in products obtained from such cells should be determined. In the case of rodent-derived cells,

one can use a murine leukemia virus as a model virus. Manufacturers using human-derived B-lymphocyte cells immortalized by infection with Epstein-Barr virus should demonstrate the ability of the manufacturing process to clear a herpesvirus. In this instance, pseudorabies virus or another herpesvirus can be used as a specific model virus.

Finally, when the goal is to characterize the capacity of the manufacturing process to remove and/or inactivate viruses in general (i.e., to characterize the robustness of the clearance process), viral clearance studies can be performed with a panel of “nonspecific model viruses” that have a wide range of physical and chemical characteristics. Data obtained from studies with relevant or specific model viruses also can contribute to this assessment. It is not necessary to test all types of viruses; preference should be given to viruses with diverse physical and chemical properties that would be representative of a wide range of viral families. The results obtained for such viruses provide useful information about the ability of the production process to remove or inactivate viruses in general. *Table 1* gives examples of model viruses that represent a range of physicochemical properties for use in viral clearance studies, including those examples already described in *〈 1050 〉* (see *Table A-1*). Additional points to consider during viral selection are: 1) ability to grow the virus of interest to a sufficiently high titer; 2) ability to create stocks with minimal aggregates; 3) availability of a qualified/validated assay system for detection of the selected virus; 4) the selected viruses are not likely to pose a health hazard to personnel performing the study or the environment; and 5) potential to address new and emerging viruses.

Table 1. Examples of Viruses Used in Viral Clearance Studies for Biotechnology Products Derived from Cell Cultures

Virus	Family	Genus	Natural Host	Genome	Enveloped	Size (nm)	Shape	Resistance ^a
Adenovirus 5	Adeno	Mastadeno-virus	Human	DNA	No	70–90	Spherical	High
BVDV	Flavi	Pestivirus	Bovine	RNA	Yes	50–70	Pleomorphic/spherical	Low
Cache valley virus	Bunya	Bunyavirus	Bovine, ovine	RNA	Yes	80–120	Spherical	Low
Encephalomyocarditis virus (EMC)	Picorna	Cardiovirus	Mouse	RNA	No	25–30	Icosahedral	Medium
Feline calicivirus	Calici	Vesivirus	Feline	RNA	No	35–40	Icosahedral	High
Herpes simplex 1	Herpes	Alpha-herpesvirus	Human	DNA	Yes	120–200	Spherical	Medium
MuLV	Retro	Type C oncovirus	Mouse	RNA	Yes	80–110	Spherical	Low
Parainfluenza virus	Paramyxo	Paramyxovirus	Various	RNA	Yes	100–200	Pleomorphic/spherical	Low
Parvoviruses (MVM, PPV, CPV, BPV)	Parvo	Parvovirus	Murine, porcine, canine, bovine	DNA	No	18–26	Icosahedral	High
Poliovirus sabin type 1	Picorna	Enterovirus	Human	RNA	No	25–30	Icosahedral	Medium
Porcine circovirus (PCV)	Circo	Circovirus	Porcine	DNA	No	15–20	Icosahedral	High
Pseudorabies virus	Herpes	—	Swine	DNA	Yes	120–200	Spherical	Medium
Reovirus 3	Reo	Orthoreovirus	Various	RNA	No	60–80	Spherical	Medium
Simian virus 5	Paramyxo	Rubulavirus	Simian	RNA	Yes	150–300	Pleomorphic/spherical	Low
Sindbis virus	Toga	Alphavirus	Human	RNA	Yes	60–70	Spherical	Low
SV40	Papova	Polyomavirus	Monkey	DNA	No	40–50	Icosahedral	Very high
Vesicular stomatitis virus	Rhabdo	Vesiculovirus	Equine, bovine	RNA	Yes	70 × 150	Bullet	Low
West Nile virus	Flavi	Flavivirus	Avian	RNA	Yes	40–70	Pleomorphic/spherical	Medium

^a Resistance to physicochemical treatments based on studies of purification processes. Resistance is relative to the specific treatment, and it is used in the context of understanding the biology of the virus and the nature of the manufacturing process. Actual results will vary according to the treatment. These viruses are examples only, their use is not mandatory, and the table is not exhaustive.

Process Clearance Capability

The viral clearance capability of a process is typically expressed as the logarithmic value of the virus reduction factor evaluated by the entire process. Although there are no specific requirements for the clearance capability that a process must achieve, the nature and origin of the cell type, raw materials used in production, as well as other factors may influence the need for greater or lower levels of viral clearance. For example, the target retroviral clearance

capability for a CHO and NS0 cell manufacturing process could be 4–6 logs of total-process retroviral clearance above the level found in the maximum therapeutic dose, based on the retroviral-like particle titer in the production bioreactor material. Clearance capability for other types of viruses might include >4 logs of clearance for additional enveloped viruses and >4 logs of clearance for nonenveloped viruses. Specific clearance targets must be established and justified on a case-by-case basis. In general, for a single virus clearance step to be considered effective, 4 logs or more of clearance must be demonstrated with 95% confidence ($\alpha = 0.05$). In contrast, a clearance step demonstrating 1–3 logs of clearance with 95% confidence ($\alpha = 0.05$) is considered a supportive step. A step can be supportive for some viruses and effective for others. To avoid overestimating reduction capacity, the analyst should perform viral clearance studies under expected worst-case conditions for the parameters defined as critical for that step (e.g., pH, temperature, and flow rate). Sufficient numbers of downstream purification process steps should be identified and evaluated so that the required overall clearance target can be reached.

Downstream Processing Steps for Viral Clearance

Downstream processing steps should employ different (orthogonal) mechanisms of viral clearance because consideration of multiple steps of the same or similar clearance mechanisms may lead to overestimation of the overall viral reduction capability. In general, a purification process should include at least one robust viral inactivation step and one robust viral removal step. For every purification step assessed, the mechanism of viral clearance should be described as inactivation, removal, or a combination of both; if both, the primary clearance mechanism should be identified. For inactivation steps, clearance studies should be planned in such a way that they can determine the kinetics of viral inactivation. Ideally, a manufacturing process includes steps that are dedicated to viral clearance; that is, they are present in the production or purification process specifically for the removal or inactivation of viruses. Examples of dedicated steps include solvent and detergent treatment, low-pH inactivation, and virus-removal filtration.

Qualification and Scale-Down of Purification Steps

Viral clearance studies using infectious virus spikes are not conducted at production scale in the manufacturing suite because this could contaminate the suite with viruses. Such studies should be performed in a segregated facility equipped for virological work and staffed with personnel who have virology expertise and familiarity with the operation of a scaled-down purification process. Each scaled-down step of the purification process must be qualified, i.e., found to be comparable to the full-scale production process by all relevant, measurable criteria. Comparability should be demonstrated using representative raw materials in intermediates from production, and equipment with process parameters through appropriate scale-down principles. The outputs should be measured with the appropriate analytical methods and statistical analyses.

In many instances, the monitored parameters are the same as those analyzed during performance of the actual manufacturing steps. For chromatography, the following parameters should be representative of the respective clinical or commercial-scale manufacturing: column bed height, residence time, linear flow rate, chromatographic matrix, buffer composition (including pH, conductivity, and operating temperature), and product pool concentrations and composition. Scaled-down and manufacturing-scale chromatography systems should produce

similar elution profiles and step yields. Similar considerations apply for other types of procedures. Any unavoidable differences between scaled-down and manufacturing-scale procedures should be investigated to determine their potential influence on the viral clearance results.

Selection of Sampling Points

Sampling points for assessing viral inactivation should include the starting materials (virus-spiked process solutions and appropriate controls) as well as samples taken at several time points during the inactivation process. This approach allows the analyst to monitor the kinetics of viral inactivation. Sampling points for virus-removal steps, such as chromatography and filtration, should include the feed-stream process solution that will be applied to the step, as well as the resulting process pool that is then processed in the subsequent steps. Chromatographic fractions (e.g., flow-through, washes, pre-peak, peak, post-peak, strip) of the mainstream, as well as pre- and post-mainstream samples, typically are evaluated in studies that support registration-enabling studies. Analyzing such side fractions might also be helpful in cases where the mechanism of partitioning is not understood. Sampling points are discussed further in the section *Performance of Viral Spiking Studies*.

Selection of Assays for Viral Quantitation

Various types of viral quantitation assays can be used. Examples include infectivity assays such as tissue-culture infectious-dose assays and plaque quantitation assays. In addition, quantitative polymerase chain reaction (qPCR) assays detect and quantify the nucleic acids of both infectious and noninfectious viruses. These assays are described in *Virology Test Methods* { 1237 }, *Nucleic Acid-Based Techniques—Extraction, Detection, and Sequencing* { 1126 }, and *Nucleic Acid Based Techniques—Amplification* { 1127 }. When using nucleic acid-based techniques (NAT), it is important to assure that the virus spike and the spiked samples to be analyzed do not contain significant amounts of free viral nucleic acids. The amount of free nucleic acids can be reduced by nuclease pretreatment of samples. It is important to characterize the impact of nuclease pretreatment on the virus spike since it could have a negative impact on the virus spike. Quantitative/quantal infectivity assays should have adequate sensitivity and reproducibility, and should be performed with a sufficient number of replicates to ensure adequate statistical validity of the results. All assays should include appropriate system suitability controls. Virus titration assays used in support of viral clearance studies must be shown to be suitable for this purpose by means of qualification or validation.

Sample Matrix Effects on Viral Quantitation Assays

When a virus is cleared by inactivation or removal (e.g., filtration or column chromatography) methods, the analysts must perform preliminary cytotoxicity and viral interference analyses to determine if their viral quantitation assays are sensitive to matrix components present in their samples. These studies should be performed before the spiking studies to confirm that the results of the viral clearance spiking studies are actually due to viral clearance and not to other intrinsic or extrinsic factors yielding a false-negative result. For example, some process intermediates may be cytotoxic to the detector (indicator) cells or may interfere with virus detection by a particular assay system. The process solutions used in these studies should be representative of those from a full-scale good manufacturing practice (GMP) manufacturing run.

The dilution of the process sample solution that does not cause cytotoxicity or interference is determined and the process solution is diluted accordingly before storage or testing samples from that manufacturing step in the virus-spiking studies.

In a cytotoxicity evaluation, the cells used in the viral quantitation assay are exposed to a series of dilutions of the sample matrix solution for that process step. Then the cells are assessed for viability and changes in morphology that could interfere with evaluation of the indicator cells for viral cytopathic effects or plaques and hence could have an impact on assay performance. The dilution of the process sample solution that does not cause cytotoxicity is determined, and the process solution is diluted accordingly before storage or testing samples from that manufacturing step in the virus-spiking studies.

The study involves the following steps:

1. Perform serial dilutions (e.g., 10-fold, 5-fold, or 2-fold) of the virus inactivation or filtration load using appropriate cell culture media as the diluent.
2. Inoculate the different dilutions of virus inactivation or filtration load alone onto the indicator cells that will be used for each virus titration.
3. Include appropriate buffer or media and cell controls.
4. Identify the dilution of the virus inactivation load that does not exhibit measurable detector cell toxicity attributable to the load matrix.

Process solutions also can interfere with virus detection by the in vitro cell-based viral infectivity assay system, either by inactivating the virus itself or by altering the indicator cells in a way that delays or prevents occurrence of a productive viral infection. To assess a process solution for viral interference, analysts prepare dilutions of the process solution and spike them with a known quantity of virus. Each dilution is subsequently titrated in the quantitative/quantal viral infectivity assay. If the difference between the known titer and the titer determined for the spiked solution in the assay (the viral interference titer) differ by more than the predetermined assay variability (typically ± 0.5 log), then viral interference is suspected.

To assess the inactivation step load for viral interference, analysts should follow these steps:

1. Perform serial dilutions (e.g., 10-fold, 5-fold, or 2-fold) of the virus stock using appropriate cell culture media as the diluent. This will serve as the positive control for the virus titer.
2. Perform serial dilutions (e.g., 10-fold, 5-fold, or 2-fold) of the virus stock using the virus inactivation load as the diluent. [Note—Information from the cytotoxicity study will determine the design of this step and the next one.]
3. Inoculate the dilution series onto the indicator cells.
4. Include assay suitability controls (positive and negative) in the test.
5. Identify the dilution of the virus inactivation load that does not result in significant changes in virus stock titer compared to the positive control virus titer.

If qPCR assays are used to quantitate the virus, each process solution should be tested for interference and recovery with the nucleic acid extraction method used, as well as for interference with the qPCR reaction itself. Each process solution must be diluted to a level that does not cause interference. Although diluting a process solution to achieve acceptable assay performance is a commonly used strategy, this reduces the maximum demonstrable VRF for the

clearance step under evaluation.

Effects of Storing and Freezing Viral Clearance Samples

If process solutions are used within their established hold times, stability studies should be performed to better understand the effect on the clearance step of holding or storing the solutions, or on the virus (e.g., aggregation). For example, bacterial growth or precipitation of proteins in the solutions may occur, particularly after prolonged storage.

If the samples from the spiking study are stored frozen before virus quantitation, analysts should perform stability studies using the viral infectivity result as an endpoint. Ideally, this should be done before the viral spiking study by spiking dilutions of each process solution with a known amount of virus and then freezing each sample. After specified times in frozen storage, the samples are removed, and the titer of each sample dilution is determined. If the difference between the known titer and the titer determined from the spiked frozen solution is greater than the assay variability (typically ± 0.5 log), this indicates that viral infectivity has been compromised by frozen storage.

Qualification of Virus Stocks and Effects on Processing Steps

The virus stocks used in viral clearance studies are critical reagents. Each virus stock should have a traceable, certified source with full documentation of the controlled production procedures. Virus stocks should meet predetermined criteria for identity, purity (e.g., sterility, mycoplasma, and adventitious virus testing), infectious titer, stability at freezing temperatures, and minimal viral aggregates. Analysts should minimize the number of passages from the master or working viral bank in order to reduce the chance of mutation. Purity of virus preparations should be taken into account in cases when virus preparation impurities may influence the performance of a certain unit operation (e.g., virus filtration).

During qualification, analysts should obtain an initial virus titer for each virus stock. Ideally, analysts should independently assay viral stocks for titer on separate days using different passage numbers of the indicator culture cells that were used in the viral quantification assay. The resulting titers should agree within the predetermined variability of the assay (typically the expectation is ± 0.5 log), and if so, the analyst can average the titers to determine the certified titer of the stock.

Analysts should perform a mock spiking study before the true spiking study. This mock spiking study evaluates the effects of the spiking virus matrix on the process step that is under evaluation. Mock spiking studies are important for all steps that will be tested for viral clearance, but they are particularly important for those steps that will be evaluated for the clearance of virus from a stock that contains impurities or additives (e.g., stabilizing protein). Mock spiking studies are conducted by adding the viral suspension matrix (including all components except the virus) to the process solution at a virus-spike to feed-stream-solution (load) ratio of NMT 10% (v/v) and then executing the processing step. The mock-spiked step is monitored for expected performance, and if results are different than expected, then reducing the virus:load spiking ratio may be helpful. This adjusted virus-spike to feed-stream-solution (load) ratio would be used in performing the actual clearance study for the processing step under study.

Performance of Viral Spiking Studies

Viral clearance studies are usually conducted either at a site where both the spiking and viral quantitation assays are performed (e.g., a biosafety testing laboratory) or at two different sites: one where the spiking studies are done and a second where the samples are quantitated. Sponsors and analysts must gain knowledge and control factors that can influence each manipulation that is not part of the manufacturing process, for example, freezing and thawing (see *Effects of Storing and Freezing Viral Clearance Samples*) as well as shipping conditions. Before initiating a clearance study, analysts should prepare a well-documented study design that clearly defines the following: the steps that will be tested, the sampling plan for each step, sample identification, sample handling and storage, sample shipment if required, critical operating parameters, process scale, rationale for worst-case conditions (if they have been established), and the appropriate controls for each step. At a minimum, the samples tested should include those used to determine: 1) the spiking virus titer, 2) the spiking virus titer after freeze-thaw (if applicable), 3) the virus titer in the production solution before processing, and 4) the virus titer after processing. The analyst may need to collect and assay additional samples, depending on the step under test and the phase of product development. In a typical biomanufacturing process, viral clearance can be accomplished by virus inactivation (e.g., pH treatment, heat treatment, or solvent and detergent treatment) or by virus removal (e.g., filtration or column chromatography). *Figures 1A* and *1B* show examples of experimental designs for virus inactivation, *Figure 2* shows virus removal by filtration, and *Figure 3* shows virus removal by column chromatography. These examples are intended for general reference and do not represent every specific condition. In developing their study designs, sponsors should consider their own process conditions in order to plan their own specific steps. The overall concept of viral clearance procedures can be expressed as follows:

Viral Clearance = Viral Inactivation + Viral Removal, where
Viral Removal = Removal by Filtration + Removal by Column Chromatography

VIRAL CLEARANCE BY VIRUS INACTIVATION

General considerations: Analysts should recognize that virus inactivation is not always a simple, first-order reaction. Typically, virus inactivation is complex and involves a fast initial phase followed by a slower phase. Thus, analysts should plan the inactivation process in such a way that samples are taken at different times in order to construct an inactivation time curve. Samples collected for inactivation studies should include the planned process time, and at a minimum, time zero; a time point or a suitable number of greater than zero but less than the minimum inactivating-agent exposure time; a time point equal to the minimum inactivating-agent exposure time; and in addition a time point beyond the minimum exposure time might be helpful in cases where there is a slow inactivation curve. Additional time points may be particularly important when there is no prior experience with virus inactivation kinetics. When viral clearance by inactivation occurs, two scenarios generally are possible: 1) the inactivating agent is not present in the load material and inactivation is initiated by addition of the inactivating agent after virus spiking (*Figure 1A*); and 2) the load material already contains the inactivating agent before the virus spike, and inactivation is initiated by addition of the virus spike (*Figure 1B*). An example of the latter is a Protein A column eluate at low pH. Whenever possible, analysts should determine the initial virus load from the virus-spiked load material before they add an inactivating agent. However, this is not possible when the inactivating agent is already present in the load material at the start of the experiment. In these situations, the initial virus load can be calculated using the certified titer value of the spiking virus preparation and the virus:load spiking ratio. When viral inactivation occurs too

rapidly to allow for plotting an inactivation time curve under normal process conditions, analysts should include appropriate controls to show that activity is indeed lost due to the inactivating agent present.

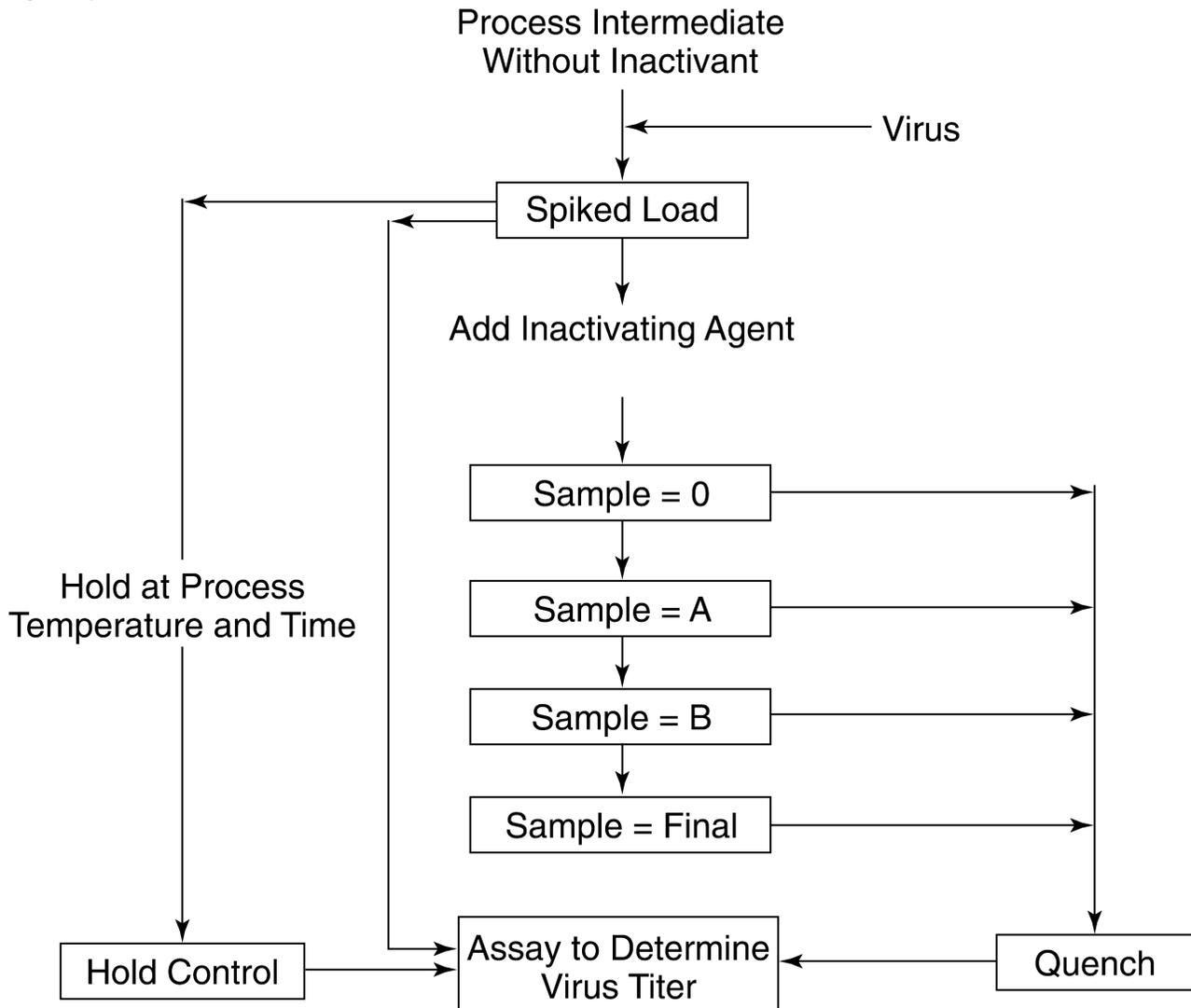


Figure 1A. Virus inactivation when load material does not contain an inactivating agent; the inactivating agent is added after viral spiking. Sampling points are indicated by "T"s in the text boxes in this figure and all subsequent figures.

Study design: The general approach to the study design for assessing viral clearance by inactivation is outlined in *Figures 1A* and *1B*. In *Figure 1A*, the virus spike is added to the load material (process intermediate) before initiation of inactivation. The virus and load material are mixed well, and the spiked load and hold control samples are pulled. The spiked load should be tested immediately to determine virus titer. The actual titer of the spiked load (obtained experimentally) should be compared to the theoretical titer of the spiked load (obtained from the certified titer of the lot of virus used and the spiking ratio) to verify performance of virus detection assay and that the appropriate amount of virus was added to the load material. The hold control should be treated the same as the spiked load (i.e., prior to neutralization of the hold control, the sample should be pH adjusted in the same manner as the spiked load and then neutralized and spiked with virus). In addition, the hold control should remain at process temperature until the final sample in the experiment is pulled. The viral titer of the hold control

then is assessed and compared to the actual titer of the spiked load to determine if any virus was inactivated in the presence of the load material over time and at the temperature of the inactivation study. This may be considered when determining the final VRF.

Inactivation should be initiated in a way that mimics the manufacturing process (e.g., with constant mixing) so that the inactivating agent (e.g., detergent, or acid utilized to lower pH) is mixed homogeneously into the solution as soon as possible. This minimizes the presence of localized high concentrations of the inactivating agent. If heat is used for inactivation, the heat should be applied in a way that mimics manufacturing (e.g., the ramp to target temperature should be identical) and controls should be kept unheated. However, the exact reproduction of addition (mixing) of inactivating agents or reproduction of a heating ramp can be very difficult at down-scale. In such cases it is advised to spike the virus directly into the inactivation agent-containing (or heated) material and to follow inactivation kinetics as described in *Figure 1B*. For control of the added virus, virus is spiked into process material without the inactivating agent or into unheated material.

Worst-case process conditions (e.g., pH, time, and temperature) should be applied if they have been established. Time points (samples) are pulled and the reaction is immediately quenched (e.g., by neutralization, dilution, or immediate cooling of the sample) so that further viral inactivation cannot occur. This allows the analyst to determine the kinetics of virus inactivation and then construct a virus inactivation versus time curve. Large-volume sampling can be applied to maximize clearance values.

In *Figure 1B*, the load material contains the inactivating agent and thus the addition of the virus spike starts the inactivation. In this scenario, it may not be possible to neutralize the process intermediate solution before the virus-spiking study because large fluctuations in pH may cause the protein product to fall out of solution. Also, the spiked load and hold control samples cannot be pulled from the virus-spiked load material, thus the control samples are pulled from a media or buffer control that does not contain the inactivating agent.

Media/Buffer Control
(Without Inactivating Agent)

Process Intermediate
(Contains Inactivating Agent)

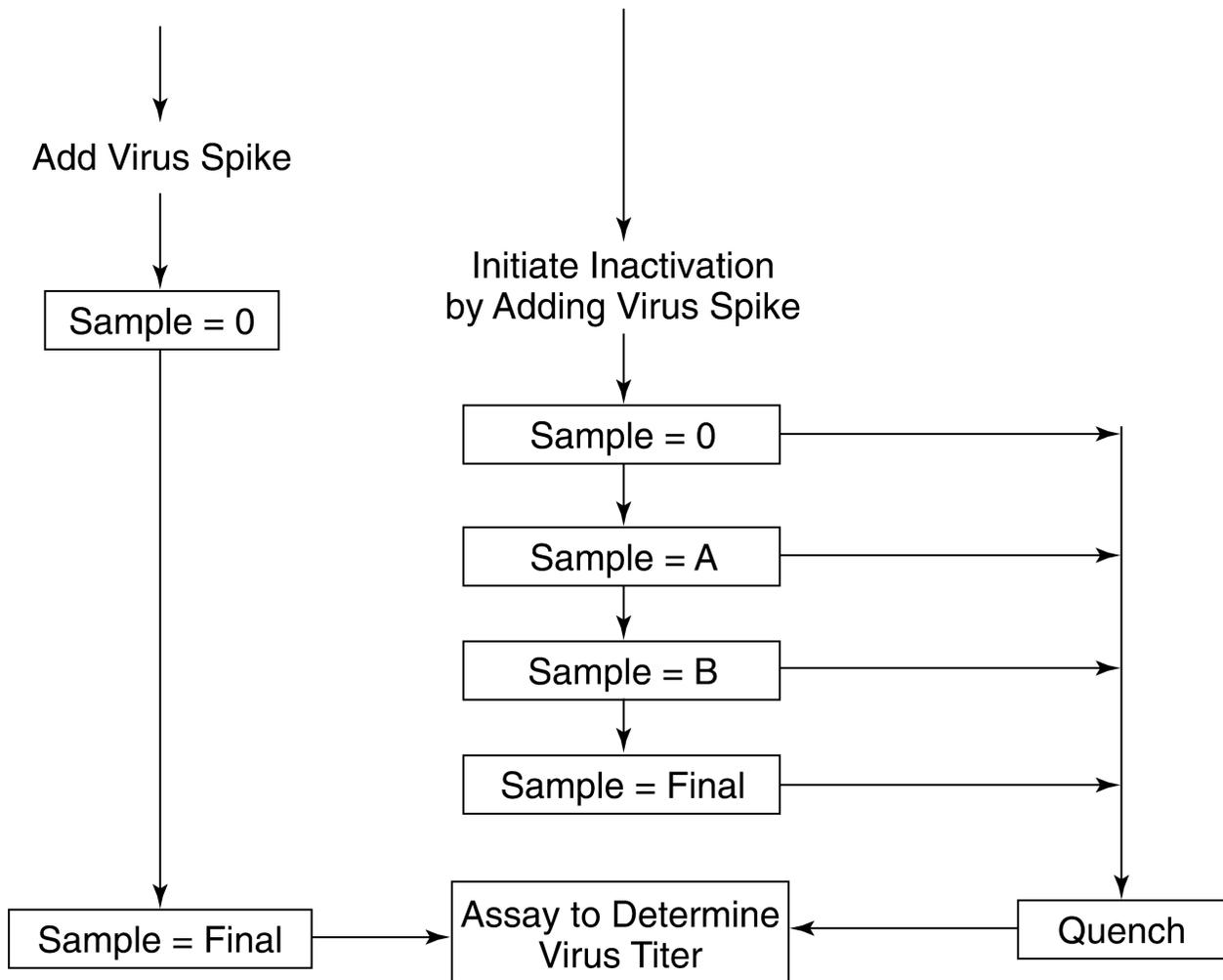


Figure 1B. Virus inactivation when the load material contains the inactivating agent before the virus spike; the addition of virus spike starts inactivation.

VIRAL CLEARANCE BY FILTRATION

Analysts must perform preliminary cytotoxicity and viral interference analyses when preparing to perform viral clearance by filtration (see *Sample Matrix Effects on Viral Quantitation Assays*). The viral filter load and pool dilution that will be used in the clearance study is that dilution shown not to cause cytotoxicity or interference.

Study design: The general approach to the study design for assessing viral clearance by filtration is outlined in *Figure 2*. Once the process intermediate is spiked with virus, the spiked load sample should be assayed immediately for virus titer. Virus spiking can be associated with aggregation effects. The aggregation status of a contaminating virus in the respective product intermediate is difficult to predict and therefore, as a worst-case approach, a mono-disperse virus preparation is advised for spiking. Filtration of virus before spiking (e.g. 0.45 µm or 0.22 µm) reduces larger virus aggregates from frozen virus stocks. A pure high-titer virus preparation can be helpful to reduce virus spike-induced effects influencing performance of virus filtration. The hold control sample should be maintained at processing conditions (temperature and time) and then assayed to evaluate the impact of these conditions, as well as the process

intermediate components, on virus titer. Worst-case process conditions should be applied, if they have been established. The virus titers obtained from the spiked load and hold control should be within the experimental variability of the virus titration assay, and if so, the analyst can average these values to obtain the initial virus load for use in calculating log reduction value. If there is a negative impact on virus titer because of the processing conditions (temperature, time, and process intermediate composition), then an investigation should begin to understand the process. If warranted, the final VRF may need to be adjusted to account for the impact of hold conditions on viral preparations. However, this approach might underestimate the removal capacity from virus filtration. Alternatively, use of another detection method (e.g., NAT) or another, more stable, model virus can be discussed.

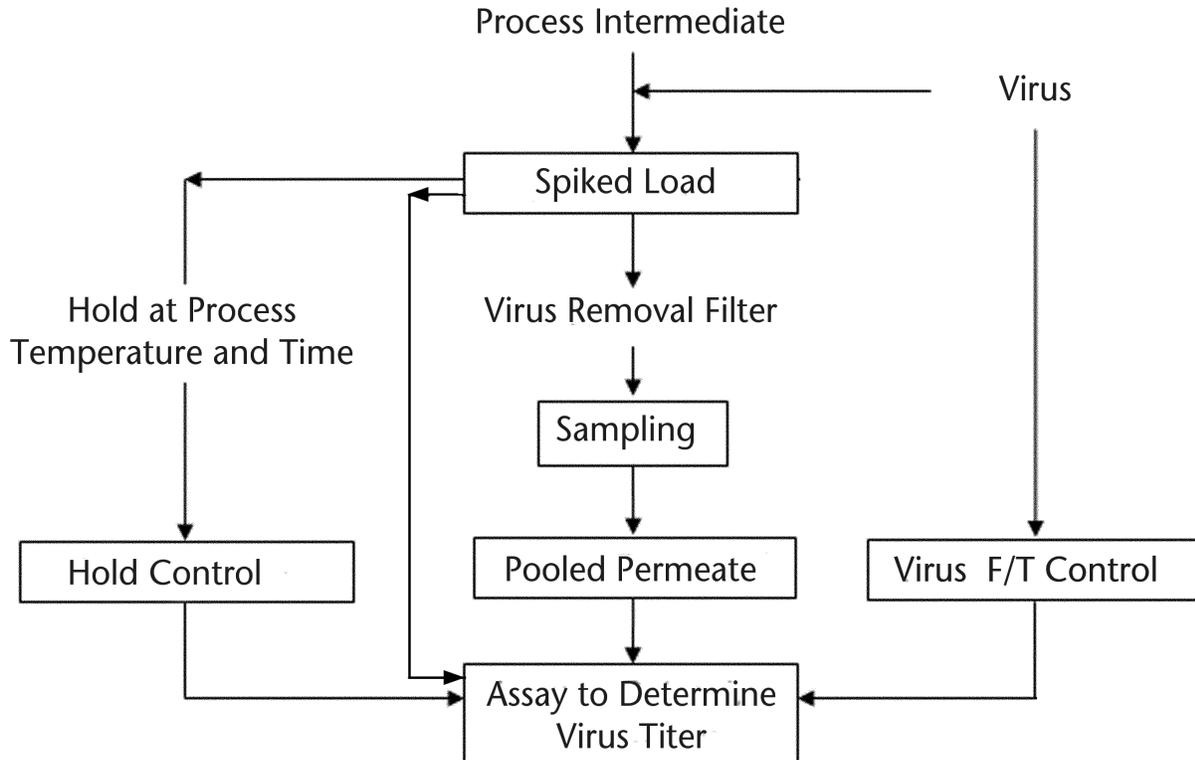


Figure 2. Clearance of virus by filtration methods.

Appropriate pre- and post-filtration controls should be used to assess removal of the virus spike by the prefiltration step. Samples are evaluated using virus titration assays. Test samples may require neutralization to appropriate pH levels for cell-culture-based titration assays. If a 0.22- μm or 0.45- μm prefilter is used during production in line with the virus-reduction filter, spiking could be performed on the intermediate before prefiltration. However, a sample should be taken from the stage after prefiltration in order to determine the VRF. Alternatively, the virus spike can be added to the intermediate after this prefiltration (using a similarly filtered virus spike) in order to determine virus reduction caused by the virus filter. To gain additional understanding of the virus clearance process, analysts can take samples at various time points throughout the run. In the event that samples are stored frozen prior to virus quantitation, a virus freeze/thaw (F/T) control is suggested to determine if there is any impact of the F/T cycle on virus titer (see also *Effects of Storing and Freezing Viral Clearance Samples*).

VIRAL CLEARANCE BY COLUMN CHROMATOGRAPHY

General considerations: For registration-enabling studies, analysts should investigate the distribution of the virus load among different chromatography fractions. Generally, analysts add virus to the starting intermediate (feed stream or load) for each step tested and determine the virus titer of the spiked load material and product pool spiked load material before and after the step. Fractions, in addition to the main product pool, may require testing at the later stages of product development (phase 3 trials and beyond). As is usual for viral clearance, these studies should be performed in duplicate. With column chromatography, the ability of the columns as well as other devices to clear virus may increase or decrease after repeated use. Evidence of constant viral clearance after multiple uses may provide support for repeated use of such columns. Typically, this evidence is obtained when analysts compare clearance on new resin versus resin that has been cycled to or slightly beyond the targeted column lifetime, i.e., the number of times the column will be used in commercial manufacturing. For registration-enabling studies, data should also be provided to show that any virus potentially retained by the resin (carry-over) will be adequately inactivated or removed by cleaning procedures before reuse of the column.

For each manufacturing step assessed for viral clearance, the probable mechanism of reduction in viral infectivity should be known and described as virus inactivation or virus removal, or both. In some situations, it may be necessary to distinguish between removal and inactivation. For example, a column chromatography step that physically separates virus from product also may use a buffer capable of inactivating virus. In such situations, it may be possible to combine the use of viral infectivity assays with NAT assays to measure the individual contributions of the inactivation and removal mechanisms. Dissection of each step to determine the relative contribution of each mechanism of clearance allows for a thorough understanding of how viral clearance is achieved. This understanding may help to identify critical variables in each clearance step that should be controlled to support reproducible clearance. The testing of each critical variable (when it is well understood) at worst-case conditions helps to evaluate the process robustness of the step.

To measure virus clearance by a column chromatography system, analysts must perform preliminary cytotoxicity and viral interference analyses (see *Sample Matrix Effects on Viral Quantitation Assays* for details). The column load dilution that will be used in the clearance study is that dilution shown not to cause cytotoxicity or interference. [Note—Interference and cytotoxicity studies also can be performed on collected fractions, if warranted.]

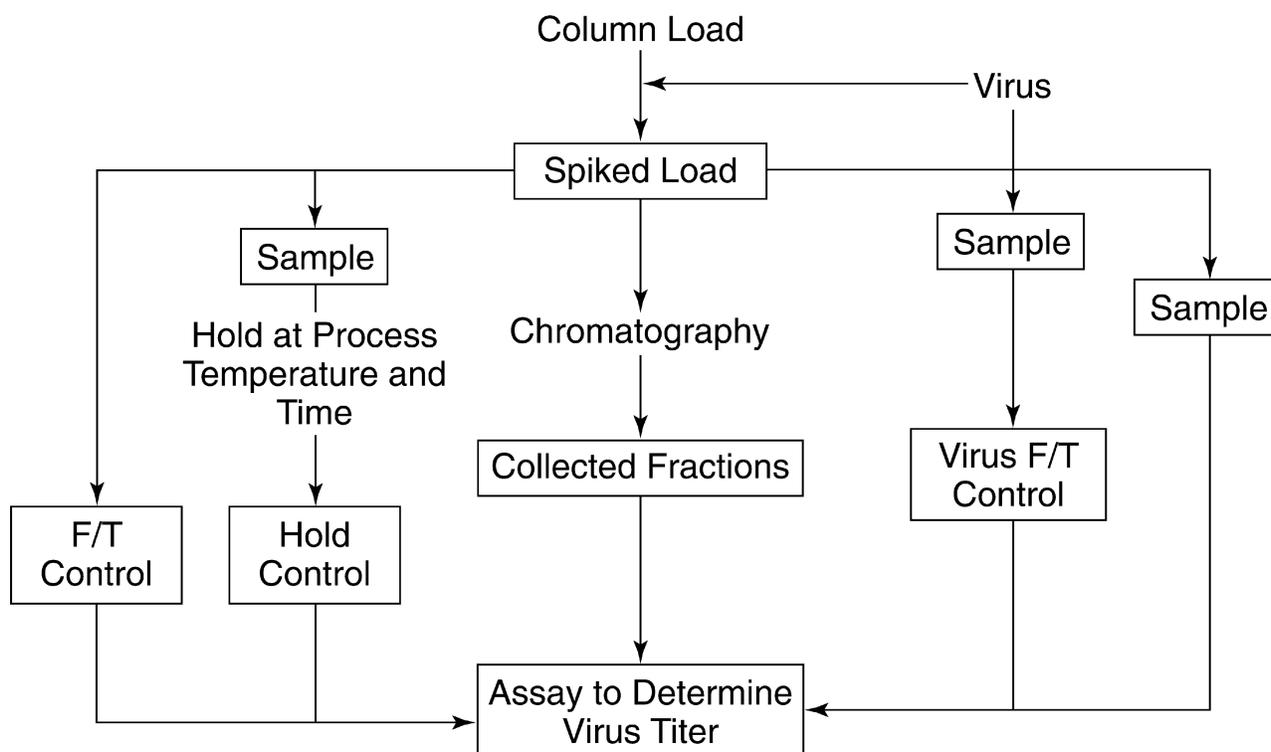


Figure 3. Clearance of virus by a column chromatography system.

Study design: *Figure 3* shows the general approach to study design for assessing viral clearance by chromatography. The column load is the material applied to the chromatography column. The load material in small-scale viral clearance studies should be comparable to material from clinical or commercial manufacturing in terms of composition and physicochemical attributes. The virus stock is a freshly thawed virus material of predetermined infectivity titer that is used to perform virus spiking of the column load material and also is used in the interference study. The virus stock can be sampled to determine the actual virus titer immediately after thaw. The spiked load control should be tested immediately for virus titer, and additional samples should be tested as soon as possible or frozen. The titer of the frozen sample and other samples will be determined at the end of the chromatography step run. In certain cases, filtration of the spiked load with a 0.22- μm or 0.45- μm prefilter may be necessary to remove any viral aggregates formed when the process intermediate was spiked with the model virus.

The chromatography step experiment for viral clearance should closely mimic the actual manufacturing process step parameters. It should be performed under worst-case conditions (e.g., flow, protein concentration, and elution conditions) if they have been established. The product pool or multiple fractions can be collected, and the virus titer can be determined in each collected fraction (such as flow-through, washes, pre-peak, peak, post-peak, strip, and others). The titers of virus in all the fractions that comprise the product pool are used to calculate the clearance factor of the step. Interference and cytotoxicity studies also can be performed on collected fractions, if warranted. For the duration of the step, the hold control (the load plus virus) should be kept at the temperature at which the chromatography is performed. At the end of the chromatography run, the hold control should be titrated along with other collected samples/fractions (these can be titrated immediately, or if not feasible, can be frozen and tested at a later time). The titer of the hold control or an average of the titers of hold control and spiked load (if within assay variability) should be used as the starting

titer in the viral clearance calculation. Samples are evaluated using virus titration assays. Test samples may require neutralization to appropriate pH levels for cell-culture-based titration assays.

GLOSSARY

(See also *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* { 1050 }, *Glossary*.)¹

Process Robustness of Viral Clearance: Ability of the downstream process or a single-unit operation to exhibit the characteristic performance and effectiveness in clearing viruses after minor or moderate changes of standard operating parameters or conditions.

Process Solutions: Production intermediates that are obtained for viral clearance studies. They should be obtained from a full-scale production facility or from a scaled-down process that has been shown to be representative of full-scale production.

Production Process: The manufacturing processes for biotechnology products are often varied and complex, and they are typically divided into upstream and downstream processing. Upstream activities produce the protein of interest, usually by cell culture or fermentation, and considerations include integrity and quality of the process, cell banks, expression systems, cultivation, media, process/product purity, impurities, and contaminants. Downstream processing involves separation and purification of the bulk bioproduct to make it suitable for its end use, e.g., purification, sterilization, and final formulation. Downstream activities include filtration, centrifugation, precipitation, numerous chromatographic separations, sterilization by terminal filtration, or lyophilization.

Purification Process: Separating and isolating the product of interest, in its desired form, from the fermentation supernatant or cell homogenate.

Viral Clearance Effectiveness: The efficacy of the entire purification process or an individual process step within a purification process to clear viruses, as determined by the viral reduction factor. In general, for a virus clearance step to be considered effective 4 logs or more of clearance must be demonstrated with 95% confidence ($\alpha = 0.05$).

Viral Load: The amount of virus added to the load material of a purification step and then subjected to an inactivation/removal treatment. The experimental determination of viral load before and after treatment enables calculation of a reduction factor that is specific to the virus and the treatment used.

Viral Reduction Factor (VRF): The VRF of an individual clearance step represents the \log_{10} of the ratio of the virus load in the pretreatment material (used to challenge the clearance step) to the virus load in the post-treatment material. The overall VRF is calculated by adding all the individual steps with a \log_{10} reduction factor >1 . The \log_{10} viral reduction factor is determined by the following equation:

$$\log_{10} \text{ initial virus load} - \log_{10} \text{ final virus load} = \log_{10} \text{ Viral Reduction Factor}$$

The \log_{10} viral reduction value expresses levels of decreased viral contamination by factors of 10 that could be easily converted to percent reduction. For instance, a 1-log reduction is equivalent to a 90% reduction, a 2-log reduction is a 99% reduction, a 3-log reduction is a 99.9% reduction, and a 4-log reduction is a 99.99% reduction.

Virus Removal/Inactivation Validation: Virus removal/inactivation validation studies evaluate the capacity of the process to eliminate and/or to inactivate the viruses. Typically, this involves spiking the product with a known virus and then subjecting the product to inactivation/removal processes. ■ 1S (USP39)

¹ Cf. glossary in 〈 1050 〉.

BRIEFING

〈 1176 〉 **Prescription Balances and Volumetric Apparatus Used in Compounding**, *USP* 38 page 1331. The USP Compounding Expert Committee is proposing a title change and revisions to *Prescription Balances and Volumetric Apparatus* 〈 1176 〉. This new title will better represent the contents of the revised chapter, which has been updated by the deletion of obsolete terminology and the addition of concise and practical information on mechanical and electronic balances as well as volumetric apparatus. The new information proposed for 〈 1176 〉 will educate the reader on testing balances and determining the minimum accurately weighable quantity on the balance. The revised chapter also contains guidance on selection and use of cylindrical graduates and additional information on other types of volumetric apparatus that are used in compounding.

The proposed chapter is available at <http://www.usp.org/usp-nf/notices/general-chapter-1176> with line numbers. Please provide the line numbers corresponding to your comments when submitting comments to CompoundingSL@usp.org.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(CMP: J. Sun.)

Correspondence Number—C151881

Comment deadline: May 31, 2015

Change to read:

〈 1176 〉 PRESCRIPTION BALANCES AND VOLUMETRIC APPARATUS

■ USED IN COMPOUNDING ■ 1S (USP39)

Change to read:

Prescription Balances

~~Note—Balances other than the type described herein may be used if these afford equivalent or better accuracy. This includes micro-, semimicro-, or electronic single-pan balances (see *Weights and Balances* 〈 417 〉). Some balances offer digital or direct-reading features. All balances should be calibrated and tested frequently using appropriate test weights, both singly and in combination.~~

~~**Description**—A prescription balance is a scale or balance adapted to weighing medicinal and other substances required in prescriptions or in other pharmaceutical compounding. It is~~

constructed so as to support its full capacity without developing undue stresses, and its adjustment is not altered by repeated weighings of the capacity load. The removable pans or weighing vessels should be of equal weight. The balance should have leveling feet or screws. The balance may feature dial-in weights and also a precision spring and dial instead of a weighbeam. A balance that has a graduated weighbeam must have a stop that halts the rider or poise at the zero reading. The reading edge of the rider is parallel to the graduations on the weighbeam. The distance from the face of the index plate to the indicator pointer or pointers should be not more than 1.0 mm, the points should be sharp, and when there are two, their ends should be separated by not more than 1.0 mm when the scale is in balance. The indicating elements and the lever system should be protected against drafts, and the balance lid should permit free movement of the loaded weighing pans when the lid is closed. The balance must have a mechanical arresting device.

Definitions—

Capacity—Maximum weight, including the weight of tares, to be placed on one pan. The *N.B.S. Handbook 44*, 4th ed., states: "In the absence of information to the contrary, the nominal capacity of a Class A balance shall be assumed to be 15.5 g (½ apothecaries' ounce)." Most of the commercially available Class A balances have a capacity of 120 g and bear a statement to that effect.

Weighbeam or Beam—A graduated bar equipped with a movable poise or rider. Metric graduations are in 0.01-g increments up to a maximum of 1.0 g.

Tare Bar—An auxiliary ungraduated weighbeam bar with a movable poise. It can be used to correct for variations in weighing glasses or papers.

Balance Indicator—A combination of elements, one or both of which will oscillate with respect to the other, to indicate the equilibrium state of the balance during weighing.

Rest Point—The point on the index plate at which the indicator or pointer stops when the oscillations of the balance cease; or the index plate position of the indicator or pointer calculated from recorded consecutive oscillations in both directions past the zero of the index plate scale. If the balance has a two-pointer indicating mechanism, the position or the oscillations of only one of the pointers need be recorded or used to determine the rest point.

Sensitivity Requirements (SR)—The maximum change in load that will cause a specified change, one subdivision on the index plate, in the position of rest of the indicating element or elements of the balance.

Class A Prescription Balance—A balance that meets the tests for this type of balance has a sensitivity requirement of 6 mg or less with no load and with a load of 10 g on each pan. The Class A balance should be used for all the weighing operations required in prescription compounding.

In order to avoid errors of 5% or more that might be due to the limit of sensitivity of the Class A prescription balance, do not weigh less than 120 mg of any material. If a smaller weight of dry material is required, mix a larger known weight of the ingredient with a known weight of dry diluent, and weigh an aliquot portion of the mixture for use.

Testing the Prescription Balance—A Class A prescription balance meets the following four basic tests. Use a set of test weights, and keep the rider on the weighbeam at zero unless

~~directed to change its position.~~

~~1. *Sensitivity Requirement*—Level the balance, determine the rest point, and place a 6-mg weight on one of the empty pans. Repeat the operation with a 10-g weight in the center of each pan. The rest point is shifted not less than one division on the index plate each time the 6-mg weight is added.~~

~~2. *Arm Ratio Test*—This test is designed to check the equality of length of both arms of the balance. Determine the rest point of the balance with no weight on the pans. Place in the center of each pan a 30-g test weight, and determine the rest point. If the second rest point is not the same as the first, place a 20-mg weight on the lighter side; the rest point should move back to the original place on the index plate scale or farther.~~

~~3. *Shift Tests*—These tests are designed to check the arm and lever components of the balance.~~

~~A. Determine the rest point of the indicator without any weights on the pans.~~

~~B. Place one of the 10-g weights in the center of the left pan, and place the other 10-g weight successively toward the right, left, front, and back of the right pan, noting the rest point in each case. If in any case the rest point differs from the rest point determined in Step A, add a 10-mg weight to the lighter side; this should cause the rest point to shift back to the rest point determined in Step A or farther.~~

~~C. Place a 10-g weight in the center of the right pan, and place a 10-g weight successively toward the right, left, front, and back of the left pan, noting the rest point in each case. If in any case the rest point is different from that obtained with no weights on the pans, this difference should be overcome by addition of the 10-mg weight to the lighter side.~~

~~D. Make a series of observations in which both weights are simultaneously shifted to off-center positions on their pans: both toward the outside, both toward the inside, one toward the outside and the other toward the inside, both toward the back, and so on until all combinations have been checked. If in any case the rest point differs from that obtained with no weights on the pan, the addition of the 10-mg weight to the lighter side should overcome this difference.~~

~~A balance that does not meet the requirements of these tests must be adjusted.~~

~~4. *Rider and Graduated Beam Tests*—Determine the rest point for the balance with no weight on the pans. Place on the left pan the 500-mg test weight, move the rider to the 500-mg point on the beam, and determine the rest point. If it is different from the zero rest point, add a 6-mg weight to the lighter side. This addition should bring the rest point back to its original position or farther. Repeat this test, using the 1-g test weight and moving the rider to the 1-g division on the beam. If the rest point is different, it should be brought back at least to the zero rest point position by the addition of 6 mg to the lighter pan. If the balance does not meet this test, the weighbeam graduations or the rider must be corrected.~~

~~*Metric or apothecaries' weights*—for use with a prescription balance should be kept in a special rigid and compartmentalized box and handled with plastic or plastic-tipped forceps to prevent scratching or soiling. For prescription use, analytical weights (Class P or better) are recommended. However, Class Q weights have tolerances well within the limits of accuracy of the prescription balance, and they retain their accuracy for a long time with proper care. Coin-type (or disk-shaped) weights should not be used.~~

~~Test weights—consisting of two 20-g or two 30-g, two 10-g, one 1-g, one 500-mg, one 20-mg, one 10-mg, and one 6-mg (or suitable combination totaling 6 mg) weights, adjusted to N.B.S. tolerances for analytical weights (Class P or better) should be used for testing the prescription balances. These weights should be kept in a tightly closed box and should be handled only with plastic or plastic-tipped forceps. The set of test weights should be used only for testing the balance or constantly used weights. If properly cared for, the set lasts indefinitely.~~

Volumetric Apparatus

~~Pharmaceutical devices for measuring volumes of liquids, including burets, pipets, and cylinders graduated either in metric or apothecary units meet the standard specifications for glass volumetric apparatus described in NTIS COM-73-10504 of the National Technical Information Service.¹ Conical graduates meet the standard specifications described in N.B.S. Handbook 44, 4th Edition, of the National Institute of Standards and Technology.² Graduated medicine droppers meet the specifications (see *Medicine Dropper* (1101)). An acceptable ungraduated medicine dropper has a delivery end 3 mm in external diameter and delivers 20 drops of water, weighing 1 g at a temperature of 15°. A tolerance of ±10% of the delivery specification is reasonable.~~

Selection and Use of Graduates—

~~*Capacity*—The capacity of a graduate is the designated volume, at the maximum graduation, that the graduate will contain, or deliver, as indicated, at the specified temperature.~~

~~*Cylindrical and Conical Graduates*—The error in a measured volume caused by a deviation of ±1 mm in reading the lower meniscus in a graduated cylinder remains constant along the height of the uniform column. The same deviation of ±1 mm causes a progressively larger error in a conical graduate, the extent of the error being further dependent upon the angle of the flared sides to the perpendicular of the upright graduate. A deviation of ±1 mm in the meniscus reading causes an error of approximately 0.5 mL in the measured volume at any mark on the uniform 100-mL cylinder graduate. The same deviation of ±1 mm can cause an error of 1.8 mL at the 100-mL mark on an acceptable conical graduate marked for 125 mL.~~

~~A general rule for selection of a graduate for use is to use the graduate with a capacity equal to or just exceeding the volume to be measured. Measurement of small volumes in large graduates tends to increase errors, because the larger diameter increases the volume error in a deviation of ±1 mm from the mark. The relation of the volume error to the internal diameters of graduated cylinders is based on the equation $V = \pi r^2 h$. An acceptable 10-mL cylinder having an internal diameter of 1.18 cm holds 109 μ L in 1 mm of the column. Reading 4.5 mL in this graduate with a deviation of ±1 mm from the mark causes an error of about ±2.5%, and the same deviation in a volume of 2.2 mL in the same graduate causes an error of about ±5%. Minimum volumes that can be measured within certain limits of error in graduated cylinders of different capacities are incorporated in the design details of graduates in N.B.S. Handbook 44, 4th ed., of the National Institute of Standards and Technology. Conical graduates having a capacity of less than 25 mL should not be used in prescription compounding.~~

1. Balances
 - 1.1 Mechanical Prescription Balances
 - 1.2 Electronic Balances
 - 1.3 Minimum Accurately Weighable Quantity on the Balance
2. Volumetric Apparatus
 - 2.1 Selection and Use of Graduates
 - 2.2 Medicine Droppers
 - 2.3 Dispensing Bottles
 - 2.4 Syringes
 - 2.5 Pipets
 - 2.6 Volumetric Flasks
 - 2.7 Additional Considerations When Using Volumetric Apparatus
3. Glossary of Terms

1. BALANCES

Pharmacies that perform materials measurements for compounding and dispensing should have access to a well-maintained National Institute of Standards and Technology (NIST) Class III torsion prescription balance or superior balance (preferably an electronic balance) to weigh masses accurately. The pharmacy should have a set of calibration weights, or the balance should have internal calibration capability to standardize the precision and accuracy of the balance.

For more information regarding standards for weights and balances, see *Balances* (41). The standards in NIST Handbook 44 called "Specifications, Tolerances, and Other Technical Requirements for Weighing and Measuring Devices" may also be useful to compounders.¹ Some balances offer digital or direct-reading features and printing capabilities, which may be desirable for ease of use. All balances should be calibrated or verified and tested frequently using appropriate test weights.

1.1 Mechanical Prescription Balances

A NIST Class III torsion prescription balance, formerly referred to as a Class A prescription balance, is a device designed to accurately weigh drugs and other substances and materials required in compounding and dispensing practices. The NIST Class III torsion balance is constructed to support its full capacity without developing undue stresses, and adjustments to the balance are not altered by repeated weighings of the capacity load. The removable pans or weighing vessels should be of equal weight. The balance should have leveling feet or screws. The balance may feature dial-in weights and also a precision spring and dial. The indicating elements (i.e., index plate) and the weighing lever system should be protected against drafts, and the balance lid should permit free movement of the loaded weighing pans with weighing papers or boats when the lid is closed. The balance must have a mechanical arresting device.

WEIGHTS

Metric or apothecaries' weights for use in testing or compounding with a NIST Class III torsion prescription balance should be kept in a special rigid and compartmentalized box and handled

with plastic or plastic-tipped forceps to prevent scratching or soiling. Weights should meet or exceed ASTM Class 4 criteria.

BALANCE TESTING PROCEDURE

Prescription balances should be tested in the location where they are used to ensure that they are operating properly. Testing should be conducted monthly or more frequently if the balance is used extensively. Additional testing should be done when the balance has been moved, unlevelled, contaminated, or otherwise damaged, misaligned, or misused.

Four tests are required to evaluate the suitability of a Class III torsion prescription balance. A balance is acceptable if it passes all four tests. If the balance fails any test, it should not be used until it has been repaired to meet specifications and retested. The four tests are:

1. Sensitivity requirement
2. Arm ratio test
3. Shift test
4. Rider and graduated beam

The index plate of this type of balance is required to determine rest points. The significance of any additional markings on the index plate is at the discretion of the balance manufacturer. The designations of the markings should be available from the manufacturer or contained in the documentation for the balance.

The markings on the index plate will be used to execute the tests by establishing a correlation between a marking and an actual test weight. The sensitivity requirement for this type of balance is 0.1 grain, or 6 mg. A 6-mg weight is not available in most weight sets, but a 10-mg weight is usually included. The testing procedure described below is a practical method used to establish the relationship between the markings on the index plate and actual test weights; the information may be used to calculate the sensitivity requirement equivalent to one marking on the index plate.

Sensitivity requirement test: The balance should have a maximum sensitivity of 6 mg with no load and with a load of 10 g on each pan. This test should be performed without the use of weighing boats or weighing papers. Because 6-mg weights are not in calibration weight sets, a 10-mg weight is used in this test.

1. With the pans clean, dry, and empty, and the lid closed, level the balance.
2. Disengage the arrest mechanism of the pan and adjust the leveling until the pointer either rests on the central zero line or the pointer oscillates the same distance of <0.5-scale unit to either side of center.
3. Place a 10-mg weight on the right-hand pan.
4. When the balance comes to rest, record the number of scale divisions visually estimated to the nearest 0.5-scale unit that the pointer was shifted. The whole- or 1-scale unit lines on the visual pointer scale of a balance are the second tallest in comparison to the tallest or zero line on the scale. The shortest lines, which occur midway between the center line and the nearest whole-unit lines and between whole-unit lines, represent 0.5-scale units.
5. Repeat steps 1–4 with a 10-g weight centered on both pans in addition to the 10-mg weight on the right-hand pan.

The balance is suitable for pharmaceutical measurement only with the following conditions:

- a. For balances with scales marked in two or more whole- and half-scale units on both sides of the center or zero line, i.e., a total of four or more marking lines, the 10-mg weight shall displace the pointer from 1.5 to less than one-half the distance between the 1.5- and 2-scale unit lines. [Note—The 10-mg pointer displacement of 1.5-scale units is proportional to a 1-scale unit displacement by 6.67 mg. A visual observation of 10 mg moving the pointer to, or to the right of, the 1.5-scale unit mark but not to the 2-scale unit mark or off-scale, is equivalent to 6 mg displacing the pointer scale 1-scale unit, because 0.67 mg > 6 mg represents a visually indeterminable pointer displacement of 0.1-scale unit past the 1-scale unit mark. The distance between 2 whole-scale units on balances ranges from 2 to 4 mm, which corresponds proportionally to 0.2–0.4 mm for 0.1-scale division.]
- b. For balances with scales marked in three or fewer total whole- and half-scale units on both sides of the center or zero line, i.e., a total of three marking lines, the 10-mg weight shall displace the pointer from 1.5 to less than the off-scale stop point at the extreme right side of the scale.

Arm ratio test: The arm ratio test will determine whether both arms of the balance are of equal length.

1. Level the balance.
2. With the pans empty, adjust the balance until the pointer is in the middle of the marker plate.
3. Place a 30-g weight in the center of each pan.
4. When the balance comes to rest, record the rest point.
5. If the rest point has changed from the middle of the marker plate, place a 30-mg weight on the lighter side.
6. When the balance comes to rest, this new rest point should either return to or go farther than the middle of the marker plate.

Shift test: The shift test checks the mechanics of the arm and lever components of the balance.

1. Level the balance.
2. With the pans empty, adjust the balance until the pointer is in the middle of the marker plate.
3. Place a 10-g weight in the center of the left pan and place another 10-g weight successively toward the right, left, front, and back side of the right pan, noting the rest point in each case.
4. In any case where the rest point has changed from the center of the marker plate, add a 10-mg weight to the lighter side.
5. When the balance comes to rest, this new rest point either should return to or go farther than the middle of the marker plate.
6. Level the balance and adjust the balance until the pointer is in the middle of the marker plate.
7. Repeat the procedure with the 10-g weight in the center of the right pan, and vary the position of the 10-g weight on the left pan.
8. Level the balance and adjust the balance until the pointer is in the middle of the marker plate. Make several observations in which both 10-g weights are shifted simultaneously

to off-center positions on their pans (i.e., both toward the inside, both toward the outside, one front and the other back). In any case where the rest point is shifted from the middle of the marker plate, the addition of a 10-mg weight on the lighter side should equalize or overcome the shift.

Rider and graduated beam test: The rider and graduated beam test checks the accuracy of the calibrated dial or rider on the balance.

1. Level the balance.
2. With the pans empty, adjust the balance until the pointer is in the middle of the index plate.
3. Place a 500-mg weight on the left pan and move the dial or rider to the 500-mg point.
4. When the balance comes to rest, record the rest point.
5. If the rest point has changed from the middle of the index plate, place a 10-mg weight on the lighter side.
6. When the balance comes to rest, this new rest point should either return to or go farther than the middle of the index plate.
7. Follow the same procedure using a 1-g weight on the left pan and the dial or rider on the 1-g point. If the new rest point is shifted from the middle of the index plate, a 10-mg weight to the lighter side should equalize or overcome the shift.

1.2 Electronic Balances

A typical electronic prescription balance is an instrument that provides essential readability for materials weighed within the range of capacities for the balance. The display should have prompts to guide users through the balance function, as well as an output port for printing if necessary. Most balances sold for prescription compounding meet or exceed Class I or II accuracy requirements according to NIST Handbook 44 and come with certificates issued under the National Type Evaluation Program (NTEP) of the National Conference on Weights and Measures.

Calibration/certification of the balance should be performed according to the standard operating procedures of the facility. Many electronic balances contain internal calibration programs that automatically calibrate the balance daily. If there is no internal calibration feature, external calibration may be conducted using a calibration weight, according to the procedure supplied by the manufacturer. Weights for use in calibrating an electronic balance should be kept in a special rigid and compartmentalized box and handled with plastic or plastic-tipped forceps, or gloves that are provided with the weights, to prevent scratching or soiling. These calibration weights should meet or exceed ASTM Class 1 criteria. In addition, there are companies that offer calibration services to certify that the balance is performing adequately. For more information regarding the use of electronic balances, see [41](#) and *Weighing on an Analytical Balance* [1251](#).

1.3 Minimum Accurately Weighable Quantity on the Balance

The minimum accurately weighable quantity (MAWQ) is the smallest weight or mass that will produce no greater than a predetermined fraction of error on a properly calibrated, situated, and operated balance. The predetermined weighing error is assigned based on either a professional standard (such as NMT 0.05 or 5% error in the weight of any prescription ingredient) or scientific rigor, for example, NMT 0.005 or 0.5% error in the weight of an

ingredient that is in limited supply. The compounder should use professional judgment when assigning the acceptable error for each process.

The formula for determining MAWQ for a typical Class III torsion balance is:

$$\text{MAWQ} = \text{Sensitivity requirement/Acceptable error}$$

Example: Calculate the MAWQ for a Class III torsion balance with a sensitivity requirement of 6 mg and an acceptable error of 5% or 0.05.

$$\text{MAWQ} = 6 \text{ mg}/0.05 = 120 \text{ mg}$$

For electronic balances, the MAWQ is calculated using the linearity or the absolute error over the range of the balance. This value is provided by the balance manufacturer. Note that the balance linearity and the readability of the smallest mass unit may not be the same.

Example: Calculate the MAWQ for an electronic balance with a linearity of 0.002 g and an acceptable error of 5% or 0.05.

$$\text{MAWQ} = 0.002 \text{ g}/0.05 = 0.04 \text{ g or } 40 \text{ mg}$$

2. VOLUMETRIC APPARATUS

An assortment of appropriate volumetric devices should also be available to accurately measure fluids and liquids of different volumes and densities. Pharmaceutical devices approved for measuring volumes of liquids, including burets, pipets, and cylindrical graduates marked in metric or metric and apothecary units, are to meet the standard specifications for glass volumetric apparatus described in "Specifications and Tolerances for Reference Standards and Field Standard Weights and Measures, 2. Specifications and Tolerances for Field Standard Measuring Flasks".² Conical graduates are to meet the standard specifications described in NIST Handbook 44.¹ There are ASTM standards (ASTM E542) for the calibration of laboratory volumetric apparatus that may be useful to compounders as well.³

2.1 Selection and Use of Graduates

CAPACITY

The capacity of a cylindrical graduate is the volume at the maximum graduation mark at the specified temperature. Volumes for prescription compounding and dispensing that are measured in cylindrical graduates should be adequate to not exceed 5% error.

CYLINDRICAL AND CONICAL GRADUATES

The error in a measured volume caused by a deviation of ± 1 mm in reading the lower meniscus in a graduated cylinder remains constant along the height of the uniform column. The same deviation of ± 1 mm causes a progressively larger error in a conical graduate, the extent of the error being further dependent upon the angle of the flared sides to the perpendicular of the upright graduate. A deviation of ± 1 mm in the meniscus reading causes an error of approximately 0.5 mL in the measured volume at any mark on the uniform 100-mL cylinder graduate. The same deviation of ± 1 mm can cause an error of 1.8 mL at the 100-mL mark on an acceptable conical graduate marked for 125 mL.

A general rule for selection of a graduate for use is to use the graduate with a capacity equal to or just exceeding the volume to be measured. Measurement of small volumes in large

graduates tends to increase errors, because the larger diameter increases the volume error in a deviation of ± 1 mm from the mark. The relation of the volume error to the internal diameters of graduated cylinders is based on the equation:

$$V = \pi r^2 h$$

V = volume

r = radius

h = height

An acceptable 10-mL cylinder having an internal diameter of 1.18 cm holds 109 μL in 1 mm of the column. Reading 4.5 mL in this graduate with a deviation of ± 1 mm from the mark causes an error of about $\pm 2.5\%$, and the same deviation in a volume of 2.2 mL in the same graduate causes an error of about $\pm 5\%$. *Table 1* shows the accuracy limits for cylindrical graduates.

Table 1. Tolerance or Accuracy Limits for Class A Cylindrical Graduates^a

Capacity (mL)	Smallest Graduation Mark Interval (mL)	Tolerance (\pm mL) ^b	Minimum Volume for 5% Error (mL) ^c
5	0.1	0.05	1.0
10	0.1 or 0.2	0.10	2.0
25	0.2 or 0.5	0.17	3.4
50	1	0.25	5.0
100	1	0.50	10.0
250	2	1.00	20.0
500	5	2.00	40.0
1000	10	3.00	60.0
2000	20	6.00	120.0
4000	50	14.50	290.0

^a ASTM E1272—Standard specification for laboratory glass graduated cylinders (<http://www.astm.org/Standards/E1272.htm>). Some brands exceed the ASTM limits; for example, one source of a class A 10-mL graduate lists the tolerance as ± 0.08 mL.

^b The constant volume error in each measurement.

^c The minimum volume for $N\%$ error = [Tolerance (mL) \times 100]/ N (%); e.g., for $N = 5\%$ for a 10-mL graduate, the minimum volume is 2.0 mL. (The minimum volume for 5% error = $(0.1 \text{ mL} \times 100)/5 = 2.0$.)

There is an inverse relationship between the temperature and density of liquids. For compounding and dispensing purposes, deviations will be negligible when volume measurements are performed at temperatures NMT 5° from that specified on the particular cylindrical graduate, which is usually 20° . For example, the densities in g/mL of water, ethanol, and glycerin vary, respectively, from 0.999 to 0.997, 0.791 to 0.785, and 1.265 to 1.262 over the temperature range of 15° – 25° . The accuracy of each cylindrical graduate used in pharmacy

practice is recommended to be verified as follows, at a measuring temperature of 20°–25° with the assumption that deviations follow a normal or Gaussian distribution:

1. Tare a clean, dry graduate on a properly calibrated balance of adequate capacity, linearity, and readability for the volumes to be measured.
2. Fill the tared, dry graduate identically five or more times on a level surface to the smallest, a mid-range, and the maximum capacity graduation marks with *Purified Water* or deionized water, precluding and wiping spills and splashes from the exterior and interior above the target fill line.
3. Record the weight of each filling.
4. Calculate the mean weight of each set of fillings.
5. Calculate the percent deviation of each weight from the theoretical weight as follows:

$$\text{Percent deviation weight} = \frac{[(\text{Actual weight} - (\text{intended volume} \times 0.9975)) / (\text{Intended volume} \times 0.9975)] \times 100\%}{}$$

6. Calculate the percent mean deviation weight as follows:

$$\text{Percent mean deviation weight} = \frac{[(\text{Mean actual weight} - (\text{intended volume} \times 0.9975)) / (\text{Intended volume} \times 0.9975)] \times 100\%}{}$$

Deviations for cylindrical graduates used in compounding and dispensing should not exceed 5.0% for individual weights or 2.5% for mean weights of the corresponding volumes of *Purified Water*. *Table 1* and *Table 2* show that a wider range of volumes can be measured in Class A cylindrical graduates compared to Class B. According to ASTM standards, Class A cylindrical graduates must be marked with the letter "A" to designate compliance with applicable construction and accuracy requirements. Class B cylindrical graduates are the same basic design as Class A cylindrical graduates and are considered to be for general purpose use. However, volumetric tolerances may be up to twice the allowable range for Class A cylindrical graduates.

Table 2. Tolerance or Accuracy Limits for Class B Cylindrical Graduates^a

Capacity (mL)	Smallest Graduation Mark Interval (mL)	Tolerance (\pm mL) ^b	Minimum Volume for 5% Error (mL) ^c
5	0.1	0.10	2.0
10	0.1 or 0.2	0.2	4.0
25	0.2 or 0.5	0.34	6.8
50	1	0.50	10.0
100	1	1.00	20.0
250	2	2.00	40.0
500	5	4.00	80.0
1000	10	6.00	120.0
2000	20	12.00	240.0
4000	50	29.00	580.0

^a ASTM E1272—Standard Specification for Laboratory Glass Graduated Cylinders (<http://www.astm.org/Standards/E1272.htm>).

b The constant volume error in each measurement.

c The minimum volume for $N\%$ error = $[\text{Tolerance (mL)} \times 100]/N (\%)$; e.g. for $N = 5\%$ for a 5-mL graduate, the minimum volume is 2.0 mL. (The minimum volume for 5% error = $(0.1 \text{ mL} \times 100)/5 = 2.0$.)

2.2 Medicine Droppers

Medicine droppers meet the specifications in *Packaging and Storage Requirements* (659). Medicine droppers should be used only for qualitative purposes, such as pH adjustment with an acid, alkali, or buffer, and visual identification testing with reagents. Medicine droppers are not approved for volumetric measurements for compounding. Calibrated medicine droppers have markings to guide delivery of the prescribed quantity of medication. Their purpose should be limited to the measurement of a dose for administration.

2.3 Dispensing Bottles

Some dispensing bottles may be supplied by the manufacturer as part of the packaging, whereas others may be selected and supplied by the pharmacist. These containers are not necessarily accurately calibrated and should not be used for measurement during compounding and dispensing unless they are appropriately calibrated by the compounder.

2.4 Syringes

Syringes are available in a variety of sizes with calibrated increments used for measuring and may be used to accurately measure and deliver a wide range of liquid volumes. For viscous liquids, measurements made with syringes are usually more accurate than those made with cylindrical graduates.

ORAL SYRINGES

Oral syringes are available as a device for accurately providing a dose of liquid medication to a patient. The performance of the syringes may be user dependent. For this reason, it is important that suitable operating procedures are documented and followed, and that operators are specifically trained in the correct use of the instruments. Users should be cautious about relying on manufacturers' performance figures. It is more appropriate to perform calibration, taking into account the variation between different users. Oral syringes provide an improvement in dosing accuracy for viscous medications, compared with medication droppers.

SYRINGE CALIBRATION

Syringe calibration is based on the gravimetric determination of the quantity of water either contained or delivered, and the conversion of this value to true volume at the standard temperature of 20° . At 80% of the nominal syringe volume, 5–10 measurements should be performed.

Calibration procedure:

1. Aspirate and dispense an exact volume of deionized water that is 80% of the nominal syringe volume.
2. Determine the mass of the dispensed water.

3. Calculate the volume of the dispensed water using mass and density.
4. Document the measurement values.
5. Calculate the accuracy (R).

Calibration formula:

$$\text{Accuracy } (R) = [(\text{Average value} - \text{target value})/\text{Target value}] \times 100$$

2.5 Pipets

Pipets are thin glass tubes used to deliver volumes <25 mL. The two types of pipets are the single-volume pipet and the calibrated pipet. The single-volume pipet is the most accurate and the simplest to use, but the single-volume pipet is limited to the measurement of a single fixed volume; it is not capable of partial volume measurements.

The calibrated pipet has graduation marks from a point near the tip of the pipet to the capacity of the pipet. In addition to delivering its entire contents, the calibrated pipet can be used to deliver partial volumes with good volumetric precision.

Micropipets generally are used when very small volumes (<1 mL) are required; micropipets are available in a variety of sizes. Each micropipet can be adjusted, usually by turning a dial, to deliver a volume within a limited range. For example, one micropipet may deliver volumes of 0–20 μL , another delivers 0–100 μL , and yet another delivers 0–1000 μL . The pipet selected should provide the greatest accuracy for the volume to be measured. Pipets should be calibrated/certified periodically as specified in the facility's standard operating procedures. Calibrating micropipets is a very specialized process that requires adequate training and appropriate equipment. There are companies that offer contract calibration services to certify that pipets are performing correctly.

2.6 Volumetric Flasks

Volumetric flasks have a slender neck and wide, bulb-like base. They are single-volume glassware and come in a variety of sizes. Only one calibration mark is etched on the neck of the flask. When the flask is filled to that mark, the flask contains the volume indicated on the flask. Volumetric flasks are difficult to use if dissolving solids in liquid because of the narrowness of the neck. If solids are to be dissolved, the flask should be partially filled with liquid, the solid material added and completely dissolved, and then the flask should be filled to the calibration mark.

2.7 Additional Considerations When Using Volumetric Apparatus

The use of volumetric apparatus requires some working knowledge of viscosity, density, surface tension, and adhesion. Each of these properties may affect measurement accuracy. For example, higher-viscosity liquids will be drawn into the vessel at a slower rate, and the operator should allow time for complete filling. Delivery can also be slow, because higher-viscosity fluids travel through the orifice at a slower rate. Density can affect filling and emptying in a manner similar to viscosity. In addition, liquids with very low surface tension may tend to "crawl up" the vessel wall or leak from the tip of a syringe or pipet. Adhesion can affect accuracy by resulting in a slow rate of vessel emptying. The operator should confirm that all of the liquid has been drained from the vessel if performing a quantitative transfer.

Understanding the terms To Contain (TC) and To Deliver (TD) is important, because they apply to glassware. TC and TD glassware consists of vessels that range from 1 to about 100 mL and

are individually marked to indicate whether they are TC or TD vessels. A TC vessel is designed to deliver the entire measured content of the operation, and it may require forced air to expel the final quantity. A TD vessel is designed to deliver the entire measured amount via gravity flow.

3. GLOSSARY OF TERMS

Accuracy: The closeness of the displayed weight, as measured by the balance, to the true weight, as known by the use of a calibration weight or weights.

Capacity: The maximum weight, including containers and tares, that should be placed on a balance pan. The maximum capacity should be stated in the manufacturer's specifications for the balance.

Linearity: The ability to maintain the same sensitivity over the entire weighing range of the balance. This is the constant weighing error in every amount weighed within the capacity range of the balance. This term is used only with electronic balances. Analogous terms are absolute error or linear accuracy. The linearity is determined by the balance manufacturer.

Minimum accurately weighable quantity (MAWQ): The smallest weight or mass that will produce no greater than a predetermined fraction of error on a properly calibrated, situated, and operated balance.

Precision: The reproducibility of the weighing measurement as expressed by a standard deviation. A similar term, repeatability, is sometimes used in specifications for electronic balances.

Readability: The smallest division at which the balance increments.

Repeatability: An instrument's ability to consistently deliver the same weight reading for a given object, and to return to a zero reading after each weighing cycle. This is tested by repeatedly weighing the same test weight.

Rest point: The point on the index plate at which the indicator or pointer stops when the oscillations of the balance cease. Index plates are found on mechanical balances.

Sensitivity: Also referred to as "Sensitivity requirement", is the smallest weight that gives a perceptible change in the indicating element (e.g., one subdivision marking on the index plate of a mechanical balance or one number change on the digital display of an electronic balance). A similar term, linearity, is sometimes used in specifications for electronic balances. ■ 1S (USP39)

¹ ~~NTIS COM 73 10504 is for sale by the National Technical Information Service, Springfield, VA 22151.~~

² ~~N.B.S. Handbook 44, 4th ed. (1971), is for sale by the Superintendent of Documents, U. S. Government Printing Office, Washington, DC 20402.~~

■ 1

NIST Handbook 44—Specifications, Tolerances, and Other Technical Requirements for Weighing and Measuring Devices (<http://www.nist.gov/pml/wmd/pubs/h44-11.cfm>). ■ 1S (USP39)

■ 2

Harris GL. Specifications and tolerances for reference standards and field standard weights and

measures. 2. Specifications and tolerances for field standard measuring flasks. 1996. NTIS Order Number: PB96-178926. National Technical Information Service, Alexandria, Virginia 22312 (<http://www.ntis.gov>). ■1S (USP39)

■3

ASTM E542 standard practice for calibration of laboratory volumetric apparatus (<http://www.astm.org/Standards/E542.htm>). ■1S (USP39)

BRIEFING

《1229.5》 **Biological Indicators for Sterilization.** The General Chapters–Microbiology Expert Committee proposes this new general information chapter as an addition to the 《1229》 family of chapters. The content on the use of biological indicators (BIs) in this proposal is consistent with that presented in that series of chapters. This proposed chapter contains information on BIs with respect to their types, characterization, and selection for specific types of sterilization, as well as responsibilities of the BI manufacturer and user. This chapter will replace *Biological Indicators for Sterilization* 《1035》, which will be proposed for deletion in the next issue of *Pharmacopeial Forum*.

(GCM: R. Tirumalai.)

Correspondence Number—C154738

Comment deadline: May 31, 2015

Add the following:

■ 《1229.5》 BIOLOGICAL INDICATORS FOR STERILIZATION

INTRODUCTION

A biological indicator (BI) is a well-characterized preparation of a specific microorganism that has known resistance to a specific sterilization process.¹ BIs are used to demonstrate the effectiveness of processes that render a product sterile in its final package or container, as well as the effectiveness of the sterilization of equipment, product contact materials, and packaging components as required. BIs may also be used to monitor established sterilization cycles and are used for periodic reassessment of sterilization process effectiveness. BIs are process aids and can support the correlation of physical parameters to microbiological destruction. Microorganisms recognized as suitable for BIs are spore-forming bacteria, because the spores of these microorganisms are significantly more resistant than the vegetative cells that comprise the majority of normal bioburden.

PROPER USE OF BIOLOGICAL INDICATORS

BIs provide microbiological evidence of process effectiveness that should be correlated to physical measurements (see *Sterilization of Compendial Articles* 《1229》). Microbiological

resistance to sterilization varies with the physical conditions; however, there are no established means for accurately predicting microbial destruction based solely on physical measurements, with the exception of radiation sterilization. BI placement locations within or on materials to be sterilized are chosen to confirm that the desired sterilizing conditions have been attained. BIs are typically spore-forming bacteria of the genera *Geobacillus*, *Bacillus*, and *Clostridium*. BIs are chosen for their greater resistance to a specific sterilization process as compared to the expected bioburden. Preference in BI selection should be given to well-characterized strains.

RESPONSIBILITIES

BI Manufacturer's Responsibility

The responsibility for determining the performance characteristics of each BI lot resides with the BI manufacturer. The manufacturer should provide, with each lot of BIs, a certificate of analysis that attests to the validity of BI performance claims. The manufacturer should provide information concerning the microbial population and resistance (*D* and *z* values, respectively, where appropriate) as well as storage and expiry information. The resistance of the BI should be determined by the manufacturer under defined conditions. The manufacturer should provide directions for use, including the medium and conditions used for the recovery of microorganisms after exposure to the sterilization process. Disposal instructions also should be provided by the manufacturer of the BI.

BI User's Responsibility

When BIs are purchased, their suitability for use in a specific sterilization process must be established. The BI user should obtain a certificate of analysis for each lot of BI and verify the manufacturer's label claims for spore population. When a BI is used in accordance with the BI manufacturer's directions, the resistance of the BI need not be reconfirmed.

User-Prepared Biological Indicators

A user of BIs may elect to propagate spore crops of a single species for use as a suspension. Alternatively, these spore suspensions may be purchased from a BI manufacturer. When liquid suspensions are applied to a substrate, it is the user's responsibility to determine the population and resistance of the microorganism used. The resistance determined for liquid suspensions relates only to other lots of the same suspension and is not representative of how that microorganism will perform on a substrate or in a different suspending medium. In these circumstances, the BI resistance and population should be re-established (see *Biological Indicators—Resistance Performance Tests* (55)).

CHARACTERIZATION OF BIOLOGICAL INDICATORS

The use of BIs should include procedures for their acceptance and control. The following elements outline the major considerations. Resistance performance is addressed separately in (55), which provides methods for evaluating BI resistance.

Packaging and Storage

Store under the conditions recommended on the label or under validated conditions, and protect from light, toxic substances, excessive heat, and moisture.

Expiration Date

Use within the BI's labeled or determined expiration date.

Identification

Where identification of the BI species is deemed appropriate or necessary, use either a phenotypic or genotypic identification method (see *Microbial Characterization, Identification, and Strain Typing* { 1113 } for additional information).

Purity

By examination of the colonies derived from the spores on a suitable plate culture medium, determine that there is no evidence of contamination with other microorganisms.

Disposal

Prior to discarding used spores, sterilize using a method recommended by the BI manufacturer or other equivalent means.

TYPES OF BIOLOGICAL INDICATORS

A BI is a well-characterized preparation of a specific bacterial spore of known resistance to a specific sterilization process. Some BIs may contain two different species and concentrations of bacterial spores for use in the evaluation of two different sterilization processes.

One form of BI preparation includes spores that are placed on a carrier (e.g., a disk or strip of paper, glass, plastic, metal, or other material) and may be packaged to maintain the integrity and viability of the spores inoculated onto the carrier. The carrier and primary packaging should not be damaged or degraded by the specific sterilization process. Another preparation of BI is a spore suspension that is inoculated on or into representative units of the article to be sterilized. A surrogate article may be used if it is not practical to inoculate the actual article. A surrogate article is a preparation that differs in one or more ways from the actual article but performs as the actual article during cycle development, validation, and routine use. The physical design of actual or surrogate articles can affect the resistance of spore suspensions that are inoculated on or into an article (see { 55 }). In the case of liquid inoculated products, it is essential to determine the population, *D* value (and, in terminal sterilization applications, *z* value) of the relevant BI spore in the liquid product, and any simulated product substrate (if utilized).

A third form of BI is a sealed system that includes the growth medium (either in direct contact with the BI during the sterilization or placed in contact with the BI after sterilization) for recovery of process-exposed BI microorganisms. Some BI systems may contain a growth

indicator or sensor in addition to growth media.

SELECTION FOR SPECIFIC STERILIZATION PROCESSES

The selection of a BI requires knowledge of the resistance of the BI system to the specific sterilization process. It must be established that the BI system provides a challenge to the sterilization process greater than the resistance of the native bioburden. The recommendations for BI with each sterilization process are not exclusive; they represent only the more common choices.

Steam Sterilization by Direct Contact

For steam sterilization by direct contact, the commonly used BI contains spores of *G. stearothermophilus* (ATCC 12980 or ATCC 7953), a thermophilic microorganism with a moist heat resistance substantially greater than that of most vegetative microorganisms (see *Steam Sterilization by Direct Contact* (1229.1)).

Moist Heat Sterilization of Aqueous Liquids

Heat-resistant spore-forming microorganisms such as *C. sporogenes* (ATCC 7955), *B. subtilis* (ATCC 35021), or *B. atrophaeus* (ATCC 9372) are used. *B. subtilis*, *B. atrophaeus*, and *C. sporogenes* are preferred for use in sterilization of aqueous solutions or where their lower thermal resistance is more appropriate.

Dry Heat Sterilization

For dry heat sterilization, spores of *B. atrophaeus* (ATCC 9372) are typically used (see *Gaseous Sterilization* (1229.7)). Where dry heat depyrogenation has been demonstrated (*Dry Heat Depyrogenation* (1228.1)), sterilization by dry heat need not be reconfirmed, and a BI is not required.

Ionizing Radiation

The use of a resistant BI is unnecessary for the evaluation of radiation sterilization processes. Dose setting involves the evaluation of preirradiation bioburden as well as dosimetric evaluation and allied tests as defined in ISO 11137-1, -2, and -3, as well as in *Radiation Sterilization* (1229.10).

Gas Sterilization

For ethylene oxide sterilization, spores of *B. atrophaeus* are commonly used. For other gaseous agents, spores of *G. stearothermophilus* or *B. atrophaeus* are commonly used (see (1229.7)).

Chemical Sterilization

The sterilization of items using a liquid sterilant is accomplished using spores of an appropriate

strain such as *B. atrophaeus*, *B. subtilis*, or other appropriate spore-forming species, as determined by the user. Whichever strain is chosen for this purpose should have greater resistance than does the bioburden.

Vapor Phase Sterilization

The biphasic nature of these materials precludes the accurate determination of specific lethal conditions (for *D* values, see *Vapor Phase Sterilization* 〈 1229.11 〉). BIs using either *G. stearothermophilus* or *B. atrophaeus* have been utilized in the evaluation of these processes.

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¹ Microbial retention challenges as described in *Sterilizing Filtration of Liquids* 〈 1229.4 〉 are not BIs.

BRIEFING

〈 1229.9 〉 **Physicochemical Integrators and Indicators for Sterilization.** The General

Chapters—Microbiology Expert Committee proposes this new general chapter as an addition to the *Sterilization of Compendial Articles* 〈 1229〉 family of chapters. This general chapter will replace *Sterilization—Chemical and Physicochemical Indicators and Integrators* 〈 1209〉 and will provide additional information on the use of chemical and physicochemical integrators and indicators under the 〈 1229〉 parent chapter, consistent with the 〈 1229〉 sterilization series of chapters. General chapter 〈 1209〉 will be omitted in the next issue of *Pharmacoepial Forum*.

(GCM: R. Tirumalai.)

Correspondence Number—C154739

Comment deadline: May 31, 2015

Add the following:

■ 〈 1229.9〉 **PHYSICOCHEMICAL INTEGRATORS AND INDICATORS FOR STERILIZATION**

INTRODUCTION

Physicochemical integrators provide some assessment of sterilization process efficacy and may be used in cases where validation of a sterilization process is not required—an exception is the validation and monitoring of radiation sterilization with dosimetry. The physicochemical indicator provides an immediate visual confirmation that an item has been exposed to a sterilization process. Performance standards both within and between lots of physicochemical integrators or indicators from a given manufacturer should be consistent. Integrators or indicators should not interact physically or chemically with any container or product when placed in the sterilizer load, and should not alter the strength, quality, or purity of the sterilized article. The integrator or indicator should be positioned such that it does not alter the effectiveness of the sterilization process.

PHYSICOCHEMICAL INTEGRATORS

A physicochemical integrator is defined as a device that responds to one or more sterilization process critical parameters, which results in a measurable value that can be correlated to microbial lethality. The manufacturers of physicochemical integrators should provide data to demonstrate that the labeled performance characteristics tests of the integrators are met. Physicochemical integrators require precautions for use and the appropriate interpretive criteria to define their performance characteristics. Performance of the sterilization apparatus must be ascertained from records generated by calibrated instruments (temperature, pressure, exposure time, gas concentration, and others, as applicable). The integrator can demonstrate only inadequate or adequate exposure to a combination of sterilization parameters.

Physicochemical integrators for radiation sterilization are designed to react predictably to the delivered radiation dose and can provide primary evidence of sterilization process effectiveness. The use of dosimeters in radiation sterilization cycle development and routine process control is addressed in ANSI/AAMI/ISO 11137-3—Sterilization of health care products—Radiation—Part 3: Guidance on dosimetric aspects (1).

PHYSICOCHEMICAL INDICATORS

A physicochemical indicator is defined as a device that provides visual evidence of exposure to one or more critical sterilization parameters. Physicochemical indicators cannot provide primary evidence of sterilization efficacy.

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1. ANSI/AAMI/ISO 11137-3:2006/(R)2010—Sterilization of health care products—Radiation—Part 3: guidance on dosimetric aspects. New York: American National Standards Institute; 2010. ■1S (USP39)

BRIEFING

《1229.12》 **New Sterilization Methods.** The General Chapters—Microbiology Expert Committee proposes this new general chapter as an addition to the *Sterilization of Compendial Articles* 《1229》 family of sterilization chapters. Commonly available sterilization processes are intended for the elimination of microorganisms from the materials subjected to them. However, certain situations may arise in which the user may need to develop and implement a new or novel sterilization process. This chapter provides information on the important steps to be considered for such a process.

(GCM: R. Tirumalai.)

Correspondence Number—C154825

Comment deadline: May 31, 2015

Add the following:

■ 《1229.12》 NEW STERILIZATION METHODS

INTRODUCTION

Sterilization processes are developed for the elimination of viable microorganisms while preserving the essential physical, chemical, and biological properties of the materials subjected to them. Where this cannot be accomplished by the sterilization methods described in *Sterilization of Compendial Articles* 《1229》, it may be possible to sterilize by using a proposed method not commonly used. When doing so, it is the end user's responsibility to demonstrate that the proposed new method can be used safely.

POINTS TO CONSIDER FOR A NEW STERILIZATION METHOD

The major steps in the implementation of a new sterilization method include the following:

- A literature review to identify supportive information on the proposed method

- Elimination of any established method through experimental evidence and/or comprehensive literature review of any materials used
- Identification and confirmation of reproducible lethality against a broad range of microorganisms, including bacterial spore-formers
- Identification and definition of critical process parameters necessary to ensure sufficient lethality. The effective range of these parameters should be explored to identify necessary conditions for the proposed sterilization process. Among the parameters to be considered, depending upon the nature of the process under consideration, are process dwell time, temperature, concentration, energy or power, and relative humidity. This evaluation should include positive and negative controls to ensure that the proposed method is in fact responsible for microbial destruction.
- The selection of a biological indicator (usually a spore-forming microorganism) with increased resistance to the sterilization method
- Evaluation of the proposed method against anticipated bioburden microorganisms and comparison of relative resistance of the bioburden microorganism to that of the chosen biological indicator
- The identification of in-process and/or post-process measurements and/or analysis that can reliably confirm the effectiveness of the proposed sterilization process.

Where the proposed new method is used for materials intended for human and/or veterinary use, the relevant regulatory authorities should be contacted to secure their acceptance before either investigational or clinical usage. Validation of the proposed new method should be completed before use on a commercial basis. ■ 1S (USP39)

BRIEFING

Casein, Hammarsten, *USP 38* page 1828. It is proposed to correct the name of this reagent and remove the information about a possible supplier as there are multiple possible suppliers for it.

Additionally, minor editorial changes have been made to update the reagent to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C155590

Comment deadline: May 31, 2015

Change to read:

Casein, ~~Hammersten~~

■ Hammarsten ■ 1S (USP39)

[9000-71-9][~~Note—A suitable grade is available from www.emdchemicals.com, catalog number CX0525-1.~~]

■ —Use a suitable grade. ■ 1S (USP39)

BRIEFING

Triphenylene. It is proposed to add this new reagent used as the internal standard in the *Assay* in the monograph for *Paricalcitol Capsules*.

(HDQ: M. Marques.)

Correspondence Number—C133281

Comment deadline: May 31, 2015

Add the following:

■ Triphenylene (9,10-Benzophenanthrene), $C_{18}H_{12}$ —**228.29** [217-59-4]—Use a suitable grade with a content of NLT 98%. ■ 1S (USP39)

BRIEFING

L## (Succinylcholine Chloride, Dionex IonPac CG19). It is proposed to add this new column used as a guard column in the *Limit of Choline* test in the monograph for *Succinylcholine Chloride*, which appears elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C127194

Comment deadline: May 31, 2015

Add the following:

■ L## (Succinylcholine Chloride, Dionex IonPac CG19)—Weak cation-exchange resin consisting of a highly cross-linked core of 8.0- μ m microporous particles having an average pore size of 10 Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene. Substrate is surface grafted with carboxylic acid functionalized groups. Capacity of NLT 46 μ Eq/column (4-mm \times 5-cm). ■ 1S (USP39)

BRIEFING

L## (Succinylcholine Chloride, Dionex IonPac CS19). It is proposed to add this new column used in the *Limit of Choline* test in the monograph for *Succinylcholine Chloride*, which appears elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C127194

Comment deadline: May 31, 2015

Add the following:

■ L## (Succinylcholine Chloride, Dionex IonPac CS19)—Weak cation-exchange resin consisting of a highly cross-linked core of 5.5- μ m porous particles having a pore size of 2000 Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene. Substrate is surface grafted with carboxylic acid functionalized groups. Capacity NLT 2400 μ Eq/column (4-mm \times 25-cm). ■ 1S (USP39)

BRIEFING

L##. It is proposed to add this new packing.

(HDQ: M. Marques.)

Correspondence Number—C112847

Comment deadline: May 31, 2015

Add the following:

■ L#—Alkyl chain, reversed-phase bonded totally or superficially to porous silica designed to retain hydrophilic and other polar compounds when using highly aqueous mobile phase, including 100% aqueous, 1.5–10 µm in diameter. ■ 1S (USP39)

CONTAINERS FOR DISPENSING CAPSULES AND TABLETS**BRIEFING**

Container Specifications for Capsules and Tablets, USP 38 page 1907.

(HDQ.)

Correspondence Number—C106488; C126560; C129432; C142335; C142336

The following table is provided as a reminder for the pharmacist engaged in the typical dispensing situation who already is acquainted with the *Packaging and Storage* requirements set forth in the individual monographs. It lists the capsules and tablets that are official in the *United States Pharmacopeia* and indicates the relevant tight (T), well-closed (W), and light-resistant (LR) specifications applicable to containers in which the drug that is repackaged should be dispensed.

This table is not intended to replace, nor should it be interpreted as replacing, the definitive requirements stated in the individual monographs.

Container Specifications for Capsules and Tablets

Monograph Title	Container Specification
Add the following: ■ Desloratadine Tablets	T ■ 1S (USP39)
Add the following: ■ Desloratadine Orally Disintegrating Tablets	T ■ 1S (USP39)
Add the following: ■ Diphenhydramine Hydrochloride and Ibuprofen Capsules	T ■ 1S (USP39)
Add the following: ■ Zolmitriptan Tablets	W, LR ■ 1S (USP39)
Add the following: ■ Zolmitriptan Orally Disintegrating Tablets	W, LR ■ 1S (USP39)

BRIEFING

Description and Relative Solubility of USP and NF Articles, USP 38 page 1917.

(HDQ.)

Correspondence Number—C93995; C111969

Add the following:

■ **Teniposide:** White to off-white, crystalline powder. Very soluble in acetone and in dimethylformamide; slightly soluble in methanol; insoluble in water and in ether. ■ 1S (USP39)

Add the following:

■ **Zolmitriptan:** White to off-white, crystalline powder. Freely soluble to soluble in methanol; soluble in water; sparingly soluble in dichloromethane; practically insoluble in toluene.

■ 1S (USP39)

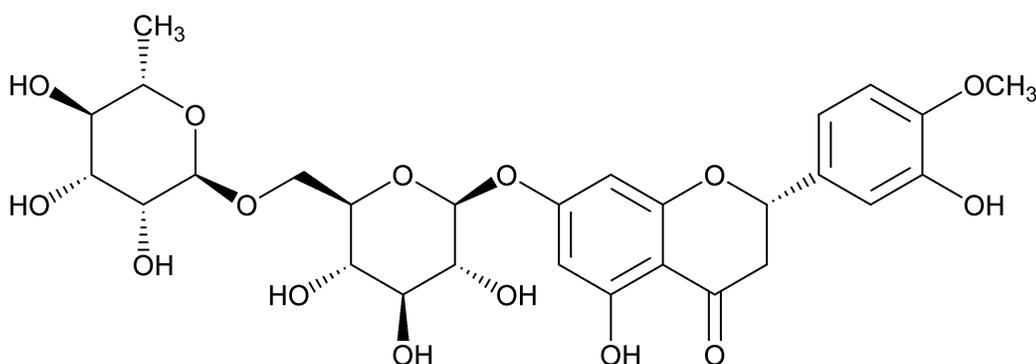
BRIEFING

Hesperidin. Because there is no existing *USP* monograph for this dietary ingredient, a new monograph is proposed. The liquid chromatographic procedures in the *Assay* and the test for *Related Compounds* are based on analyses performed with the Waters Nova-Pak C18 brand of L1 column. Typical retention times for eriocitrin, isonaringin, hesperidin, neohesperidin, diosmin, naringenin, didymin, and hesperetin are 3.2, 5.2, 7.3, 8.6, 10.9, 18.9, 22.4, and 27.5 min, respectively.

(DS: H. Dinh.)

Correspondence Number—C141757

Comment deadline: May 31, 2015

Add the following:■ **Hesperidin**

$C_{28}H_{34}O_{15}$ 610.57

4*H*-1-Benzopyran-4-one, 7-[[6-*O*-(6-deoxy- α -L-mannopyranosyl)- β -D-glucopyranosyl]oxy]-2,3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-, (2*S*)-; (*S*)-5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxo-3,4-dihydro-2*H*-chromen-7-yl 6-*O*-(α -L-rhamnopyranosyl)- β -D-glucopyranoside [520-26-3].

DEFINITION

Hesperidin contains NLT 90.0% and NMT 102.0% of hesperidin ($C_{28}H_{34}O_{15}$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY● **Procedure**

Solution A: Dilute 5 mL of glacial acetic acid with water to 1000 mL.

Mobile phase: Methanol and *Solution A* (30:70)

System suitability solution: 0.2 mg/mL each of USP Isonaringin RS, USP Hesperidin RS, USP Neohesperidin RS, USP Diosmin RS, and USP Didymin RS in dimethyl sulfoxide

Standard solution: 1.0 mg/mL of USP Hesperidin RS in dimethyl sulfoxide

Sample solution: 1.0 mg/mL of Hesperidin in dimethyl sulfoxide

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 284 nm

Column: 3.9-mm × 15-cm; 4-μm packing L1

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 10 μL

Run time: At least 5 times the retention time of hesperidin

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for hesperidin and its related compounds are shown in *Table 1*.]

Suitability requirements

Resolution: NLT 1.8 between hesperidin and neohesperidin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of hesperidin (C₂₈H₃₄O₁₅) in the portion of Hesperidin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Hesperidin RS in the *Standard solution* (mg/mL)

C_U concentration of Hesperidin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–102.0% on the dried basis

IMPURITIES● **Residue on Ignition** 〈 281 〉

Sample: 1.0 g

Acceptance criteria: NMT 0.2%

● **Related Compounds**

Solution A, Mobile phase, System suitability solution, Chromatographic system, and

System suitability: Proceed as directed in the *Assay*.

Standard solution: 0.05 mg/mL of USP Hesperidin RS in dimethyl sulfoxide

Sample solution: 1.0 mg/mL of Hesperidin in dimethyl sulfoxide

Analysis**Samples:** *Standard solution and Sample solution*

Calculate the percentage of each impurity in the portion of Hesperidin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_S = peak response of hesperidin from the *Standard solution* C_S = concentration of USP Hesperidin RS in the *Standard solution* (mg/mL) C_U = concentration of Hesperidin in the *Sample solution* (mg/mL) F = correction factor for each individual impurity (see *Table 1*)**Acceptance criteria:** See *Table 1*. [Note—Disregard any impurity less than 0.1%.]**Table 1**

Name	Relative Retention Time	Correction Factor (F)	Acceptance Criteria, NMT (%)
Eriocitrin ^a	0.4	1.00	1.0
Isonaringin ^b	0.7	1.07	4.0
Hesperidin	1.0	—	—
Neohesperidin ^c	1.2	0.91	1.0
Diosmin ^d	1.5	1.67	1.0
Naringenin ^e	2.6	0.51	1.0
Didymin ^f	3.0	1.02	3.0
Hesperetin ^g	3.8	0.45	1.0
Any unspecified impurity	—	1.00	1.0
Total unspecified impurities	—	—	3.0
Total impurities	—	—	10.0

^a (S)-5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxo-3,4-dihydro-2H-chromen-7-yl 6-O-(α -l-rhamnopyranosyl)- β -d-glucopyranoside.

^b (S)-5-Hydroxy-2-(4-hydroxyphenyl)-4-oxo-3,4-dihydro-2H-chromen-7-yl 6-O-(α -l-rhamnopyranosyl)- β -d-glucopyranoside.

^c (S)-5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxo-3,4-dihydro-2H-chromen-7-yl 2-O-(α -l-rhamnopyranosyl)- β -d-glucopyranoside.

^d 7-[[6-O-(6-Deoxy- α -l-mannopyranosyl)- β -d-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-one.

^e (S)-5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one.

^f (S)-5-Hydroxy-2-(4-methoxyphenyl)-4-oxo-3,4-dihydro-2H-chromen-7-yl 6-O-(α -l-

rhamnopyranosyl)- β -d-glucopyranoside.

9 (S)-5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one.

SPECIFIC TESTS

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 5.0%

- **Microbial Enumeration Tests** 〈 2021 〉: The total bacterial count does not exceed 10³ cfu/g, and the total combined molds and yeasts count does not exceed 10² cfu/g.

- **Absence of Specified Microorganisms** 〈 2022 〉: It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, tight containers.

- **USP Reference Standards** 〈 11 〉

USP Didymin RS

USP Diosmin RS

USP Hesperidin RS

USP Isonaringin RS

USP Neohesperidin RS

■ 1S (USP39)

BRIEFING

Krill Oil, USP 38 page 6115. On the basis of comments received, the following changes are proposed:

1. Add the content of myristic acid and palmitic acid, and the ratio of palmitic acid to myristic acid content to *Table 1* of the *Fatty Acid Profile* in the *Identification* section.
2. Change the upper limits and lower limits for several fatty acids listed in *Table 1* to reflect new data recently received.
3. Change the range of total phospholipids content from 28%–52% to 30%–55% and the range of phosphatidylcholine from 60%–90% (w/w) to 60%–96% (w/w) of the total phospholipids content in the *Acceptance criteria* for the *Content of Total Phospholipids* test.
4. Change the range of phosphatidylcholine from 60%–90% (w/w) to 60%–96% (w/w) of the total phospholipids content in *Identification* test *B—Phospholipid Profile*.
5. Add a table of molecular weight values and approximate chemical shift values (in reference to triphenyl phosphate) for the phospholipids of importance to krill oil analysis in the test for *Total Phospholipids* in order to clarify the proper use of the NMR data obtained. The values proposed are based on data and comments received. Additionally, equations are included for the calculations required by this test.
6. Remove the tests for *Acid Value* and *Unsaponifiable Matter* in the *Specific Tests* section that provide minimum added value to the monograph.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(DS: H. Dinh.)

Correspondence Number—C153348

Comment deadline: May 31, 2015

Krill Oil

DEFINITION

Change to read:

Krill Oil is the fixed oil extracted from Antarctic krill (*Euphausia superba* Dana) biomass using appropriate food-grade organic solvents. Krill Oil contains ~~NLT 28% (w/w) and NMT 52% (w/w) of total phospholipids, of which 60% to 90% is phosphatidylcholine.~~

■ NLT 30% (w/w) and NMT 55% (w/w) of total phospholipids, of which 60%–96% is phosphatidylcholine. ■ 1S (*USP39*)

It contains NLT 10% (w/w) of eicosapentaenoic acid (EPA) and NLT 5.0% (w/w) of docosahexaenoic acid (DHA)

■ mostly ■ 1S (*USP39*)

in the form of phospholipids. It also contains NLT 0.01% of astaxanthin.

IDENTIFICATION

Change to read:

● **A. Fatty Acid Profile**

Antioxidant solution, System suitability solution 1, and Chromatographic system:

Proceed as directed in *Fats and Fixed Oils* { 401 }, *Omega-3 Fatty Acids Determination and Profile*.

Standard solution: Prepare as directed in *Test Solution 1* in *Fats and Fixed Oils* { 401 }, *Omega-3 Fatty Acids Determination and Profile*, except use 250 mg of USP Krill Oil RS.

Sample solution: Prepare as directed in the *Standard solution*, except replace USP Krill Oil RS with Krill Oil.

System suitability

Samples: *System suitability solution 1* and *Standard solution*

Suitability requirements

Chromatogram similarity: The chromatogram obtained from the *Standard solution* is similar to the reference chromatogram provided with the lot of USP Krill Oil RS being used.

Resolution: NLT 1.0 between the methyl oleate and methyl *cis*-vaccinate peaks, *Standard solution*

Theoretical area percentages: Meets the requirements for *System suitability solution 1*

Analysis

Sample: *Sample solution*

Identify the retention times of the relevant fatty acid methyl esters in the *Sample solution*

by comparing the chromatogram of the *Sample solution* with that of the *Standard solution* and the USP reference chromatogram.

Calculate the area percentage for each fatty acid as methyl esters in the portion of Krill Oil taken:

$$\text{Result} = (r_A/r_B) \times 100$$

r_A peak area of each individual fatty acid from the *Sample solution*

r_B total area from all peaks, except the solvent and butylated hydroxytoluene peaks, from the *Sample solution*

Acceptance criteria: The fatty acids obtained from the *Sample solution* meet the limit requirements in *Table 1*.

Table 1

Fatty Acid	Shorthand Notation	Lower Limit (Area %)	Upper Limit (Area %)
■ Saturated fatty acids ■ 1S (USP39)			
■ Myristic acid	14:0	6.4	13.0
Palmitic acid	16:0	17.0	24.6
Palmitic acid:myristic acid ratio	16:0/14:0	1.6	2.8 ■ 1S (USP39)
Monounsaturated fatty acids			
Palmitoleic acid	16:1 n-7	2.5	9.0
			7.0
<i>cis</i> -Vaccenic acid	18:1 n-7	4.7	■ 8.0 ■ 1S (USP39)
Oleic acid	18:1 n-9	7.0	14.5
			1.2
Eicosenic acid	20:1 n-9	0.1	■ 1.7 ■ 1S (USP39)
			0.9
Erucic acid	22:1 n-9	0.0	■ 1.3 ■ 1S (USP39)
Polyunsaturated fatty acids			
Linoleic acid	18:2 n-6	1.4	3.0
			3.5
α-Linolenic acid	18:3 n-3	0.5	■ 5.0 ■ 1S (USP39)
			7.2
Morotic acid	18:4 n-3	1.8	■ 10.0 ■ 1S (USP39)
			22.1
Eicosapentaenoic acid	20:5 n-3	14.0	■ 24.3 ■ 1S (USP39)
Docosapentaenoic acid	22:5 n-3	0.0	0.7
			7.5
Docosahexaenoic acid	22:6 n-3	■ 7.1 ■ 1S (USP39)	■ 15.7 ■ 1S (USP39)
			13.2

Change to read:

- **B. Phospholipid Profile**

Solution A, Line shape standard (¹H), Sensitivity standard (¹H), Sensitivity standard (³¹P), Internal standard, Sample solution, Standard solution, Instrumental conditions, System suitability, and Analysis: Proceed as directed in the test for *Content of Total Phospholipids*.

Acceptance criteria: The *Sample solution* contains all of the following phospholipids: phosphatidylcholine (60%–90%

■ 96% ■ 1S (USP39)

(w/w) of the total phospholipids content), lysophosphatidylcholine (as a mixture of 1-lysophosphatidylcholine and 2-lysophosphatidylcholine), and phosphatidylethanolamine.

COMPOSITION

- **Content of EPA and DHA**

Standard solution 1a, Standard solution 1b, Standard solution 2a, Standard solution 2b, System suitability solution 1, and Chromatographic system: Proceed as directed in *Fats and Fixed Oils* { 401 }, *Omega-3 Fatty Acids Determination and Profile*.

Test solution 1: Prepare as directed in *Test Solution 1* in *Fats and Fixed Oils* { 401 }, *Omega-3 Fatty Acids Determination and Profile*, except use 250 mg of Krill Oil.

Test solution 2: Prepare as directed in *Test Solution 2* in *Fats and Fixed Oils* { 401 }, *Omega-3 Fatty Acids Determination and Profile*, except use 250 mg of Krill Oil.

Analysis: Proceed as directed in the *Analysis (for triglycerides)* in *Fats and Fixed Oils* { 401 }, *Omega-3 Fatty Acids Determination and Profile*.

Acceptance criteria: NLT 10.0% (w/w) of EPA and NLT 5.0% (w/w) of DHA

Change to read:

- **Content of Total Phospholipids**

(See *Nuclear Magnetic Resonance Spectroscopy* { 761 }, *Qualitative and Quantitative NMR Analysis*.)

[Note—All deuterated solvents used in this method should be NLT 99.8 atom % D. Whenever water is used in this method, it should be of sufficient quality to ensure that no trace metals or other contaminants that may affect the analysis are present.]

Solution A: 0.2 M EDTA, adjusted with a 1 M cesium carbonate solution to a pH of 7.2–7.5. Document the final pH and the amount of 1 M cesium carbonate solution necessary to attain the desired pH. [Note—Use cesium carbonate of a sufficient grade for trace metals analysis.]

Line shape standard (¹H): 1% Chloroform in acetone-d₆

Sensitivity standard (¹H): 0.1% Ethylbenzene in chloroform-d

Sensitivity standard (³¹P): 0.0485 M triphenyl phosphate in acetone-d₆

Internal standard: Use a suitable triphenyl phosphate NMR analytical standard with purity NLT 99.0%.

Sample solution: [Note—NMR solvents containing tetramethylsilane (TMS) are readily available. If the solvents used do not contain TMS, it must be added to the *Sample solution* at an approximate concentration of 0.05% (v/v) for use as a chemical shift scale reference.] Transfer 300–350 mg of Krill Oil to a 5-mL sealable glass vial. Add 25.0 mg of

the *Internal standard* to the vial. Add 1 mL each of deuterated chloroform (chloroform-d) and deuterated methanol (methanol-d₄) of a grade suitable for NMR analysis to the vial to dissolve the sample. Once dissolution is complete, add 1 mL of *Solution A*, seal the vial, and shake the solution for 10–20 min, then centrifuge the contents of the vial. Transfer the lower organic phase to an appropriate NMR tube. It is critical to collect the entire organic phase and transfer it to the NMR tube. It may be unavoidable to also transfer small amounts of the aqueous phase when collecting the organic phase in the NMR tube. This is acceptable practice, so long as the aqueous phase remains completely separated and atop the organic phase in the NMR tube. The entire amount of aqueous phase must be above the probe's radio frequency (RF) coil (outside the analysis area of the tube). Should the organic phase contain undissolved materials, they must remain suspended at the aqueous-organic interface and be outside the analysis area of the tube as well. The organic phase must be free of bubbles and suspended materials that may interfere with NMR data acquisition.

Standard solution: Prepare as directed in the *Sample solution*, using 300–350 mg of USP Krill Oil RS in place of the sample.

Instrumental conditions

(See *Nuclear Magnetic Resonance Spectroscopy* { 761 }.)

Magnetic field strength: NLT 300 MHz for ¹H frequency

Probe: Direct observe probe capable of tuning to the resonance frequency of ³¹P (dependent on the specific magnetic field strength used)

Instrument performance qualification

[Note—Testing for sensitivity and line shape should be performed on the interval specified by the manufacturer of the instrument used. Performing these tests on a minimum of a monthly basis is required for this method, but may be done more often, as required. Resolution testing is to be performed during each analysis and documented as a part of the analytical results.]

¹H Line shape test: Using the *Line shape standard* (¹H) and the protocol recommended by the instrument manufacturer, the instrument must achieve the line shape specifications for the probe in use, as required by the instrument manufacturer. [Note—A different standard solution may be required or recommended by the manufacturer of the instrument; 1% chloroform in acetone-d₆ is most commonly used.]

¹H Sensitivity test: Using the *Sensitivity standard* (¹H) and the protocol recommended by the instrument manufacturer, the instrument must achieve the sensitivity specifications required by the instrument manufacturer. [Note—A different standard solution may be required or recommended by the manufacturer of the instrument; 0.1% ethylbenzene in chloroform-d is most commonly used.]

~~**³¹P Resolution test:** The resolution is demonstrated using the phosphatidylcholine ether peak and the phosphatidylcholine peak. The separation of these peaks (with line broadening factor of 1.0) must be demonstrated as follows. Using the baseline as a reference, determine the total peak height of the phosphatidylcholine ether peak, and draw a line at 30% of that total peak height (intensity). The phosphatidylcholine ether peak and the neighboring phosphatidylcholine peak must be fully resolved at a point that is NMT 30% of the peak height of the phosphatidylcholine ether peak.~~

³¹P Sensitivity test: Using the *Sensitivity standard* (³¹P) and the protocol recommended by the instrument manufacturer, the instrument must achieve the

sensitivity specifications required by the instrument manufacturer. [Note—A different standard solution may be required or recommended by the manufacturer of the instrument; 0.0485 M triphenyl phosphate in acetone-d is most commonly used.]

■ 1S (USP39)

¹H Resolution test: The resolution is demonstrated by the ability to detect both of the ²⁹Si satellite signals of TMS. The satellites must be resolved from the TMS signal in the spectrum with a line broadening factor of NMT 0.5 ppm.

³¹P Resolution test: The resolution is demonstrated using the phosphatidylcholine ether peak and the phosphatidylcholine peak. The separation of these peaks (with

■ an applied ■ 1S (USP39)

line broadening factor of 1.0) must be demonstrated as follows. Using the baseline as a reference, determine the total peak height of the phosphatidylcholine ether peak

■ [Note—The PC ether signal appears just downfield from the PC signal.] ■ 1S (USP39)

, and draw a line at 30% of that total peak height (intensity). The phosphatidylcholine ether peak and the neighboring phosphatidylcholine peak must be fully resolved at a point that is NMT 30% of the peak height of the phosphatidylcholine ether peak.

Data collection: Use the parameters specified in Table 2. Use 90 degree pulses, and calibrate pulses before use according to the recommendations supplied by the instrument manufacturer.

Table 2

Parameter	³¹ P NMR Quantitative Measurement	¹ H NMR Qualitative Measurement
Pulse program	¹ H-decoupled ³¹ P ■ (inverse gated) ■ 1S (USP39)	Single pulse ¹ H
Spectral width	50 ppm (25 ppm to -25 ppm)	20 ppm (-3 ppm to 17 ppm)
Transmitter offset	Center of spectral width, 0 ppm	Center of spectral width, 7 ppm
Relaxation delay	2–5 s ■ 5–15 s ■ 1S (USP39)	2–5 s
Acquisition time	1–6 s	1–6 s
Sum of relaxation delay and acquisition time	NLT 15 s ■ 1S (USP39)	NLT 15 s ■ 1S (USP39)
Size of data set	NLT 64k (32k with zero-filling)	NLT 64k (32k with zero-filling)

[Note—The acquisition time is dependent upon the field strength and the time domain
■ the dwell time and the number of data points collected. ■ 1S (USP39)

The number of scans acquired using a 300 MHz instrument must be NLT 512.]

System suitability: Under the conditions outlined in *Data collection*, the ^{31}P NMR signal of triphenyl phosphate should be observed at -17.80 ppm, and the ^1H NMR spectrum should be referenced to the ^1H signal of TMS (0 ppm) for all spectra acquired in the *Analysis*. For quantitative analysis, a sufficient number of scans should be acquired such that the signal-to-noise ratio for the phosphatidylcholine signal in the ^{31}P spectrum of the *Sample solution* acquired in the *Analysis* is NLT 2000.

Analysis: Acquire the data outlined in *Data collection*. Minimally acquire the ^1H spectrum (fingerprint) of the *Sample solution* and the *Standard solution* as well as the quantitative ^{31}P spectrum of the *Sample solution* and the *Standard solution*. Record the resulting spectra, and perform integration by hand or automated means on the quantitative ^{31}P NMR spectrum of the *Sample solution*. Integration of the peaks contained in the spectrum of the *Sample solution* must be performed such that the complete set of phospholipid peaks (as identified by comparison to the spectrum of the *Standard solution* and its reference spectrum) is included in the integration. The integration region for each signal must extend ± 0.05 ppm on either side of the ^{31}P signal. Quantify the total phospholipids present, the phosphatidylcholine ether content, and the phosphatidylcholine content in the *Sample solution* using comparison to the concentration of the *Internal standard*.

Compare the ^1H spectrum of the *Sample solution* to that of the *Standard solution* to determine the similarity of fingerprints according to which phospholipids identified in the reference spectrum of the *Standard solution* are present in the spectrum of the *Sample solution*.

■ **Calculations:** Use the following equations and molecular weights listed in *Table 3* to determine the phospholipids content in the sample taken:

$$\text{mmol}_{IS} = (W_{IS} \times C_{IS}) / (MW_{IS} \times 100)$$

mmol_{IS}

= millimoles of the *Internal standard* in the *Sample solution* (mmol)

W_{IS}

= weight of the *Internal standard* added to the *Sample solution* (mg)

C_{IS} = purity value of the *Internal standard*, based on quantitative ^{31}P NMR analysis (% by weight)

MW_{IS} = molecular weight of the *Internal standard* (326.28 g/mol for triphenyl phosphate)

$$\text{mmol}_{PL} = (I_{PL} \times A_{IS} \times \text{mmol}_{IS}) / (I_{IS} \times A_{PL})$$

mmol_{PL} = millimoles of the phospholipid of interest in the *Sample solution* (mmol)

I_{PL} = integrated area under the phospholipid signal of interest obtained from the spectrum of the *Sample solution*

A_{IS} = number of phosphorus atoms per molecule expected from the *Internal standard* (1 for triphenyl phosphate)

mmol_{IS} = millimoles of the *Internal standard* in the *Sample solution*

I_{IS} = integrated area under the *Internal Standard* obtained from the spectrum of the *Sample solution*

A_{PL} = number of phosphorus atoms per molecule expected from the phospholipid of interest (1 for any phospholipid listed in *Table 3*)

$$C_{PL} = (MW_{PL} \times \text{mmol}_{PL} \times 100) / W_S$$

C_{PL} = concentration of the phospholipid of interest in the *Sample solution* (% w/w)

MW_{PL} = molecular weight of the phospholipid of interest (g/mol, from *Table 3*) in the *Sample solution* (mg)

mmol_{PL} = millimoles of the phospholipid of interest in the *Sample solution* (mmol)

W_S = weight of sample present in the *Sample solution* (mg)

[Note—Use the molecular weight specified in *Table 3* for the calculations.]

Table 3

Component	Approximate chemical shift (ppm) in reference to triphenyl phosphate	Molecular weight (g/mol)
Triphenyl phosphate (Internal standard)	−17.8	—
Phosphatidylcholine (PC)	−0.89	791
1-Lysophosphatidylcholine (1-LPC) ^a	−0.48	534.5
2-Lysophosphatidylcholine (2-LPC) ^a	−0.4	534.5
Phosphatidylethanolamine (PE)	−0.24	770
<i>N</i> -Acylphosphatidylethanolamine (NAPE)	0	1032
Lysophosphatidylethanolamine (LPE)	0.25	492.5
Other	—	800

^a Ability to resolve the signals of 1-lysophosphatidylcholine and 2-lysophosphatidylcholine will depend upon the applied magnetic field strength of the NMR spectrometer used for the test procedure.

■ 1S (USP39)

Acceptance criteria

Total phospholipids: 28%–52%

■ 30%–55% ■ 1S (USP39)

(w/w)

Phosphatidylcholine: 60%–90%

■ 96% ■ 1S (USP39)

(w/w) of the *Total phospholipids* content● **Content of Astaxanthin**

[Note—Perform this analysis in subdued light, using low-actinic glassware.]

Sample solution: 0.005 g/mL in chloroform. [Note—If the solution is not clear, centrifuge it with an appropriate centrifuge to obtain a clear supernatant.]**Instrumental conditions**(See *Spectrophotometry and Light-Scattering* { 851 } .)**Analytical wavelength:** 486 nm**Cell path:** 1 cm**Blank:** Chloroform**Analysis****Sample:** *Sample solution*

Calculate the percentage of astaxanthin in the portion of Krill Oil taken:

$$\text{Result} = A / (F \times C)$$

A = absorbance of the *Sample solution*F = coefficient of extinction ($E_{1\%}$) of pure astaxanthin in chloroform (100 mL·g₋₁·cm₋₁),
1692C = concentration of the *Sample solution* (g/mL)**Acceptance criteria:** NLT 0.01%**CONTAMINANTS****Delete the following:**● **Fats and Fixed Oils** { 401 } : NMT 0.1 ppm each of Pb, Cd, inorganic As, and Hg ■ 1S (USP39)● **Limit of Dioxins, Furans, and Polychlorinated Biphenyls****Analysis:** Determine the content of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by method No. 1613 revision B of the Environmental Protection Agency. Determine the content of polychlorinated biphenyls (PCBs) by method No. 1668 revision A of the Environmental Protection Agency.**Acceptance criteria:** The sum of PCDDs and PCDFs is NMT 2.0 pg/g of WHO toxic equivalents. The sum of PCDDs, PCDFs, and dioxin-like PCBs (polychlorinated biphenyls, non-ortho IUPAC congeners PCB-77, PCB-81, PCB-126, and PCB-169, and mono-ortho IUPAC congeners PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, and PCB-189) is NMT 3.0 pg/g of WHO toxic equivalents.**SPECIFIC TESTS**● **Astaxanthin Esterification****Standard solution A:** 10 mg/mL of USP Astaxanthin Esters from *Haematococcus pluvialis* RS in acetone**Standard solution B:** 10 mg/mL of USP Astaxanthin (Synthetic) RS in acetone

Sample solution: 250 mg/mL of Krill Oil in acetone

Chromatographic system

(See *Chromatography* { 621 }, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel. [Note—Dry silica gel at 110° for 1 h before use.]

Application volume: 5 µL

Developing solvent system: Hexane and acetone (70:30)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about 15 cm of the length of the plate. Remove the plate from the chamber, and allow to dry.

Acceptance criteria: The principal spot from *Standard solution B*, located in the bottom half of the plate, is free astaxanthin. The *Sample solution* may exhibit a light, minor spot, in the same location. The principal spots from *Standard solution A* are from monoesters (primary spot, located slightly above the middle of the plate) and diesters (secondary spot, located in the top third of the plate). The principal spot from the *Sample solution* should correspond in color and R_F value to the diester spot from *Standard solution A*. The secondary spot from the *Sample solution* should correspond in color and approximately the same R_F value to the monoester spot from *Standard solution A*. [Note—Slight differences in R_F values within monoester spots and within diester spots may exist because of different intensities.]

Delete the following:

■ ~~Fats and Fixed Oils, Acid Value { 401 } : 170–190 ■ 1S (USP39)~~

● **Fats and Fixed Oils, Peroxide Value { 401 } :** NMT 5.0 mEq peroxide/kg

Delete the following:

■ ~~Fats and Fixed Oils, Unsaponifiable Matter { 401 } : NMT 1.5% ■ 1S (USP39)~~

ADDITIONAL REQUIREMENTS

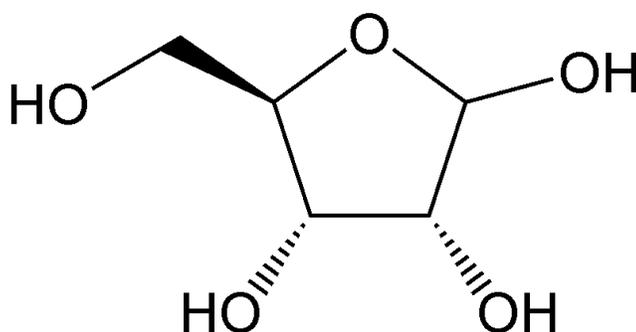
- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature. It may be bottled or otherwise packaged in containers from which air has been expelled by production of a vacuum or by an inert gas.
- **Labeling:** The label states the average content of DHA and EPA in mg/g. It also states the name and concentration of any added antioxidant.
- **USP Reference Standards { 11 }**
 - USP Astaxanthin Esters from *Haematococcus pluvialis* RS
 - USP Astaxanthin (Synthetic) RS
 - USP Docosahexaenoic Acid Ethyl Ester RS
 - USP Eicosapentaenoic Acid Ethyl Ester RS
 - USP Krill Oil RS

USP Methyl Tricosanoate RS **BRIEFING**

Ribose. Because there is no existing *USP* monograph for this dietary ingredient, a new monograph is being proposed. The liquid chromatographic procedures in the *Assay* and the test for *Related Compounds* are based on analyses performed with the Shodex Sugar KS-801 brand of L22 column. Typical retention times for arabinose and ribose are 9.0 and 9.8 min, respectively.

(DS: H. Dinh.)

Correspondence Number—C151188

Comment deadline: May 31, 2015**Add the following:****■ Ribose**C₅H₁₀O₅ 150.13

(2*S*,3*R*,4*S*,5*R*)-5-(Hydroxymethyl)oxolane-2,3,4-triol;
d-Ribose [50-69-1].

DEFINITION

Ribose contains NLT 98.0% and NMT 102.0% of d-ribose (C₅H₁₀O₅), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** { 197K }
- **B.** It meets the requirements in *Specific Tests for Optical Rotation* { 781S }, *Specific Rotation*.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **Procedure****Mobile phase:** Degassed water**System suitability solution:** 20 mg/mL of USP Ribose RS and 0.2 mg/mL of USP Arabinose RS in *Mobile phase*

Standard solution: 20 mg/mL of USP Ribose RS in *Mobile phase*

Sample solution: 20 mg/mL of Ribose in *Mobile phase*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 8.0-mm × 30-cm; 6-μm packing L22

Temperatures

Detector: 40°

Column: 80°

Flow rate: 1.0 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for arabinose and ribose are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.2 between ribose and arabinose, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Column efficiency: NLT 2500 theoretical plates for the ribose peak, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of d-ribose in the portion of Ribose taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ribose RS in the *Standard solution* (mg/mL)

C_U = concentration of Ribose in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.2%
- **Chloride and Sulfate, Chloride** 〈 221 〉
Standard: 0.10 mL of 0.020 N hydrochloric acid
Sample: 3.6 g of Ribose
Acceptance criteria: NMT 0.002%
- **Chloride and Sulfate, Sulfate** 〈 221 〉
Standard: 0.10 mL of 0.020 N sulfuric acid
Sample: 3.3 g of Ribose
Acceptance criteria: NMT 0.003%
- **Related Compounds**

Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Standard solution: 0.02 mg/mL of USP Arabinose RS in *Mobile phase*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of arabinose in the portion of Ribose taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of arabinose from the *Sample solution*

r_S peak response of arabinose from the *Standard solution*

C_S concentration of USP Arabinose RS in the *Standard solution* (mg/mL)

C_U concentration of Ribose in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Ribose taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of any unspecified impurity from the *Sample solution*

r_T sum of all the peak responses from the *Sample solution*

Acceptance criteria

Arabinose: NMT 1.0%

Unspecified impurity: NMT 0.1%

Total unspecified impurities: NMT 1.0%

SPECIFIC TESTS

- **Optical Rotation, Specific Rotation** 〈 781S 〉

Sample solution: 20 mg/mL in water

Acceptance criteria: -18.0° to -22.0°

- **Color of Solution**

Sample solution: Dissolve 5.0 g of Ribose in 50 mL of water. Centrifuge or filter, if necessary, to obtain a clear solution.

Blank solution: Water

Analysis: Absorbance at 430 nm in a 1-cm cell

Acceptance criteria: NMT 0.2 AU

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight light-resistant containers.

- **USP Reference Standards** 〈 11 〉

USP Arabinose RS

USP Ribose RS

■ 1S (USP39)

BRIEFING

meso-Zeaxanthin, *USP 38* page 6479. On the basis of comments received, it is proposed to modify the *Stereoisomeric Composition* test to improve the resolution of three zeaxanthin isomers: (3*S*,3'*S*)-zeaxanthin, (3*R*,3'*S*)-*meso*-zeaxanthin, and (3*R*,3'*R*)-zeaxanthin.

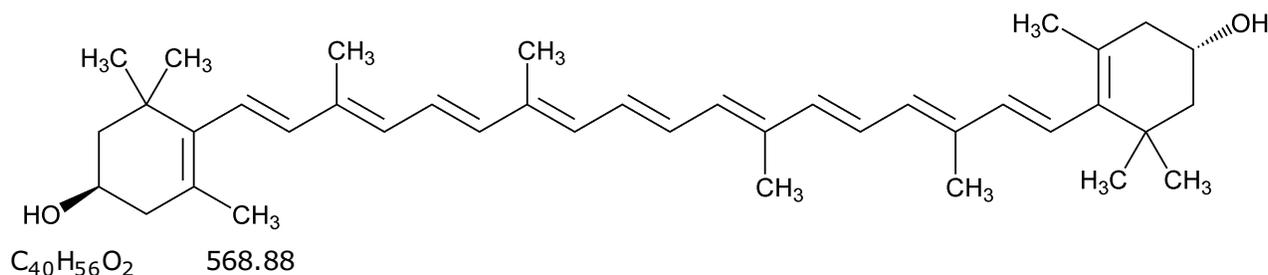
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(DS: H. Dinh.)

Correspondence Number—C155285

Comment deadline: May 31, 2015

meso-Zeaxanthin



β,β -Carotene-3,3'-diol (3*R*,3'*S*)-;
(3*R*,3'*S*)-*meso*-Zeaxanthin [31272-50-1].

DEFINITION

meso-Zeaxanthin consists chiefly of the 3*R*,3'*S*-isomer of zeaxanthin. It contains NLT 80.0% of total carotenoids calculated as zeaxanthin ($C_{40}H_{56}O_2$) and NLT 74.0% of zeaxanthin ($C_{40}H_{56}O_2$) on the anhydrous basis.

IDENTIFICATION

- **A.**
Sample solution: Use the *Sample solution* of the test for *Content of Total Carotenoids*.
Analysis: Record the UV-Vis spectrum from 300–600 nm.
Acceptance criteria: The *Sample solution* shows a shoulder at about 427 nm and a maximum absorption at about 453 nm and at about 480 nm.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Content of Zeaxanthin*.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of (3*R*,3'*S*)-*meso*-zeaxanthin from the *Standard solution*, as obtained in the test for *Stereoisomeric Composition*.

COMPOSITION

• **Content of Total Carotenoids**

[Note—Use low-actinic glassware.]

Sample stock solution: Transfer 25.0 mg of *meso*-Zeaxanthin to a 100-mL volumetric flask, add 20 mL of chloroform, and place the flask in an ultrasonic bath at 30° for 2–5 min

to obtain a clear solution. Dilute with cyclohexane to volume to obtain a solution containing 250 µg/mL.

Sample solution: 2.5 µg/mL of the *Sample stock solution* in cyclohexane

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* { 851 } .)

Analytical wavelength: 453 nm

Cell: 1 cm

Blank: Cyclohexane

Analysis

Sample: *Sample solution*

Calculate the percentage of total carotenoids as zeaxanthin (C₄₀H₅₆O₂) in the portion of *meso*-Zeaxanthin taken:

$$\text{Result} = A / (C \times F)$$

A = absorbance of the *Sample solution*

C = concentration of the *Sample solution* (g/mL)

F = coefficient of extinction (E_{1%}) of zeaxanthin in cyclohexane (100 mL·g₋₁·cm₋₁), 2540

Acceptance criteria: NLT 80.0% of total carotenoids (*T*) as zeaxanthin (C₄₀H₅₆O₂) on the anhydrous basis

• **Content of Zeaxanthin**

[Note—Use low-actinic glassware.]

Mobile phase: Hexane and ethyl acetate (75:25)

Standard solution: 150 µg/mL of USP *meso*-Zeaxanthin RS prepared as follows. Dissolve 15.0 mg of USP *meso*-Zeaxanthin RS in 10 mL of chloroform, swirling briefly, and dilute with *Mobile phase* to 100 mL.

Sample solution: Transfer 15.0 mg of sample to a 100-mL volumetric flask, add 10 mL of chloroform, and place the flask in an ultrasonic bath at 30° for 2–5 min to obtain a clear solution. Dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: 453 nm

Column: 4.6-mm × 25-cm; 3-µm packing L3

Column temperature: 25°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[Note—The approximate relative retention times for lutein and zeaxanthin are 0.95 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.0 between zeaxanthin and lutein

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis: Inject the *Sample solution*, and measure the peak areas. [Note—The peak response of zeaxanthin is NLT 90.0% of the sum of all the peak responses.]

Calculate the percentage of zeaxanthin ($C_{40}H_{56}O_2$) in the sample taken:

$$\text{Result} = (r_U/r_T) \times T$$

r_U peak response of zeaxanthin

r_T sum of all the peak responses

T = percentage of total carotenoids as determined in the test for *Content of Total Carotenoids*

Acceptance criteria: NLT 74.0% of zeaxanthin ($C_{40}H_{56}O_2$) on the anhydrous basis

• **Lutein and Other Related Compounds**

[Note—Use low-actinic glassware.]

Mobile phase, Standard solution, Sample solution, and Chromatographic system:

Proceed as directed in the test for *Content of Zeaxanthin*.

Analysis

Sample: *Sample solution*

[Note—The peak response of lutein is NMT 9.0% of the sum of all the peak responses.]

Calculate the percentage of lutein in the portion of *meso*-Zeaxanthin taken:

$$\text{Result} = (r_U/r_T) \times T$$

r_U peak response of lutein

r_T sum of all the peak responses

T = percentage of total carotenoids as determined in the test for *Content of Total Carotenoids*

Calculate the percentage of other related compounds in the portion of *meso*-Zeaxanthin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U individual peak response of any other peak (excluding zeaxanthin and lutein)

r_T sum of all the peak responses

Acceptance criteria

Lutein: NMT 8.5%

Other related compounds: NMT 1.0% of any other individual related compound

Change to read:

• **Stereoisomeric Composition**

■ [

Note—Use low-actinic glassware.] ■ 1S (USP39)

Mobile phase: Hexane, alcohol, and isopropanol (80:5:5)

■ Gradient elution (see *Table 1*)

Table 1

Time (min)	<i>n</i> -Hexane (%)	2-Propanol (%)
0.0	95	5
50	95	5
55	50	50
63	50	50
65	95	5
75	95	5

■ 1S (USP39)

Standard solution: 0.1 mg/mL of USP *meso*-Zeaxanthin RS in alcohol and hexane (1:1).

Dissolve in 50% of the final volume with alcohol in an ultrasonic bath at 60° for 2–5 min, cool the flask, and dilute with hexane to volume. Pass through a 0.45-µm pore size membrane filter.

■ Transfer 2.5 mg of USP *meso*-Zeaxanthin RS to a 50-mL volumetric flask, add 25 mL of dehydrated alcohol, and sonicate at 60° for 2–5 min to dissolve the substance. Cool the flask to room temperature and dilute with *n*-hexane to volume. Transfer 1.0 mL of the resultant solution to a 15-mL test tube, and evaporate with a stream of nitrogen to dryness. Dissolve the residue in a 10.0-mL mixture of 2-propanol and *n*-hexane (5:95). Pass through a membrane filter of 0.45-µm pore size. ■ 1S (USP39)

Sample solution: Weigh 10 mg of *meso*-Zeaxanthin into a 100-mL volumetric flask, add 50 mL of alcohol, and place the flask in an ultrasonic bath at 60° for 2–5 min to dissolve. Cool the flask, and dilute with hexane to volume. Pass the solution through a 0.45-µm pore size membrane filter.

■ Transfer 2.5 mg of *meso*-Zeaxanthin to a 50-mL volumetric flask, add 25 mL of dehydrated alcohol, and sonicate at 60° for 2–5 min to dissolve the substance. Cool the flask to room temperature and dilute with *n*-hexane to volume. Transfer 1.0 mL of the resultant solution to a 15-mL test tube, and evaporate with a stream of nitrogen to dryness. Dissolve the residue in a 10.0-mL mixture of 2-propanol and *n*-hexane (5:95). Pass through a membrane filter of 0.45-µm pore size. ■ 1S (USP39)

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: HPLC

Detector: 453 nm

Column: 4.6-mm × 25-cm; 5-µm packing L51

Column temperature: 35°

■ 30° ■ 1S (USP39)

Flow rate: 0.5 mL/min

■ 0.8 mL/min ■ 1S (USP39)

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

[Note—The approximate relative retention times for (3*S*,3'*S*)-zeaxanthin, (3*R*,3'*S*-*meso*)-zeaxanthin, (3*R*,3'*R*)-zeaxanthin, and (3*R*,3'*R*,6'*R*)-lutein are 0.94, 1.00, 1.06, and 1.11, respectively.]

Suitability requirements

Resolution: NLT 1.5 between each pair of peaks due to (3*S*,3'*S*)-zeaxanthin, (3*R*,3'*S*-*meso*)-zeaxanthin, (3*R*,3'*R*)-zeaxanthin, and (3*R*,3'*R*,6'*R*)-lutein

Chromatogram similarity: The chromatogram from the *Standard solution* is similar to the reference chromatogram provided with the lot of USP *meso*-Zeaxanthin RS being used.

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the peaks of the relevant analytes from the *Standard solution* by comparison with the reference chromatogram provided with the USP Reference Standard being used.

Calculate the percentages of (3*S*,3'*S*)-zeaxanthin, (3*R*,3'*S*-*meso*)-zeaxanthin, and (3*R*,3'*R*)-zeaxanthin:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of the corresponding analyte

r_T sum of all the peak responses

Acceptance criteria

(3*R*,3'*S*-*meso*)-Zeaxanthin: NLT 85.0%

(3*R*,3'*R*)-Zeaxanthin: NMT 15.0%

(3*S*,3'*S*)-Zeaxanthin: NMT 1.0%

IMPURITIES

- **Lead** $\langle 251 \rangle$: NMT 1 ppm
- **Residue on Ignition** $\langle 281 \rangle$: NMT 1.0%

SPECIFIC TESTS

- **Water Determination, Method I** $\langle 921 \rangle$: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

- **USP Reference Standards** $\langle 11 \rangle$

USP *meso*-Zeaxanthin RS

β, β -Carotene-3,3'-diol (3*R*,3'*S*)-;
(3*R*,3'*S*-*meso*)-Zeaxanthin.

$\text{C}_{40}\text{H}_{56}\text{O}_2$ 568.88

BRIEFING

Guar Gum, NF 33 page 6689. As part of the USP monograph modernization effort, it is proposed to making the following revisions:

1. Update the *Definition*.
2. Replace the test for *Content of Galactomannans*, which is based on subtracting from 100.0 the total percentages from the tests for *Articles of Botanical Origin*, *Total Ash*; *Acid-Insoluble Matter*; *Protein*; and *Loss on Drying*, with a liquid chromatographic method. Change the title to *Content of Galactomannans and Ratio of Constituting Mannose and Galactose*. The HPLC method was validated using the Shodex Sugar SP 0810 brand of L22 column. The glucose, xylose, galactose, and mannose peaks elute at approximately 11.4, 12.1, 12.9, and 14.2 min, respectively.
3. Add the *Acceptance criteria* of "1.4–2.2" in the test for *Ratio of Constituting Mannose and Galactose*.
4. Add USP Dextrose RS and USP Xylose RS to the *USP Reference Standards* section.

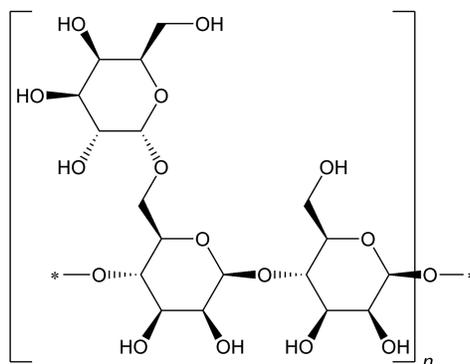
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(EXC: H. Wang.)

Correspondence Number—C122232

Comment deadline: May 31, 2015

Guar Gum



[9000-30-0].

DEFINITION

Change to read:

Guar Gum is the flour obtained by grinding the endosperms of seeds of *Cyamopsis tetragonolobus* (L.) Taub. (Fam. Leguminosae). It consists chiefly of high molecular weight hydrocolloidal polysaccharides composed of galactomannans

galactomannan. The content of galactomannan is NLT 66.0%. Galactomannan consists of a linear main chain of β -(1→4)-glycosidically linked mannopyranoses and single α -(1→6)-glycosidically linked galactopyranoses, and the ratio of the mannose and galactose is from 1.4:1 to 2.2:1. 1S (NF34)

IDENTIFICATION

• **A. Indication for a Polymeric Compound and Distinction from Locust Bean Gum**

Sample: 2 g

Analysis 1: Place the *Sample* in a 400-mL beaker, and moisten it with 4 mL of isopropyl

alcohol. Add 200 mL of cold water with vigorous stirring, and continue stirring until the *Sample* is completely and uniformly dispersed.

Acceptance criteria 1: An opalescent, viscous dispersion results.

Analysis 2: Transfer 100 mL of the sample dispersion prepared above to a 400-mL beaker, heat in a boiling water bath for about 10 min, and then cool to room temperature.

Acceptance criteria 2: No appreciable increase in viscosity is produced (distinction from locust bean gum: see *Reagents, Indicators, and Solutions—Reagent Specifications*).

• **B. Identification of Constituting Mannose and Galactose by Thin-Layer Chromatography**

Mobile phase: Acetonitrile and water (85:15)

Standard solution: Dissolve 10 mg of USP Galactose RS and 10 mg of USP Mannose RS in 2 mL of water, and dilute with methanol to 20 mL.

Sample solution: Transfer 20 mg of Guar Gum to a test tube, add 4 mL of a 100 mg/mL solution of trifluoroacetic acid, and shake vigorously to dissolve the forming gel. Stopper the tube, and heat the mixture at 115° for 1 h 20 min in a dry bath (heating block) or oil bath. Cool, transfer the hydrolysate to a centrifuge tube, and centrifuge. Some suspended particles/gel are formed. Pass the supernatant solution through a 0.45- μ m disc filter. Wash the test tube and the centrifuge tube with two 5-mL portions of water, and filter. Combine the washing filtrate with the filtered supernatant of the hydrolysate. Transfer the combined clear filtrate to a 50-mL flask, and evaporate the solution to dryness under reduced pressure. To the resulting residue add 0.2 mL of water and 1.8 mL of methanol.

Chromatographic system

(See *Chromatography* { 621 }, *Thin-Layer Chromatography*.)

Mode: TLC

Absorbent layer: 0.25-mm silica gel 60 F₂₅₄

Application volume: 5 μ L, as 9-mm bands, using an automated apparatus

Spray reagent: Dissolve 3 g of phthalic acid and 0.3 g of aminohippuric acid in ethyl alcohol, and dilute with ethyl alcohol to 100 mL.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop over a path of 15 cm. Spray with *Spray reagent*, and dry at 120° for 5 min.

Acceptance criteria: The chromatogram from the *Standard solution* shows, in the lower region, two clearly separated brownish or yellowish zones due to galactose and mannose in order of increasing R_F value. The chromatogram from the *Sample solution* shows two zones due to galactose and mannose.

ASSAY

Change to read:

• **Content of Galactomannans**

■ **Galactomannan and Ratio of Constituting Mannose and Galactose** ■ 1S (NF34)

Analysis: Subtract from 100.0 the total percentages from the tests for *Articles of Botanical Origin, Total Ash, Acid-Insoluble Matter, Protein, and Loss on Drying*.

■ **Mobile phase:** Water

System suitability solution: 5 mg/mL of USP Galactose RS, 5 mg/mL of USP Mannose RS, 5 mg/mL of USP Xylose RS, and 5 mg/mL of USP Dextrose RS in *Mobile phase*

Standard solution: 10 mg/mL of USP Galactose RS and 10 mg/mL of USP Mannose RS in *Mobile phase*

Sample solution A: Transfer 100 mg of Guar Gum to a glass test tube. Add 2.0 mL of water and 2.0 mL of 1 M trifluoroacetic acid to the tube, and mix on a vortex mixer for 30 s.

Incubate the solution at 105° in an oil-bath heating module for 6 h. After the first 15 min of incubation, mix on a vortex mixer for 30 s. After the 30 min of incubation, mix on a vortex mixer for 30 s. [Note—This ensures that gum doesn't stick to the bottom of the test tube and burn.] Before HPLC analysis, mix on a vortex mixer for 30 s, and pass the solution through a 0.45-µm PES (polyethersulfone) membrane syringe filter.

Sample solution B: 5 mg/mL of Guar Gum in *Mobile phase*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 8.0-mm × 30-cm; 7-µm packing L22

Temperatures

Detector: 55°

Column: 80°

Flow rate: 0.75 mL/min

Injection volume: 10 µL

Detector purge time: 1 min

Run time: 17 min

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for glucose, xylose, galactose, and mannose are 0.88, 0.94, 1.00, and 1.10, respectively.]

Suitability requirements

Resolution: NLT 0.9 between dextrose and xylose, NLT 1.0 between xylose and galactose, and NLT 1.5 between galactose and mannose, *System suitability solution*

Tailing factor: 0.8–1.8 for the galactose and mannose peaks, *Standard solution*

Relative standard deviation: NMT 2.0% for the galactose and mannose peaks, *Standard solution*

Analysis

Samples: *Standard solution*, *Sample solution A*, and *Sample solution B*

In the chromatogram of *Standard solution B*, no galactose and mannose peaks are observed.

Calculate the percentage of galactose (C_G) or mannose (C_M) in the portion of Guar Gum taken:

$$\text{Result } (C_G \text{ or } C_M) = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of galactose or mannose in *Sample solution A*

r_S

= peak response of galactose or mannose in the *Standard solution*

C_S

= concentration of USP Galactose RS or USP Mannose RS in the *Standard solution* (mg/mL)

C_U

= concentration of Guar Gum in *Sample solution A* (mg/mL)

Calculate the content of galactomannans in the portion of Guar Gum taken:

$$\text{Result} = C_M + C_G$$

Calculate the ratio of constituting mannose and galactose in the portion of Guar Gum taken:

$$\text{Result} = C_M/C_G$$

■ 1S (NF34)

Acceptance criteria NLT 66.0%

■ **Content of Galactomannan:** NLT 66.0%

Ratio of Constituting Mannose and Galactose: 1.4–2.2 ■ 1S (NF34)

IMPURITIES

● **Arsenic, Method II** 〈 211 〉 : NMT 3 µg/g

● **Lead** 〈 251 〉

Analysis: Prepare a *Test Preparation* as directed in the chapter, and use 10 mL of *Diluted Standard Lead Solution* (10 µg of Pb) for the test.

Acceptance criteria: NMT 10 µg/g

Delete the following:

●

● **Heavy Metals, Method II** 〈 231 〉

: NMT 20 µg/g • (Official 1-Dec-2015)

SPECIFIC TESTS

● **Articles of Botanical Origin, Total Ash** 〈 561 〉 : NMT 1.5%

● **Acid-Insoluble Matter**

Sample: 1.5 g

Analysis: Transfer the *Sample* to a 250-mL beaker containing 150 mL of water and 1.5 mL of sulfuric acid. Cover the beaker with a watch glass, and heat the mixture on a steam bath for 6 h, rubbing down the wall of the beaker frequently with a rubber-tipped stirring rod and replacing any water lost by evaporation. At the end of the 6 h heating period, add 500 mg, accurately weighed, of a filter aid, and pass through a tared, ashless filter. Wash the residue several times with hot water, dry the filter and its contents at 105° for 3 h, cool in a desiccator, and weigh. Determine the amount of acid-insoluble matter by subtracting the weight of the filter aid from that of the residue.

Acceptance criteria: NMT 7.0%

● **Microbial Enumeration Tests** 〈 61 〉 and **Tests for Specified Microorganisms** 〈 62 〉 : The

total aerobic microbial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g. It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*. It is recommended that the enrichment broth contain a 1% cellulase solution additive to optimize the recovery of *Salmonella* from this material.

- **Protein**

Sample: 1.0 g

Analysis: Transfer the *Sample* to a 500-mL Kjeldahl flask, and proceed as directed in

Nitrogen Determination 〈 461 〉, *Method I*. Determine the percentage of nitrogen.

Calculate the amount of protein by multiplying the percentage of nitrogen by 6.25.

Acceptance criteria: NMT 10.0%

- **Starch**

Analysis: To a dispersion (1 in 10) of Guar Gum add a few drops of iodine TS.

Acceptance criteria: No blue color is produced.

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 5 h.

Acceptance criteria: NMT 15.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

■ USP Dextrose RS ■_{1S} (NF34)

USP Galactose RS

USP Mannose RS

■ USP Xylose RS ■_{1S} (NF34)

BRIEFING

Acetaminophen and Caffeine Tablets, *USP 38* page 2013. As part of the USP monograph modernization initiative and based on correspondence from the FDA regarding modernization, the addition of an HPLC procedure for monitoring 4-aminophenol, based on general chapter *4-Aminophenol in Acetaminophen-Containing Drug Products* 〈 227 〉, is being proposed. This liquid chromatographic procedure is based on analyses performed with the Dionex Acclaim Mixed Mode WCX-1 brand of L85 column. The typical retention time for 4-aminophenol is about 4.2–5.3 min. The proposed limit of this nephrotoxin is 0.15% and is based on input provided by the FDA and the pharmaceutical industry to the USP Acetaminophen Expert Panel, which reports to the Small Molecules-2 Expert Committee.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: C. Anthony.)

Correspondence Number—C153892

Comment deadline: May 31, 2015

Acetaminophen and Caffeine Tablets

DEFINITION

Acetaminophen and Caffeine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of acetaminophen ($C_8H_9NO_2$) and caffeine ($C_8H_{10}N_4O_2$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, relative to the internal standard, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

Solution A: Methanol and glacial acetic acid (95:5)

Mobile phase: Methanol, glacial acetic acid, and water (28:3:69)

Internal standard solution: 6 mg/mL of benzoic acid in methanol

Standard stock solution: 0.25 mg/mL of USP Acetaminophen RS and 0.25*J* mg/mL of USP Caffeine RS in *Solution A*; *J* being the ratio of the labeled amount, in mg, of caffeine to the labeled amount, in mg, of acetaminophen per Tablet

Standard solution: 0.1 mg/mL of USP Acetaminophen RS and 0.1*J* mg/mL of USP Caffeine RS, prepared by transferring 20.0 mL of *Standard stock solution* and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and diluting with *Solution A* to volume

Sample stock solution: Nominally 2.5 mg/mL of acetaminophen in *Solution A*, prepared as follows. Transfer a portion of the powder equivalent to 250 mg of acetaminophen, from NLT 20 finely powdered Tablets, to a 100-mL volumetric flask. Add 75 mL of *Solution A*, and shake by mechanical means for 30 min. Dilute with *Solution A* to volume.

Sample solution: Transfer 2.0 mL of the *Sample stock solution* and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Solution A* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 10-cm; 5- μ m packing L1

Column temperature: 45 ± 1°

Flow rate: 2 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times for acetaminophen, caffeine, and benzoic acid are about 0.3, 0.5, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.4 between any of the analyte and internal standard peaks

Tailing factor: NMT 1.2 for each analyte peak

Relative standard deviation: NMT 2.0%

Analysis**Samples:** *Standard solution* and *Sample solution*

Calculate individually the percentages of

■ the labeled amounts of ■ 1S (USP39)

acetaminophen ($C_8H_9NO_2$) and caffeine ($C_8H_{10}N_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

 R_U = peak response ratio of the analyte

■ acetaminophen or caffeine ■ 1S (USP39)

to the internal standard from the *Sample solution* R_S = peak response ratio of the analyte

■ acetaminophen or caffeine ■ 1S (USP39)

to the internal standard from the *Standard solution* C_S = concentration of the corresponding USP Reference Standard

■ USP Acetaminophen RS or USP Caffeine RS ■ 1S (USP39)

in the *Standard solution* (mg/mL) C_U = nominal concentration of the corresponding analyte

■ acetaminophen or caffeine ■ 1S (USP39)

in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0% of acetaminophen ($C_8H_9NO_2$) and caffeine ($C_8H_{10}N_4O_2$)**PERFORMANCE TESTS****Change to read:**● **Dissolution** 〈 711 〉**Medium:** Water; 900 mL**Apparatus 2:** 100 rpm**Time:** 60 min**Solution A, Mobile phase, Internal standard solution, Standard stock solution, Chromatographic system,**■ and **System suitability:** ■ 1S (USP39)Proceed as directed in the *Assay*.**Standard solution:** Transfer 20.0 mL of the *Standard stock solution*, 3.0 mL of *Internal standard solution*, and 20 mL of water to a 50-mL volumetric flask, and allow to stand for 30 s. Dilute with *Solution A* to volume. Use within 8 h.**Sample solution:** Transfer an aliquot of a filtered portion of the solution under test to a 50-mL volumetric flask to obtain an expected concentration of 0.1 mg/mL of acetaminophen and 0.1J mg/mL of caffeine, where J is defined for the *Standard stock solution*. Add 3.0 mL of *Internal standard solution* and 20 mL of *Solution A*, and allow to stand for 30 s. Dilute with *Solution A* to volume.**Analysis:** Proceed as directed in the *Assay*, using the *Standard solution* and *Sample solution* prepared within the *Dissolution* test.Calculate the percentages of the labeled amounts of acetaminophen ($C_8H_9NO_2$) and caffeine ($C_8H_{10}N_4O_2$) dissolved:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_T peak response ratio of the corresponding analyte

■ acetaminophen or caffeine ■ 1S (USP39)

to the internal standard from the *Sample solution* R_S = peak response ratio of the corresponding analyte

■ acetaminophen or caffeine ■ 1S (USP39)

to the internal standard from the *Standard solution* C_S = concentration of the corresponding USP Reference Standard

■ USP Acetaminophen RS or USP Caffeine RS ■ 1S (USP39)

in the *Standard solution* (mg/mL) C_U = nominal concentration of the corresponding analyte

■ acetaminophen or caffeine ■ 1S (USP39)

in the *Sample solution* (mg/mL)

Tolerances: NLT 75% (Q) of the labeled amounts of acetaminophen ($C_8H_9NO_2$) and caffeine ($C_8H_{10}N_4O_2$) is dissolved.

- **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

IMPURITIES

Add the following:

- **4-Aminophenol in Acetaminophen-Containing Drug Products** 〈 227 〉: Meet the requirements ■ 1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
- **USP Reference Standards** 〈 11 〉
 USP Acetaminophen RS
 USP Caffeine RS

BRIEFING

Acetylcysteine Compounded Solution. Because of a need for an acetylcysteine compounded solution in the event of manufactured product unavailability or drug shortage, a new compounded preparation monograph is proposed based on a validated stability-indicating method used to assess stability. The liquid chromatographic procedure in the Assay is based on analyses validated using the Zorbax SB-Aq brand of L## column. The typical retention time for acetylcysteine is about 3.8 min.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C154508

Comment deadline: May 31, 2015

Add the following:

- **Acetylcysteine Compounded Solution**

DEFINITION

Acetylcysteine Compounded Solution contains NLT 90.0% and NMT 100.0% of the labeled amount of acetylcysteine ($C_5H_9NO_3S$). Prepare Acetylcysteine Compounded Solution 20% as follows (see *Pharmaceutical Compounding—Sterile Preparations* 〈 797 〉).

Acetylcysteine	2 g
Edetate Disodium Dihydrate	5.5 mg
Sodium Hydroxide 10% Solution	To adjust pH to 6.5–7.5
Sterile Water for Injection, a sufficient quantity to make	10 mL ^a

^a It is necessary to adjust the formula and compound an additional amount to completely fill each single-dose container to minimize exposure to oxygen because the preparation is susceptible to oxidation.

Dissolve *Edetate Disodium Dihydrate* in 7 mL of *Sterile Water for Injection*. Slight heating may be necessary. Allow to cool. Dissolve *Acetylcysteine* in the edetate disodium solution. Add *Sodium Hydroxide 10% Solution* dropwise with mixing to adjust the pH to between 6.5 and 7.5. Bring to final volume with *Sterile Water for Injection* and mix well. Pass through a sterile filter of 0.22- μ m pore size into single-dose sterile containers. It is necessary to completely fill the container to minimize the amount of oxygen present because the preparation is susceptible to oxidation.

ASSAY

• Procedure

Mobile phase: Acetonitrile, phosphoric acid, and water (3: 0.5: 96.5)

Standard solution: 0.4 mg/mL of acetylcysteine prepared from USP Acetylcysteine RS in *Mobile phase*

Sample solution: Transfer 0.4 mL of Solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix well.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV-Vis 200 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L##

Column temperature: 15^o

Flow rate: 2.0 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

[Note—The retention time for acetylcysteine is about 3.8 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetylcysteine ($C_5H_9NO_3S$) in the

portion of Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of acetylcysteine from the *Sample solution*

r_S peak response of acetylcysteine from the *Standard solution*

C_S concentration of USP Acetylcysteine RS in the *Standard solution* (mg/mL)

C_U nominal concentration of acetylcysteine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–100.0%

SPECIFIC TESTS

- **pH** $\langle 791 \rangle$: 6.5–7.5
- **Sterility Tests** $\langle 71 \rangle$: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in single-dose sterile glass containers and store at controlled room temperature.
- **Beyond-Use Date:** In the absence of performing and completing a sterility test, the storage conditions for *High-Risk Level CSPs in Pharmaceutical Compounding—Sterile Preparations* $\langle 797 \rangle$ apply. After successful completion of sterility testing, the *Beyond-Use Date* is NMT 60 days after the date on which it was compounded when stored at controlled room temperature.
- **Labeling:** Label it to state the *Beyond-Use Date*. The label indicates that the Solution is not to be used if it contains a precipitate. Label it to state that it is a single-dose container, that it is overfilled with an excess that should be discarded after a measured single dose is used, and to store at controlled room temperature. Label it for inhalation or oral administration only. Label to state that the preparation may have a disagreeable odor and light purple color that is a result of a chemical reaction which does not affect the strength of the preparation.
- **USP Reference Standards** $\langle 11 \rangle$
USP Acetylcysteine RS
- 1S (USP39)

BRIEFING

Adapalene Gel. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is proposed.

1. The liquid chromatographic procedures in the *Assay* and the test for *Organic Impurities* are based on analyses performed with the Hypersil BDS C18 brand of L1 column manufactured by Thermo Scientific. The typical retention time for adapalene is about 6.5 min in the *Assay* and about 39 min in the test for *Organic Impurities*.

(SM3: F. Mao.)

Correspondence Number—C141748

Comment deadline: May 31, 2015

Add the following:

■ **Adapalene Gel**

DEFINITION

Adapalene Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of adapalene ($C_{28}H_{28}O_3$).

IDENTIFICATION

- **A. Ultraviolet Absorption** (197U)
 - Diluent:** Use *Mobile phase* in the *Assay*.
 - Sample stock solution:** Use *Sample stock solution* in the *Assay*.
 - Sample solution:** Nominally equivalent to 0.4 µg/mL of adapalene, prepared as follows. Dilute 2.0 mL of *Sample stock solution* with *Diluent* to 100.0 mL. Pass a portion through a Teflon filter of 0.45-µm pore size and use the filtrate.
 - Acceptance criteria:** Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

● **Procedure**

Mobile phase: Acetonitrile, tetrahydrofuran, trifluoroacetic acid, and water (43: 36: 0.02: 21)

Standard stock solution: 0.25 mg/mL of USP Adapalene RS, prepared as follows. Transfer USP Adapalene RS to a suitable volumetric flask, add tetrahydrofuran equivalent to 1% of the final volume, and sonicate to dissolve. Dilute with *Mobile phase* to volume.

Standard solution: 20 µg/mL of USP Adapalene RS in *Mobile phase*, from *Standard stock solution*

Sample stock solution: Nominally equivalent to 20 µg/mL of adapalene, prepared as follows. Transfer 2.0 g of Gel to a 100-mL volumetric flask, add 25 mL of tetrahydrofuran, and sonicate to dissolve. Add 25 mL of acetonitrile and sonicate for 20 min. Cool to room temperature and dilute with *Diluent* to volume.

Sample solution: Pass a portion of *Sample stock solution* through a Teflon filter of 0.45-µm pore size and use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of adapalene ($C_{28}H_{28}O_3$) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Adapalene RS in the *Standard solution* (mg/mL)

C_U nominal concentration of adapalene in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• Organic Impurities

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

Solution A: Use *Mobile phase* in the *Assay*.

Solution B: *Buffer* and *Solution A* (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	0	100
4	0	100
30	55	45
65	55	45
68	0	100
80	0	100

Diluent: Acetonitrile and tetrahydrofuran (3:2)

System suitability stock solution: 0.5 mg/mL of USP Adapalene RS, prepared as follows.

Transfer USP Adapalene RS to a suitable volumetric flask, add tetrahydrofuran equivalent to 40% of the final volume, and sonicate to dissolve. Dilute with acetonitrile to volume.

System suitability solution: 0.2 mg/mL of USP Adapalene RS in *Diluent*, from *System suitability stock solution*

Standard solution: 1.0 µg/mL of USP Adapalene RS in *Diluent*, from *System suitability solution*

Sample solution: Nominally equivalent to 0.2 mg/mL of adapalene, prepared as follows.

Transfer 5.0 g of Gel to a 25-mL volumetric flask. Add 10 mL of tetrahydrofuran and sonicate to disperse for 10 min. Add 10 mL of acetonitrile and sonicate for 10 min. Cool to room temperature and dilute with acetonitrile to volume. Pass a portion through a Teflon filter of 0.45-µm pore size and use the filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution and Standard solution*

Suitability requirements

Tailing factor: NMT 2.0, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of each individual impurity in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak area of each impurity from the *Sample solution*

r_S peak area of adapalene from the *Standard solution*

C_S concentration of USP Adapalene RS in the *Standard solution* (mg/mL)

C_U nominal concentration of adapalene in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Adapalene related compound A ^{a,b}	0.5	—
Adapalene	1.0	—
Adapalene related compound B ^{b,c}	1.3	—
Any unspecified impurity	—	0.2
Total impurities	—	1.0

^a Methyl 6-bromo-2-naphthoate.

^b This process impurity is controlled in the drug substance monograph. It is included in the table for identification only and it is not to be reported in the total impurities.

^c Methyl 6-[3-(adamant-1-yl)-4-methoxyphenyl]-2-naphthoate.

SPECIFIC TESTS

- **pH** 〈 791 〉: 4.0–6.0
- **Minimum Fill** 〈 755 〉: Meets the requirements
- **Microbial Enumeration Tests** 〈 61 〉 and **Tests for Specified Microorganisms** 〈 62 〉: The total aerobic microbial count is NMT 10² cfu/g. The total yeasts and molds count is NMT

10^1 cfu/g. It meets the requirements of the tests for the absence of *Escherichia coli*, *Salmonella species*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa species*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature and protect from freezing.

- **USP Reference Standards** { 11 }

USP Adapalene RS

■ 1S (USP39)

BRIEFING

Alendronate Sodium, USP 38 page 2082.

1. In preparation for the omission of the flame tests from general chapter *Identification Tests—General* { 191 }, proposed in PF 41(2) [Mar.–Apr. 2015], it is proposed to replace the use of the flame test in *Identification* test B with the pyroantimonate precipitation test currently described in this general chapter. This test is also consistent with test (a) in 2.3.1 *Identification reactions of ions and functional groups* in the *European Pharmacopoeia*, and is employed in the *European Pharmacopoeia* monograph for *Sodium Alendronate Trihydrate*.
2. In the *Assay*, the requirement for *Column efficiency* is deleted from the system suitability requirements as the remaining requirements are adequate to evaluate the *System suitability*.

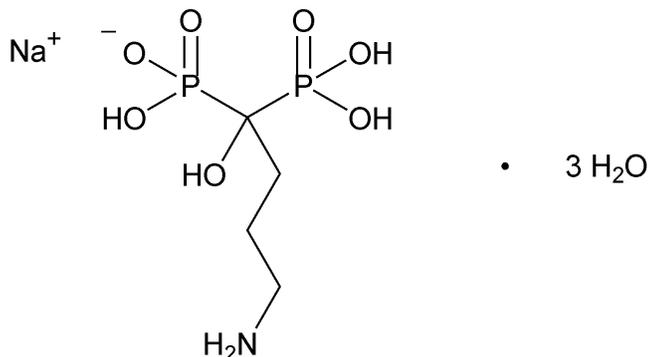
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM3: R.S. Prasad.)

Correspondence Number—C154662

Comment deadline: May 31, 2015

Alendronate Sodium



C₄H₁₂NNaO₇P₂·3H₂O 325.12

Phosphonic acid, (4-amino-1-hydroxybutylidene) bis-, monosodium salt, trihydrate;
Sodium trihydrogen (4-amino-1-hydroxybutylidene)diphosphonate, trihydrate [121268-17-5].

DEFINITION

Alendronate Sodium contains NLT 98.0% and NMT 102.0% of alendronate sodium ($C_4H_{12}NNaO_7P_2$), calculated on the dried basis.

IDENTIFICATION

• A. Infrared Absorption \langle 197M \rangle

Change to read:

- **B. Identification Tests—General, Sodium \langle 191 \rangle :** Meets the requirements of the flame pyroantimonate precipitation \blacksquare_{1S} (USP39)

test

ASSAY

Change to read:

• Procedure

Buffer solution: 14.7 g/L of sodium citrate dihydrate and 7.05 g/L of anhydrous dibasic sodium phosphate. Adjust with phosphoric acid to a pH of 8.

Mobile phase: Acetonitrile, methanol, and *Buffer solution* (25:5:70)

Diluent: 29.4 g/L of sodium citrate dihydrate

Borate solution: 19.1 g/L of sodium borate

Solution A: 0.5 mg/mL of 9-fluorenylmethyl chloroformate in acetonitrile. [Note—Prepare this solution fresh just before use.]

Standard stock solution: 0.1 mg/mL of USP Alendronate Sodium RS in *Diluent*

Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of *Solution A*, and shake for 30 s. Allow to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min. Use a portion of the clear upper aqueous layer.

Reagent blank: Transfer 5.0 mL of *Diluent* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of *Solution A*, and shake for 30 s. Allow to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min. Use a portion of the clear upper aqueous layer.

Sample stock solution: 0.1 mg/mL of Alendronate Sodium in *Diluent*

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of *Solution A*, and shake for 30 s. Allow to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min. Use a portion of the clear upper aqueous layer.

Chromatographic system

(See *Chromatography \langle 621 \rangle , System Suitability.*)

Mode: LC

Detector: UV 266 nm

Column: 4.1-mm \times 25-cm; packing L21

Column temperature: 35 $^{\circ}$

Flow rate: 1.2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: ~~NLT 1500 theoretical plates~~

■ ~~1S (USP39)~~

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution, Reagent blank, and Sample solution*

Calculate the percentage of alendronate sodium (C₄H₁₂NNaO₇P₂) in the portion of Alendronate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak area from the *Sample solution*

r_S peak area from the *Standard solution*

C_S concentration of USP Alendronate Sodium RS in the *Standard stock solution* (mg/mL)

C_U concentration of Alendronate Sodium in the *Sample stock solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Delete the following:

•

• **Heavy Metals, Method III** (231)

: 0.001% (Official 1-Dec-2015)

• **Organic Impurities**

Buffer solution: 2.94 g/L of sodium citrate dihydrate and 1.42 g/L of anhydrous dibasic sodium phosphate. Adjust with phosphoric acid to a pH of 8 and pass through a filter of 0.5-µm or finer pore size.

Solution A: Acetonitrile and *Buffer solution* (3:17)

Solution B: Acetonitrile and *Buffer solution* (7:3)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	50	50
25	0	100
27	100	0

Time (min)	Solution A (%)	Solution B (%)
32	100	0

Diluent and Borate solution: Proceed as directed in the *Assay*.

Solution C: 4 mg/mL of 9-fluorenylmethyl chloroformate in acetonitrile. [Note—Prepare this solution fresh just before use.]

Standard stock solution: 0.6 mg/mL of USP Alendronate Sodium RS in *Diluent*

Standard solution A: Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of acetonitrile and 5 mL of *Solution C*, and shake for 45 s. Allow to stand at room temperature for 30 min. Add 20 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min, and use a portion of the clear upper aqueous layer.

Standard solution B: 0.6 µg/mL of USP Alendronate Sodium RS in *Diluent* from *Standard stock solution*. Transfer 5 mL of this diluted solution (0.6 µg/mL) to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of acetonitrile and 5 mL of *Solution C*, and shake for 45 s. Allow to stand at room temperature for 30 min. Add 20 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min, and use a portion of the clear upper aqueous layer.

Reagent blank: Transfer 5.0 mL of *Diluent* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of acetonitrile and 5 mL of *Solution C*, and shake for 45 s. Allow to stand at room temperature for 30 min. Add 20 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min, and use a portion of the clear upper aqueous layer.

Sample stock solution: 0.6 mg/mL of Alendronate Sodium in *Diluent*

Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of acetonitrile and 5 mL of *Solution C*, and shake for 45 s. Allow to stand at room temperature for 30 min. Add 20 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min, and use a portion of the clear upper aqueous layer.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 266 nm

Column: 4.1-mm × 25-cm; packing L21

Column temperature: 45°

Flow rate: 1.8 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Tailing factor: NMT 2.0 for the main peak, *Standard solution A*

Signal-to-noise ratio: NLT 3 for the main peak, *Standard solution B*

Analysis

Samples: *Reagent blank* and *Sample solution*

[Note—Disregard any peak corresponding to those obtained from the *Reagent blank*.]

Calculate the percentage of each impurity in the portion of Alendronate Sodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak area of each impurity

r_T sum of all impurity peaks and the main peak

Acceptance criteria

Individual impurities: NMT 0.1%

Total impurities: NMT 0.5%

SPECIFIC TESTS

- **Loss on Drying** 〈 731 〉

Sample: Dry at a pressure of NMT 5 mm of mercury at 140° to constant weight.

Acceptance criteria: 16.1%–17.1%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, and store at room temperature.
- **USP Reference Standards** 〈 11 〉

USP Alendronate Sodium RS

BRIEFING

Alprazolam Extended-Release Tablets, *USP 38* page 2100. It is proposed to clarify the monograph by replacing the dashes for two impurities within *Table 7* with the corresponding limits and to acknowledge that these impurities may not be possible from all manufacturing processes.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: H. Joyce.)

Correspondence Number—C155151

Comment deadline: May 31, 2015

Alprazolam Extended-Release Tablets

DEFINITION

Alprazolam Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam (C₁₇H₁₃CIN₄).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

Mobile phase: Acetonitrile, water, and phosphoric acid (350:650:1)

Standard solution: 0.05 mg/mL of USP Alprazolam RS in methanol

Sample solution: Transfer an appropriate number of Tablets into a suitable volumetric flask to obtain a nominal concentration of about 0.05 mg/mL of alprazolam. Sonicate in 80% of the flask volume of methanol for 15 min, and shake mechanically for 30 min. Dilute with methanol to final volume, filter a portion of the solution, and discard the first 3 mL of filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for alprazolam

■ ■ 1S (USP39)

Column efficiency: NLT 3000 theoretical plates for alprazolam

■ ■ 1S (USP39)

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of alprazolam ($C_{17}H_{13}ClN_4$), based on the label claim, in the portion of Tablets taken:

■ Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) in the portion of Tablets taken: ■ 1S (USP39)

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of alprazolam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **Dissolution** { 711 }

Test 1

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water; adjust with phosphoric acid or potassium hydroxide to a pH of 6.0 ± 0.1); 500 mL

Apparatus 1: 100 rpm

Times: 1, 4, 8, and 12 h

Mobile phase: Acetonitrile, tetrahydrofuran, and *Medium* (7:1:12)

Standard stock solution: 0.5 mg/mL of USP Alprazolam RS in acetonitrile

Standard solution: ($L/500$) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* 〈621〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 10-cm; 5- μ m packing L7

Flow rate: 1 mL/min

Injection volume: 100 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 3000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 500 mL

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved		
	0.5-mg Tablet	2-mg Tablet	3-mg Tablet
1	NMT 25%	NMT 20%	NMT 20%
4	40%–60%	30%–55%	30%–55%
8	70%–90%	65%–90%	65%–90%
12	NLT 85%	NLT 85%	NLT 85%

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Acceptance Table 2 in Dissolution < 711 >*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water; adjust with phosphoric acid or potassium hydroxide to a pH of 6.0 ± 0.1); 500 mL

Apparatus 1: 100 rpm

Times: 1, 4, 8, and 16 h

Mobile phase: Acetonitrile, tetrahydrofuran, and *Medium* (35:5:60)

Standard stock solution: 0.05 mg/mL of USP Alprazolam RS in methanol

Standard solution: ($L/500$) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography < 621 >*, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 7.5-cm; 5- μ m packing L7

Flow rate: 1.3 mL/min

Injection volume: 80 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of alprazolam ($C_{17}H_{13}ClN_4$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U peak response of alprazolam from the *Sample solution* at each time point

r_S peak response of alprazolam from the *Standard solution*

C_S concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

V_s = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (<i>i</i>)	Time (h)	Amount Dissolved			
		0.5-mg Tablet	1-mg Tablet	2-mg Tablet	3-mg Tablet
1	1	NMT 25%	NMT 25%	NMT 20%	NMT 20%
2	4	45%–60%	40%–55%	30%–50%	25%–45%
3	8	70%–90%	65%–85%	55%–75%	50%–70%
4	16	NLT 85%	NLT 85%	NLT 85%	NLT 80%

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Acceptance Table 2* in *Dissolution* 〈 711 〉.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water; adjust with phosphoric acid or potassium hydroxide to a pH of 6.0 ± 0.1); 500 mL, deaerated

Apparatus 1: 100 rpm

Times: 1, 4, and 8 h for Tablets labeled to contain 0.5 mg or 1 mg; 1, 4, 8, and 16 h for Tablets labeled to contain 2 mg or 3 mg

Mobile phase: Acetonitrile and *Medium* (40:60)

Standard stock solution: 0.5 mg/mL of USP Alprazolam RS in methanol

Standard solution: ($L/500$) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 1- μ m pore size.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 10-cm; 3- μ m or 5- μ m packing L7

Flow rate: 1 mL/min

Injection volume: 100 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of alprazolam ($C_{17}H_{13}ClN_4$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of alprazolam from the *Sample solution* at each time point

r_S = peak response of alprazolam from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (<i>i</i>)	Time (h)	Amount Dissolved			
		0.5-mg Tablet	1-mg Tablet	2-mg Tablet	3-mg Tablet
1	1	15%–35%	10%–30%	10%–30%	5%–25%
2	4	50%–75%	45%–65%	30%–55%	25%–50%
3	8	NLT 75%	NLT 70%	60%–80%	50%–75%
4	16	—	—	NLT 85%	NLT 80%

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Acceptance Table 2* in *Dissolution* $\langle 711 \rangle$.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water; adjust with phosphoric acid or potassium hydroxide to a pH of 6.0); 500 mL

Apparatus 1 (20-mesh basket): 100 rpm

Times: 1, 4, 8, and 16 h

Mobile phase: Acetonitrile and *Medium* (32:68)

Standard stock solution: 0.4 mg/mL of USP Alprazolam RS in methanol

Standard solution: ($L/500$) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet. Pass through a suitable filter of

0.45- μm pore size, and use the filtrate.

Sample solutions: At the end of specified time intervals, withdraw a known volume (V_S) of the solution from the dissolution vessel, and replace an equal volume of fresh *Medium* into the dissolution vessel. Pass the withdrawn sample through a suitable filter of 0.45- μm pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 15-cm; 5- μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 100 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the concentration (C_i) of alprazolam ($\text{C}_{17}\text{H}_{13}\text{ClN}_4$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of alprazolam from the *Sample solution* at each time point

r_S = peak response of alprazolam from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alprazolam ($\text{C}_{17}\text{H}_{13}\text{ClN}_4$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times V] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (<i>i</i>)	Time (h)	Amount Dissolved			
		0.5-mg Tablet	1-mg Tablet	2-mg Tablet	3-mg Tablet
1	1	NMT 40%	NMT 35%	NMT 35%	NMT 35%
2	4	50%–75%	45%–65%	35%–55%	30%–55%
3	8	NLT 75%	70%–90%	55%–75%	50%–70%
4	16	NLT 85%	NLT 85%	NLT 85%	NLT 75%

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Acceptance Table 2 in Dissolution* 〈 711 〉.

- **Test 5:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water; adjust with phosphoric acid to a pH of 6.0); 500 mL

Apparatus 1: 100 rpm

Times: 1, 4, 8, and 16 h

Mobile phase: Acetonitrile, water, and phosphoric acid (350:650:1)

Standard stock solution: 0.5 mg/mL of USP Alprazolam RS in methanol

Standard solution: ($L/500$) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet

Sample solutions: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Column temperature: 30 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 50 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the concentration (C_i) of alprazolam ($C_{17}H_{13}ClN_4$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U peak response of alprazolam from the *Sample solution* at each time point

r_S peak response of alprazolam from the *Standard solution*

C_S concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 5*.

Table 5

Time Point (i)	Time (h)	Amount Dissolved
1	1	NMT 25%
2	4	40%–65%
3	8	65%–95%
4	16	NLT 85%

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Acceptance Table 2 in Dissolution* { 711 }.

*(RB 1-Apr-2014)

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

Change to read:

- **Organic Impurities**

Buffer: 5.4 g/L of monobasic potassium phosphate (KH_2PO_4) in water. Adjust with phosphoric acid to a pH of 3.4.

Solution A: Acetonitrile, methanol, and *Buffer* (27:10:63)

Solution B: Acetonitrile, methanol, and *Buffer* (7:3:10)

Mobile phase: See *Table 6*.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	95	5
22	95	5
25	15	85

Time (min)	Solution A (%)	Solution B (%)
60	15	85
60.1	95	5
70	95	5

System suitability solution: 1 µg/mL each of USP Chlordiazepoxide Related Compound A RS, USP Alprazolam Related Compound A RS, and USP Nordazepam RS; and 0.4 µg/mL of USP Alprazolam RS in methanol

Standard solution: 0.4 µg/mL of USP Alprazolam RS in methanol

Sample solution: From NLT 20 Tablets ground to a fine powder, transfer an amount of powder to a suitable flask to obtain a nominal concentration of 0.2 mg/mL of alprazolam in methanol. [Note—Sonicate for 15 min to dissolve the contents.] Filter a portion, and discard the first 1 mL of filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times are listed in *Table 6*.]

Suitability requirements

Resolution: NLT 1.5 between nordazepam and alprazolam; NLT 1.5 between chlordiazepoxide related compound A and alprazolam related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for the alprazolam peak, *System suitability solution*

Relative standard deviation: NMT 5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of alprazolam in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 7*)

Acceptance criteria: See *Table 7*.

Table 7

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Chlordiazepoxide related compound A ^a	0.36	1.0	— ■ 0.2 ■ 1S (USP39)
Alprazolam related compound A	0.45	0.7	0.5
Nordazepam ^{a,b}	0.8	1.0	— ■ 0.2 ■ 1S (USP39)
Alprazolam	1.0	—	—
2-Amino-5-chloro-benzophenone	1.8	0.9	0.5
Amino-derivative ^c	2.2	1.2	0.5
Any other individual degradation product	—	1.0	0.2
Total impurities	—	—	2.0

a If present meets the requirement for any other individual degradation product.

b ~~7-Chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one (reported as unspecified impurity).~~

■

a If possible from the manufacturing process.

b 7-Chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one.

■ 1S (USP39)

c 7-Chloro-1-methyl-5-phenyl[1,2,4]triazolo[4,3-a]quinolin-4-amine.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at room temperature.

Change to read:

- **Labeling:** When more than one *Dissolution* test is given, the

■ The ■ 1S (USP39)

labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

- **USP Reference Standards** { 11 }

USP Alprazolam RS

USP Alprazolam Related Compound A RS

2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3H-1,4-benzodiazepine.

■

C₁₇H₁₅ClN₄O 326.78 ■ 1S (USP39)

USP Chlordiazepoxide Related Compound A RS

7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.

C₁₅H₁₁ClN₂O₂ 286.72

USP Nordazepam RS **BRIEFING**

Aminobenzoate Potassium, *USP 38* page 2174. As part of the USP monograph modernization effort, the following revisions are proposed:

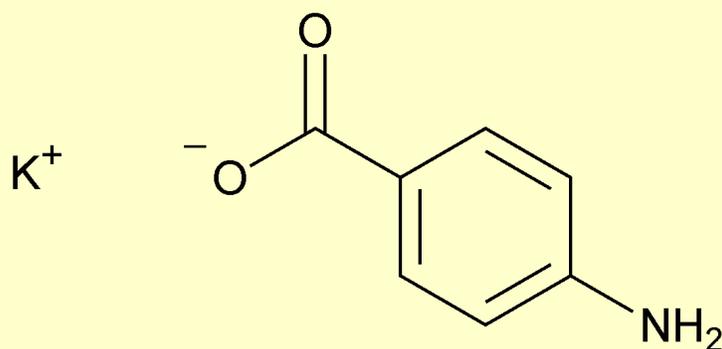
1. Revise the *Definition* from NLT 98.5% and NMT 101.0% to NLT 98.0% and NMT 102.0%, which is typical for chromatographic procedures as proposed in the *Assay*.
2. Replace *Identification test A* based on *Ultraviolet Absorption* with the more specific *Infrared Absorption* test.
3. Replace *Identification test B* based on melting temperature with the retention time agreement of the chromatographic peaks in the proposed *Assay* method.
4. Replace the existing titrimetric procedure in the *Assay* with a validated stability-indicating HPLC procedure. The proposed liquid chromatographic procedure is based on an analysis performed with the Eclipse XBD-Phenyl brand of L11 column. The typical retention time for aminobenzoic acid is about 6.0 min.
5. Delete the tests for *Chloride* and *Sulfate* because they do not add any additional value to ensure the quality of the drug substance.
6. Replace the spectrometric *Volatile Diazotizable Substances* procedure with a gas chromatographic method based on analyses performed with the DB-5MS fused silica capillary brand of G27 column. The typical retention times for aniline and *p*-toluidine are about 4.1 and 5.1 min, respectively.
7. Add a test for *Organic Impurities* based on the HPLC procedure in the *Assay* using the Eclipse XBD-Phenyl brand of L11 column. The typical retention time for aminobenzoic acid is about 5.4 min.
8. Add three new Reference Standards and an existing Reference Standard, introduced by the proposed HPLC procedure, to the *USP Reference Standards* section: USP 4-Nitrobenzoic Acid RS, USP Aniline RS, USP *p*-Toluidine RS, and USP Benzocaine RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: C. Anthony.)

Correspondence Number—C137588

Comment deadline: May 31, 2015

Aminobenzoate Potassium**Add the following:**

C₇H₆KNO₂

175.23

Benzoic acid, 4-amino-, potassium salt;
Potassium 4-aminobenzoate [138-84-1]. ■1S (USP39)

DEFINITION

Change to read:

Aminobenzoate Potassium contains NLT 98.5%

■98.0% ■1S (USP39)

and NMT 101.0%

■102.0% ■1S (USP39)

of aminobenzoate potassium (C₇H₆KNO₂), calculated on the dried basis.

IDENTIFICATION

Delete the following:

■● ~~A. Ultraviolet Absorption~~ (197U)

~~**Solution:** 10 µg/mL in 0.001 N sodium hydroxide~~

~~**Acceptance criteria:** Meets the requirements.~~

■1S (USP39)

Add the following:

■● **A. Infrared Absorption** (197K) ■1S (USP39)

Delete the following:

■● ~~B.~~

~~**Sample:** 400 mg~~

~~**Analysis:** Dissolve the *Sample* in 10 mL of water, add 1 mL of 3 N hydrochloric acid, filter, and wash the precipitate with two 5 mL portions of cold water. Recrystallize the precipitate from alcohol, and dry at 110 ° for 1 h.~~

~~**Acceptance criteria:** The resulting *p*-aminobenzoic acid melts between 186 ° and 189 °.~~

■1S (USP39)

Add the following:

■● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

● **C. Identification Tests—General, Potassium** (191): A solution (1 in 100) meets the requirements of the flame test.

ASSAY

Change to read:

● **Procedure**

Sample: ~~500 mg~~

Titrimetric system:

Mode: ~~Direct titration~~

Titrant: ~~0.1 M sodium nitrite VS~~

Endpoint detection: ~~Potentiometric~~

Analysis: ~~Add 25 mL of water and 25 mL of 3 N hydrochloric acid to the *Sample*, and cool in an ice bath. Titrate with *Titrant* using a calomel–platinum electrode system. Each mL of 0.1 M sodium nitrite is equivalent to 17.52 mg of aminobenzoate potassium ($C_7H_6KNO_2$).~~

Acceptance criteria: ~~98.5%–101.0%, on the dried basis~~

■ **Solution A:** 1.5% Acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid and passing through a suitable filter of 0.45- μ m pore size

Mobile phase: Methanol and *Solution A* (15:85)

Standard solution: 0.1 mg/mL of USP Aminobenzoate Potassium RS in *Mobile phase*. Sonicate to dissolve.

Sample solution: 0.1 mg/mL of Aminobenzoate Potassium in *Mobile phase*. Sonicate to dissolve.

Chromatographic system

(See *Chromatography* \langle 621 \rangle , *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.0-mm \times 15-cm; 3.5- μ m packing L11

Flow rate: 0.35 mL/min

Injection volume: 5 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoate potassium ($C_7H_6KNO_2$) in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Aminobenzoate Potassium RS in the *Standard solution* (mg/mL)

C_U = concentration of Aminobenzoate Potassium in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the dried basis ■ 1S (USP39)**IMPURITIES****Delete the following:**

- ~~Chloride and Sulfate, Chloride~~ (221)

Sample: 1.4 g**Acceptance criteria:** Shows no more chloride than corresponds to 0.4 mL of 0.020 N hydrochloric acid (0.02%). ■ 1S (USP39)**Delete the following:**

- ~~Chloride and Sulfate, Sulfate~~ (221)

Sample: 1.4 g**Acceptance criteria:** Shows no more sulfate than corresponds to 0.3 mL of 0.020 N sulfuric acid (0.02%). ■ 1S (USP39)**Delete the following:**

●

- ~~Heavy Metals, Method II~~ (231)

: NMT 0.002% ● (Official 1-Dec-2015)

Delete the following:

- ~~Volatile Diazotizable Substances~~

Standard stock solution: 0.1 mg/mL of *p*-toluidine, prepared by dissolving a quantity of *p*-toluidine in 5% of the flask volume of methanol, and diluting with water to volume**Standard solution:** 1 µg/mL of *p*-toluidine from the *Standard stock solution***Sample solution:** Transfer 5.0 g of Aminobenzoate Potassium to a suitable flask, and add a volume of 1.25 N sodium hydroxide that is just sufficient to dissolve the sample and to render the solution just alkaline to phenolphthalein TS. Dilute with water to 50 mL, and steam-distill the solution, collecting 95 mL of the distillate in a 100 mL volumetric flask. Dilute with water to volume.**Blank:** Water**Instrumental conditions****Mode:** Vis**Analytical wavelength:** Wavelength of maximum absorbance at about 405 nm**Analysis****Samples:** *Standard solution*, *Sample solution*, and *Blank*Transfer 20.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to three separate 100 mL beakers, and treat each as follows. Add 5.0 mL of 1 N hydrochloric acid, and cool in an ice bath. Add 2.0 mL of 0.1 M sodium nitrite dropwise, with stirring. Allow to

stand for 5 min for the diazotization reaction to be complete, add quickly to 10.0 mL of a cold solution of guaiacol (freshly prepared by dissolving 0.20 g of guaiacol in 100 mL of 1 N sodium hydroxide), mix and allow to stand for 30 min. Concomitantly determine the absorbances of the solutions.

Acceptance criteria: The absorbance of the solution from the *Sample solution* does not exceed that of the solution from the *Standard solution*, corresponding to NMT 0.002% of volatile diazotizable substances as *p*-toluidine. ■1S (USP39)

Add the following:

■ • Limit of Aniline and *p*-Toluidine

Diluent: Methylene chloride

Standard stock solution: 0.1 mg/mL each of USP Aniline RS and USP *p*-Toluidine RS in *Diluent*

Standard solution: 1 µg/mL each of USP Aniline RS and USP *p*-Toluidine RS in *Diluent* from *Standard stock solution*

Sample solution: 100 mg/mL of Aminobenzoate Potassium in *Diluent* prepared as follows. Add an appropriate quantity of Aminobenzoate Potassium to a suitable volumetric flask and dilute with *Diluent* to volume. Agitate for 10 min on a shaker and centrifuge at 3000 rpm for 5 min. Use the supernatant.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: GC

Detectors

Flame ionization: 300°

Hydrogen: 40 mL/min

Air: 400 mL/min

Column: 30-m × 0.32-mm fused silica capillary; coated with 0.5-µm film of phase G27

Temperatures

Injection port: 280°

Detector: 300°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	—	130	4
130	20	180	5

Carrier gas: Helium

Flow rate: 1.0 mL/min

Injection volume: 2 µL

Injection type: Split ratio, 1:10

System suitability

Sample: *Standard solution*

[Note—The relative retention times of aniline and *p*-toluidine are about 4.1 and 5.1 min,

respectively.]

Suitability requirements

Resolution: NLT 7.0 between aniline and *p*-toluidine

Tailing factor: NMT 1.5 for aniline and *p*-toluidine

Relative standard deviation: NMT 6.0% for aniline and *p*-toluidine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of *p*-toluidine or aniline in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of *p*-toluidine or aniline from the *Sample solution*

r_S = peak response of *p*-toluidine or aniline from the *Standard solution*

C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

C_U = concentration of Aminobenzoate Potassium in the *Sample solution* (mg/mL)

Acceptance criteria

Aniline: NMT 10 ppm

***p*-Toluidine:** NMT 20 ppm

■ 1S (USP39)

Add the following:

■ • Organic Impurities

Solution A: 1.5% Acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid

Solution B: Methanol

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	85	15
4.0	85	15
4.1	45	55
10.0	45	55
10.1	85	15
13	85	15

Diluent: Methanol and water (85:15)

System suitability solution: 1 mg/mL of USP Aminobenzoate Potassium RS, 0.01 mg/mL of USP 4-Nitrobenzoic Acid RS, and 0.01 mg/mL of USP Benzocaine RS in *Diluent* prepared as follows. Transfer 1 mL each of 0.1 mg/mL of USP 4-Nitrobenzoic Acid RS in methanol and 0.1 mg/mL USP of Benzocaine RS in *Diluent* to a 10-mL volumetric flask containing the appropriate amount of USP Aminobenzoate Potassium RS, and dilute with *Diluent* to volume.

Standard solution: 1 µg/mL each of USP Aminobenzoate Potassium RS, USP 4-Nitrobenzoic

Acid RS, and USP Benzocaine RS in *Diluent*

Sample solution: 1 mg/mL of Aminobenzoate Potassium in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.0-mm × 15-cm; 3.5-μm packing L11

Flow rate: 0.4 mL/min

Injection volume: 5 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between benzocaine and 4-nitrobenzoic acid, *System suitability solution*

Relative standard deviation: NMT 3% for the aminobenzoate potassium, 4-nitrobenzoic acid, and benzocaine peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 4-nitrobenzoic acid or benzocaine in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of 4-nitrobenzoic acid or benzocaine from the *Sample solution*

r_S peak response of 4-nitrobenzoic acid or benzocaine from the *Standard solution*

C_S concentration of USP 4-Nitrobenzoic Acid RS or USP Benzocaine RS in the *Standard solution* (mg/mL)

C_U concentration of Aminobenzoate Potassium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any unspecified impurity from the *Sample solution*

r_S peak response of aminobenzoate from the *Standard solution*

C_S concentration of USP Aminobenzoate Potassium RS in the *Standard solution* (mg/mL)

C_U concentration of Aminobenzoate Potassium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	1.0	—
Benzocaine	2.0	0.2
4-Nitrobenzoic acid	2.1	0.2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	0.10

■ 1S (USP39)

SPECIFIC TESTS

- **pH** 〈 791 〉
Sample solution: 50 mg/mL
Acceptance criteria: 8.0–9.0
- **Loss on Drying** 〈 731 〉
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Aminobenzoate Potassium RS

■ USP Aniline RS

Aniline.

C₆H₇N 93.13

USP Benzocaine RS

USP 4-Nitrobenzoic Acid RS

4-Nitrobenzoic acid.

C₇H₅NO₄ 167.12

USP *p*-Toluidine RS

4-Methylaniline.

C₇H₉N 107.15

■ 1S (USP39)

BRIEFING

Aminobenzoate Sodium, USP 38 page 2176. As part of the USP monograph modernization effort, the following revisions are proposed:

1. Revise the *Definition* from NLT 98.5% and NMT 101.0% to NLT 98.0% and NMT 102.0%, which is typical for chromatographic procedures as proposed in the *Assay*.
2. Replace *Identification test A* based on *Ultraviolet Absorption* with the more specific *Infrared Absorption* test.
3. Replace *Identification test B* based on melting temperature with the retention time agreement of the chromatographic peaks in the proposed *Assay* method.
4. Replace the existing titrimetric procedure in the *Assay* with a validated stability-indicating HPLC procedure. The proposed liquid chromatographic procedure is based on an analysis performed with the Eclipse XBD-Phenyl brand of L11 column. The

typical retention time for aminobenzoic acid is about 6.0 min.

5. Delete the tests for *Chloride* and *Sulfate* because they do not add any additional value to ensure the quality of the drug substance.
6. Replace the spectrometric *Volatile Diazotizable Substances* procedure with a gas chromatographic method based on analyses performed with the DB-5MS fused silica capillary brand of G27 column. The typical retention times for aniline and *p*-toluidine are about 4.1 and 5.1 min, respectively.
7. Add a test for *Organic Impurities* based on the HPLC procedure in the *Assay* using the Eclipse XBD-Phenyl brand of L11 column. The typical retention time for aminobenzoic acid is about 5.4 min.
8. Add three new Reference Standards and an existing Reference Standard, introduced by the proposed HPLC procedure, to the *USP Reference Standards* section: USP 4-Nitrobenzoic Acid RS, USP Aniline RS, USP *p*-Toluidine RS, and USP Benzocaine RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: C. Anthony.)

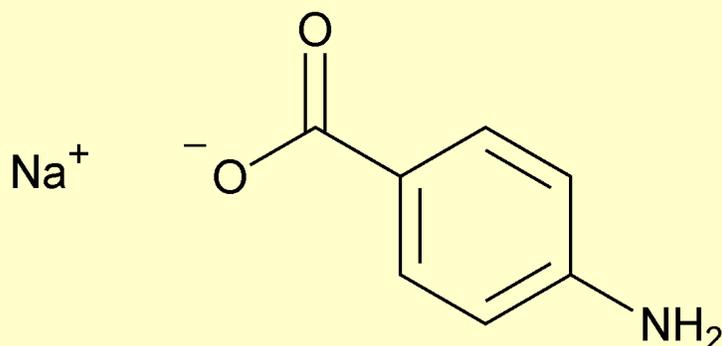
Correspondence Number—C137590

Comment deadline: May 31, 2015

Aminobenzoate Sodium

Add the following:

■



C₇H₆NNaO₂ 159.12

Benzoic acid, 4-amino-, sodium salt;

Sodium 4-aminobenzoate [555-06-6]. ■1S (USP39)

DEFINITION

Change to read:

Aminobenzoate Sodium contains NLT 98.5%

■98.0% ■1S (USP39)

and NMT 101.0%

■102.0% ■1S (USP39)

of aminobenzoate sodium (C₇H₆NNaO₂), calculated on the dried basis.

IDENTIFICATION**Delete the following:**

- ~~● A. Ultraviolet Absorption $\langle 197U \rangle$~~

~~**Sample solution:** 10 $\mu\text{g/mL}$ in 0.001 N sodium hydroxide~~

~~**Acceptance criteria:** Meets the requirements.~~

■ 1S (USP39)

Add the following:

- ● A. Infrared Absorption $\langle 197K \rangle$ ■ 1S (USP39)

Delete the following:

- ● ~~B.~~

~~**Sample:** 400 mg~~

~~**Analysis:** Dissolve the *Sample* in 10 mL of water, add 1 mL of 3 N hydrochloric acid, filter, and wash the precipitate with two 5 mL portions of cold water. Recrystallize the precipitate from alcohol, and dry at 110° for 1 h.~~

~~**Acceptance criteria:** The resulting *p*-aminobenzoic acid melts between 186° and 189°.~~

■ 1S (USP39)

Add the following:

- ● B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

- C. **Identification Tests—General, Sodium $\langle 191 \rangle$:** A solution (1 in 100) meets the requirements of the flame test.

ASSAY**Change to read:**

- **Procedure**

~~**Sample:** 500 mg~~

~~**Titrimetric system**~~

~~**Mode:** Direct titration~~

~~**Titrant:** 0.1 M sodium nitrite VS~~

~~**Endpoint detection:** Potentiometric~~

~~**Analysis:** Add 25 mL of water and 25 mL of 3 N hydrochloric acid to the *Sample* in a suitable vessel, and cool in an ice bath. Titrate with *Titrant* using a calomel–platinum electrode system. Each mL of 0.1 M sodium nitrite is equivalent to 15.91 mg of aminobenzoate sodium ($\text{C}_7\text{H}_6\text{NNaO}_2$).~~

~~**Acceptance criteria:** 98.5%–101.0%, on the dried basis~~

■ **Solution A:** 1.5% Acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid, and passing through a suitable filter of 0.45- μm pore size

Mobile phase: Methanol and *Solution A* (15:85)

Standard solution: 0.1 mg/mL of USP Aminobenzoate Sodium RS in *Mobile phase*. Sonicate to dissolve.

Sample solution: 0.1 mg/mL of Aminobenzoate Sodium in *Mobile phase*. Sonicate to dissolve.

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.0-mm \times 15-cm; 3.5- μ m packing L11

Flow rate: 0.35 mL/min

Injection volume: 5 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoate sodium ($C_7H_6NNaO_2$) in the portion of Aminobenzoate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Aminobenzoate Sodium RS in the *Standard solution* (mg/mL)

C_U

= concentration of Aminobenzoate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ■_{1S} (USP39)

IMPURITIES

Delete the following:

- ~~Chloride and Sulfate, Chloride $\langle 221 \rangle$~~

~~**Sample:** 1.4 g~~

~~**Acceptance criteria:** Shows no more chloride than corresponds to 0.4 mL of 0.020 N hydrochloric acid (0.02%). ■_{1S} (USP39)~~

Delete the following:

Delete the following:

- **Chloride and Sulfate, Sulfate** ~~(221)~~

Sample: 1.4 g

Acceptance criteria: Shows no more sulfate than corresponds to 0.3 mL of 0.020 N sulfuric acid (0.02%). ■1S (USP39)

Delete the following:

-

- **Heavy Metals, Method II** ~~(231)~~

: NMT 0.002% ●(Official 1-Dec-2015)

Delete the following:

- **Volatile Diazotizable Substances**

Standard stock solution: 0.1 mg/mL of *p*-toluidine, prepared by dissolving a quantity of *p*-toluidine in 5% of the volumetric flask volume of methanol, and diluting with water to volume

Standard solution: 1 µg/mL of *p*-toluidine from the *Standard stock solution*

Sample solution: Transfer 5.0 g of Aminobenzoate Sodium to a suitable flask, and add a volume of 1.25 N sodium hydroxide that is just sufficient to dissolve the sample and to render the solution just alkaline to phenolphthalein TS. Dilute with water to 50 mL, and steam distill the solution, collecting 95 mL of the distillate in a 100 mL volumetric flask. Dilute with water to volume.

Blank: Water

Instrumental conditions

Mode: Vis

Analytical wavelength: Wavelength of maximum absorbance at about 405 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Transfer 20.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to three separate 100 mL beakers, and treat each as follows. Add 5.0 mL of 1 N hydrochloric acid, and cool in an ice bath. Add 2.0 mL of 0.1 M sodium nitrite dropwise, with stirring. Allow to stand for 5 min for the diazotization reaction to be complete, add quickly to 10.0 mL of a cold solution of guaiacol (freshly prepared by dissolving 0.20 g of guaiacol in 100 mL of 1 N sodium hydroxide), and allow to stand for 30 min. Concomitantly determine the absorbances of the solutions.

Acceptance criteria: The absorbance of the solution from the *Sample solution* does not exceed that of the solution from the *Standard solution*, corresponding to NMT 0.002% of volatile diazotizable substances as *p*-toluidine. ■1S (USP39)

Add the following:

- **Limit of Aniline and *p*-Toluidine**

Diluent: Methylene chloride

Standard stock solution: 0.1 mg/mL each of USP Aniline RS and USP *p*-Toluidine RS in
Diluent

Standard solution: 1.0 µg/mL each of USP Aniline RS and USP *p*-Toluidine RS in *Diluent* from *Standard stock solution*

Sample solution: 100 mg/mL of Aminobenzoate Sodium in *Diluent* prepared as follows. Add an appropriate quantity of Aminobenzoate Sodium to a suitable volumetric flask and dilute with *Diluent* to volume. Agitate for 10 min on a shaker and centrifuge at 3000 rpm for 5 min. Use the supernatant.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: GC

Detectors

Flame ionization: 300°

Hydrogen: 40 mL/min

Air: 400 mL/min

Column: 30-m × 0.32-mm fused silica capillary; coated with 0.5-µm film of phase G27

Temperatures

Injection port: 280°

Detector: 300°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	—	130	4
130	20	180	5

Carrier gas: Helium

Flow rate: 1 mL/min

Injection volume: 2 µL

Injection type: Split ratio, 1:10

System suitability

Sample: *Standard solution*

[Note—The relative retention times of aniline and *p*-toluidine are about 4.1 and 5.1 min, respectively.]

Suitability requirements

Resolution: NLT 7.0 between aniline and *p*-toluidine

Tailing factor: NMT 1.5 for aniline and *p*-toluidine

Relative standard deviation: NMT 6.0% for aniline and *p*-toluidine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of *p*-toluidine or aniline in the portion of Aminobenzoate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of *p*-toluidine or aniline from the *Sample solution*

r_S = peak response of *p*-toluidine or aniline from the *Standard solution*

C_s concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

C_T concentration of Aminobenzoate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria

Aniline: NMT 10 ppm

p-Toluidine: NMT 20 ppm

■ 1S (USP39)

Add the following:

■ • Organic Impurities

Solution A: 1.5% Acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid

Solution B: Methanol

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	85	15
4.0	85	15
4.1	45	55
10.0	45	55
10.1	85	15
13	85	15

Diluent: Methanol and water (85:15)

System suitability solution: 1 mg/mL of USP Aminobenzoate Sodium RS, 0.01 mg/mL of USP 4-Nitrobenzoic Acid RS, and 0.01 mg/mL of USP Benzocaine RS in *Diluent* prepared as follows. Transfer 1 mL each of 0.1 mg/mL of USP 4-Nitrobenzoic Acid RS in methanol and 0.1 mg/mL of USP Benzocaine RS in *Diluent* to a 10-mL volumetric flask containing the appropriate amount of USP Aminobenzoate Sodium RS, and dilute with *Diluent* to volume.

Standard solution: 1 µg/mL each of USP Aminobenzoate Sodium RS, USP 4-Nitrobenzoic Acid RS, and USP Benzocaine RS in *Diluent*

Sample solution: 1 mg/mL of Aminobenzoate Sodium in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.0-mm × 15-cm; 3.5-µm packing L11

Flow rate: 0.4 mL/min

Injection volume: 5 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between benzocaine and 4-nitrobenzoic acid, *System suitability solution*

Relative standard deviation: NMT 3% for the aminobenzoate sodium, 4-nitrobenzoic

acid, and benzocaine peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 4-nitrobenzoic acid or benzocaine in the portion of Aminobenzoate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of 4-nitrobenzoic acid or benzocaine from the *Sample solution*

r_S peak response of 4-nitrobenzoic acid or benzocaine from the *Standard solution*

C_S concentration of USP 4-Nitrobenzoic Acid RS or USP Benzocaine RS in the *Standard solution* (mg/mL)

C_U concentration of Aminobenzoate Sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Aminobenzoate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any unspecified impurity from the *Sample solution*

r_S peak response of aminobenzoate from the *Standard solution*

C_S concentration of USP Aminobenzoate Sodium RS in the *Standard solution* (mg/mL)

C_U concentration of Aminobenzoate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	1.0	—
Benzocaine	2.0	0.2
4-Nitrobenzoic acid	2.1	0.2
Any individual unspecified impurity	—	0.10

■ 1S (USP39)

SPECIFIC TESTS

- **pH** 〈 791 〉

Sample solution: 50 mg/mL

Acceptance criteria: 8.0–9.0

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:● **USP Reference Standards** { 11 }USP Aminobenzoate Sodium RS ■ **USP Aniline RS**

Aniline.

C₆H₇N 93.13USP Benzocaine RS

USP 4-Nitrobenzoic Acid RS

4-Nitrobenzoic acid.

C₇H₅NO₄ 167.12USP *p*-Toluidine RS

4-Methylaniline.

C₇H₉N 107.16■ **1S (USP39)****BRIEFING**

Amitraz, *USP 38* page 2200. As part of the USP monograph modernization initiative, the following revisions are proposed:

1. *Identification* test *A*, currently based on infrared absorption, is revised to allow more flexibility to the users.
2. Omit TLC-based *Identification* test *B*. The remaining *Identification* tests are sufficient to establish identity of the article.
3. Replace the packed-column gas chromatographic *Assay* with a capillary-column procedure adapted from the *British Pharmacopoeia* monograph. This procedure is based on analysis performed with the Chrompack CP-Sil 5 CB brand of G9 column. The typical retention time of the amitraz peak is about 11 min.
4. Replace the TLC *Organic Impurities* procedure with a capillary-column gas chromatographic procedure adapted from the *British Pharmacopoeia* monograph. This procedure is based on analysis performed with the Chrompack CP-Sil 8 CB brand of G27 column. The typical retention time of the amitraz peak is about 32 min. The *impurities Acceptance criteria* are taken from the 2013 edition of the *British Pharmacopoeia*.
5. Revise the test for *Water Determination* to provide flexibility in the solvent system used based on comments and supporting data received.
6. Add the new Reference Standards used in the test for *Organic Impurities* to the *USP Reference Standards* section.

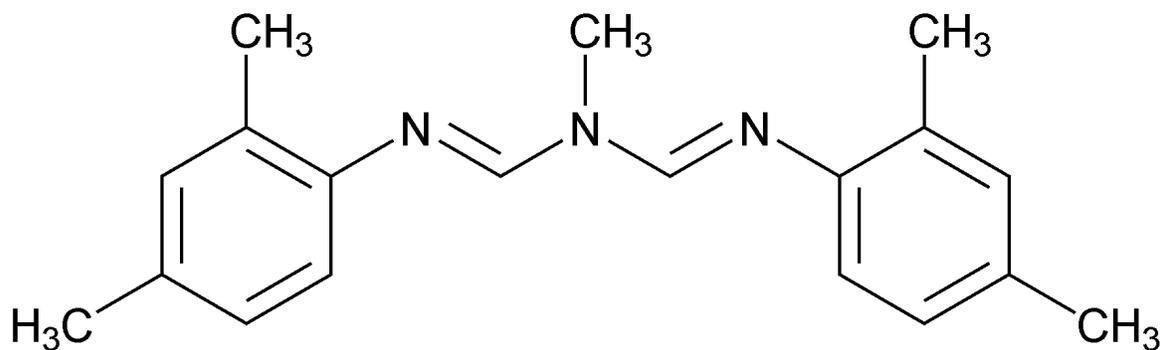
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: M. Puderbaugh.)

Correspondence Number—C119020

Comment deadline: May 31, 2015

Amitraz



$C_{19}H_{23}N_3$ 293.41

Methanimidamide, *N'*-(2,4-dimethylphenyl)-*N*-[[[(2,4-dimethylphenyl)imino]methyl]-*N*-methyl-; *N*-Methyl-*N'*-2,4-xylyl-*N*-(*N*-2,4-xylylformimidoyl)formamidine; *N*-Methylbis(2,4-xylyliminomethyl)amine [33089-61-1].

DEFINITION

Amitraz contains NLT 95.0% and NMT 101.5% of amitraz ($C_{19}H_{23}N_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. Infrared Absorption** ~~(197M)~~

- ~~(197)~~

[Note—Methods described under *Infrared Absorption* (197K), (197M), or (197A) may be used.] ■1S (USP39)

Delete the following:

- ~~**B. Thin-Layer Chromatography**~~

~~**Standard solution:** 2 mg/mL of USP Amitraz RS in toluene~~

~~**Sample solution:** 2 mg/mL of Amitraz in toluene~~

~~**Analysis:** Proceed as directed for *Organic Impurities*.~~

~~**Acceptance criteria:** The R_f value of the principal spot in the *Sample solution* corresponds to that of the *Standard solution*. ■1S (USP39)~~

Change to read:

- ~~C.~~

- ~~**B.**~~ ■1S (USP39)

~~The retention time of the major peak from the *Identification solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.~~

■ The retention time of the amitraz peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

ASSAY**Change to read:**• **Procedure****Internal standard solution:** ~~10 mg/mL of squalane in methyl acetate~~**Standard solution:** ~~8 mg/mL of USP Amitraz RS in *Internal standard solution*~~**Sample solution:** ~~8 mg/mL of Amitraz in *Internal standard solution*~~**Identification solution:** ~~8 mg/mL of Amitraz in methyl acetate~~**Chromatographic system**~~(See *Chromatography* < 621 >, *System Suitability*.)~~**Mode:** ~~GC~~**Detector:** ~~Flame ionization~~**Column:** ~~4-mm × 1.5-m column packed with 3% liquid phase G1 on support S1A~~**Temperature****Column:** ~~250°~~**Detector:** ~~250°~~**Carrier gas:** ~~Dry nitrogen~~**Flow rate:** ~~60 mL/min~~**Injection volume:** ~~5 µL~~**System suitability****Sample:** ~~*Standard solution*~~**Suitability requirements****Resolution:** ~~NLT 3.0 between squalane and amitraz~~**Relative standard deviation:** ~~NMT 2.0%~~**Analysis****Samples:** ~~*Sample solution*, *Standard solution*, and *Identification solution*~~Calculate the percentage of amitraz ($C_{19}H_{23}N_3$) in the portion of Amitraz taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

 ~~R_U ratio of the responses of the amitraz and squalane peaks from the *Sample solution*~~ ~~R_S ratio of the responses of the amitraz and squalane peaks from the *Standard solution*~~ ~~C_S concentration of USP Amitraz RS in the *Standard solution* (mg/mL)~~ ~~C_U concentration of Amitraz in the *Sample solution* (mg/mL)~~**Acceptance criteria:** ~~95.0%–101.5% on the anhydrous basis~~■ **Internal standard solution:** 0.7% v/v solution of squalane in methyl acetate**Standard solution:** 5.0 mg/mL of USP Amitraz RS in *Internal standard solution***Sample solution:** 5.0 mg/mL of Amitraz in *Internal standard solution***Chromatographic system**~~(See *Chromatography* < 621 >, *System Suitability*.)~~**Mode:** GC**Detector:** Flame ionization**Column:** 0.53-mm × 15-m fused silica; coated with a 1.5-µm layer of liquid phase G9**Temperatures**

Detector: 300°**Inlet:** 230°**Column:** 220°**Carrier gas:** Helium**Flow rate:** 12 mL/min**Injection volume:** 1 µL**System suitability****Sample:** *Standard solution*

[Note—The elution order is amitraz followed by squalane.]

Suitability requirements**Resolution:** NLT 3.0 between amitraz and squalane**Relative standard deviation:** NMT 2.0% from the peak area ratio of amitraz to squalane**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of amitraz (C₁₉H₂₃N₃) in the portion of Amitraz taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U= peak response ratio of amitraz and squalane from the *Sample solution**R_S*= peak response ratio of amitraz and squalane from the *Standard solution**C_S*= concentration of USP Amitraz RS in the *Standard solution* (mg/mL)*C_U*= concentration of Amitraz in the *Sample solution* (mg/mL)**Acceptance criteria:** 95.0%–101.5% on the anhydrous basis ■ 1S (USP39)**IMPURITIES**

- **Residue on Ignition** (281): NMT 0.2%

Change to read:

- **Organic Impurities**

Standard solution A: 2.0 mg/mL of USP Amitraz RS in toluene**Standard solution B:** 0.30 mg/mL of 2,4-dimethylaniline in toluene**Sample solution:** 100 mg/mL of Amitraz in toluene**Chromatographic system**(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 2 μ L

Developing solvent system: Cyclohexane, ethyl acetate, and triethylamine (5:3:2)

Spray reagent: 0.5% solution of *N*-(1-naphthyl) ethylenediamine dihydrochloride in methanol

Analysis

Samples: *Sample solution*, *Standard solution A*, and *Standard solution B*

Stand the plate to a depth of 3.5 cm in a solution prepared by dissolving 35 g of acetamide in 100 mL of methanol, adding 100 mL of triethylamine, and diluting to 250 mL with methanol. Allow the wet plate to stand in a current of cold air for 30 s. Immediately apply *Samples* separately to the plate, at a level about 1 cm below the top of the impregnated zone. Promptly develop the plate until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber and allow to air-dry. Examine the plate under short-wavelength UV light (*Acceptance criteria 1*). Expose the plate to the vapor of hydrochloric acid for 10 min, then expose it to the vapor of nitrogen dioxide (prepared by the reaction of nitric acid and zinc) for 10 min, remove any excess nitric oxide by air exhaust, spray the plate with *Spray reagent*, and examine the plate (*Acceptance criteria 2*).

Acceptance criteria 1: Any secondary spot from the *Sample solution* is not more intense than the corresponding spot from *Standard solution A* (2.0%).

Acceptance criteria 2: Any secondary spot from the *Sample solution* corresponding to 2,4-dimethylaniline is not more intense than the spot from *Standard solution B* (0.30%).

■ **Standard solution:** 0.05 mg/mL of 2,4-dimethylaniline, 1.0 mg/mL of USP Amitraz Related Compound A RS, 0.5 mg/mL of USP Amitraz Related Compound B RS, and 1.0 mg/mL of USP Amitraz Related Compound C RS in methyl acetate

Sample solution: 50.0 mg/mL of Amitraz in methyl acetate

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm \times 10-m fused silica; coated with a 5- μ m layer of liquid phase G27

Temperatures

Detector: 300 $^{\circ}$

Inlet: 230 $^{\circ}$

Column: See *Table 1*.

Table 1

Initial Temperature ($^{\circ}$)	Temperature Ramp ($^{\circ}$ /min)	Final Temperature ($^{\circ}$)	Hold Time at Final Temperature (min)
125	0	125	5
125	5	270	15

Carrier gas: Helium

Flow rate: 12 mL/min

Injection volume: 1 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between amitraz related compound A and amitraz related compound B

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each of amitraz related compounds A, B, and C in the portion of Amitraz taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of each individual impurity from the *Sample solution*

r_S

= peak response of the corresponding related compound from the *Standard solution*

C_S

= concentration of the corresponding related compound in the *Standard solution* (mg/mL)

C_U

= concentration of Amitraz in the *Sample solution* (mg/mL)

Calculate the percentage of 2,4-dimethylaniline and any other individual impurity in the portion of Amitraz taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of each individual impurity from the *Sample solution*

r_S

= peak response of 2,4-dimethylaniline from the *Standard solution*

C_S

= concentration of 2,4-dimethylaniline in the *Standard solution* (mg/mL)

C_U

= concentration of Amitraz in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting level for impurities is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria NMT (%)
2,4-Dimethylaniline	0.11	0.1
Amitraz related compound A	0.35	2
Amitraz related compound B	0.40	1
Amitraz related compound C	0.86	2
Amitraz	1.0	—
Any other individual impurity	—	0.1

■ 1S (USP39)

SPECIFIC TESTS

Change to read:

- **Water Determination, Method I (921)**: NMT 0.1%, ~~anhydrous pyridine being used in place of methanol in the titration vessel~~

■ 1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage**: Preserve in well-closed containers.
- **Labeling**: Label it to indicate that it is for veterinary use only.

Change to read:

- **USP Reference Standards (11)**

USP Amitraz RS

■ USP Amitraz Related Compound A RS

2,4-Dimethylphenyl formamide;
N-(2,4-Dimethylphenyl)formamide.
 $C_9H_{11}NO$ 149.19

USP Amitraz Related Compound B RS

2,4-Dimethylphenyl *N*-methyl-formamide;
N-(2,4-Dimethylphenyl)-*N*-methylformimidamide.
 $C_{10}H_{14}N_2$ 162.23

USP Amitraz Related Compound C RS

Bisformamide analog;
N,N'-Bis(2,4-dimethylphenyl)formimidamide.
 $C_{17}H_{20}N_2$ 252.35 ■ 1S (USP39)

BRIEFING

Amitraz Concentrate for Dip, USP 38 page 2201. As part of the USP monograph modernization initiative, it is proposed to make the following revisions:

1. Replace the TLC-based procedure for *Identification* test A with an IR procedure.
2. Replace the packed-column gas chromatographic *Assay* with a capillary-column

procedure adapted from the *British Pharmacopoeia* monograph. This procedure is based on analysis performed with the Chrompack CP-Sil 5 CB brand of G9 column. The typical retention time of the amitraz peak is about 11 min.

3. The test for *Water Determination* is revised to provide flexibility in the solvent system used based on comments and supporting data received.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: M. Puderbaugh.)

Correspondence Number—C119028

Comment deadline: May 31, 2015

Amitraz Concentrate for Dip

DEFINITION

Amitraz Concentrate for Dip contains amitraz in a suitable vehicle. It may contain a suitable stabilizing agent. It contains NLT 90.0% and NMT 120.0% of the labeled amount of amitraz ($C_{19}H_{23}N_3$).

IDENTIFICATION

Delete the following:

■ A. Thin-Layer Chromatography

Standard solution: 5 mg/mL of USP Amitraz RS in toluene

Sample solution: nominally 5 mg/mL of amitraz from Concentrate for Dip diluted with toluene

Chromatographic system

(See *Chromatography* ~~(621)~~, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 2 μ L

Developing solvent system: Cyclohexane, ethyl acetate, and triethylamine (5:3:2)

Spray reagent: 0.5% solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride in methanol

Analysis

Samples: *Sample solution* and *Standard solution*

Stand the plate to a depth of 3.5 cm in a solution prepared by dissolving 35 g of acetamide in 100 mL of methanol, adding 100 mL of triethylamine, and diluting to 250 mL with methanol. Allow the wet plate to stand in a current of cold air for 30 s. Immediately apply *Samples* separately to the plate, at a level about 1 cm below the top of the impregnated zone. Promptly develop the plate until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber and allow to air-dry. Examine the plate under short-wavelength UV light.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. ■ 1S (USP39)

Add the following:

- ● **A. Infrared Absorption** 〈 197 〉

Sample: Shake a quantity of Concentrate for Dip containing 0.1 g of amitraz with 10 mL of acetone for 5 min, filter, and evaporate the filtrate to dryness.

[Note—Methods described under *Infrared Absorption* 〈 197K 〉, 〈 197M 〉, or 〈 197A 〉 may be used.]

- 1S (USP39)

Change to read:

- **B.** The retention time of the major peak of the *Identification solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- The retention time of the amitraz peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

ASSAY**Change to read:**

- **Procedure**

Internal standard solution: ~~10 mg/mL of squalane in methyl acetate~~

Standard solution: ~~8 mg/mL of USP Amitraz RS in *Internal standard solution*~~

Sample solution: ~~Nominally 8 mg/mL of amitraz in *Internal standard solution*~~

Identification solution: ~~Equivalent to 8 mg/mL of amitraz in methyl acetate~~

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: ~~GC~~

Detector: ~~Flame ionization~~

Column: ~~4-mm × 1.5-m column packed with 3% liquid phase G1 on support S1A~~

Temperature

Column: ~~250°~~

Detector: ~~250°~~

Carrier gas: ~~Dry nitrogen~~

Flow rate: ~~60 mL/min~~

Injection size: ~~5 µL~~

System suitability

Sample: ~~*Standard solution*~~

Suitability requirements

Resolution: ~~NLT 3.0 between squalane and amitraz~~

Relative standard deviation: ~~NMT 2.0%~~

Analysis

Samples: ~~*Sample solution*, *Standard solution*, and *Identification solution*~~

Calculate the percentage of amitraz ($C_{19}H_{23}N_3$) in the portion of Concentrate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

~~R_U~~ ratio of the response of the amitraz peak to the response of the squalane peak from the *Sample solution*

~~R_S~~ ratio of the response of the amitraz peak to the response of the squalane peak from the *Standard solution*

~~C_S~~ concentration of USP Amitraz RS in the *Standard solution* (mg/mL)

~~C_U~~ nominal concentration of amitraz in the *Sample solution* (mg/mL)

~~**Acceptance criteria:** 90.0%–120.0%~~

■ **Internal standard solution:** 0.7% v/v solution of squalane in methyl acetate

Standard solution: 5.0 mg/mL of USP Amitraz RS in *Internal standard solution*

Sample solution: Nominally equivalent to 5.0 mg/mL of amitraz from Concentrate for Dip in *Internal standard solution*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 15-m fused silica; coated with a 1.5-μm layer of liquid phase G9

Temperatures

Column: 220°

Inlet: 230°

Detector: 300°

Carrier gas: Helium

Flow rate: 12 mL/min

Injection volume: 1 μL

System suitability

Sample: *Standard solution*

[Note—The elution order is amitraz, followed by squalane.]

Suitability requirements

Resolution: NLT 3.0 between amitraz and squalane

Relative standard deviation: NMT 2.0% from the peak area ratio of amitraz to squalane

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amitraz (C₁₉H₂₃N₃) in the portion of Concentrate for Dip taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U

= peak response ratio of amitraz and squalane from the *Sample solution*

R_S

= peak response ratio of amitraz and squalane from the *Standard solution*

C_S

= concentration of USP Amitraz RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of amitraz in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–120.0% ■ 1S (USP39)

SPECIFIC TESTS

Change to read:

- **Water Determination, Method I (921):** NMT 0.15%, ~~with anhydrous pyridine being used in place of methanol in the titration vessel~~
- 1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **Labeling:** Label it to indicate that it is for veterinary use only and that it is to be diluted before use. The label also states the name and quantity of diluent to be used, the directions for dilution, and the conditions for storage of the constituted Concentrate for Dip.
- **USP Reference Standards (11)**
USP Amitraz RS

BRIEFING

Aripiprazole, USP 38 page 2280. It is proposed to revise the monograph by replacing the dash for aripiprazole related compound F in *Table 2* with the limit for any individual unspecified impurity, and to acknowledge that this impurity may not be relevant to all manufacturing processes. It is also proposed to widen the acceptance criteria for *Loss on Drying* from NMT 0.3% to NMT 0.5% for consistency with the corresponding *European Pharmacopoeia* monograph.

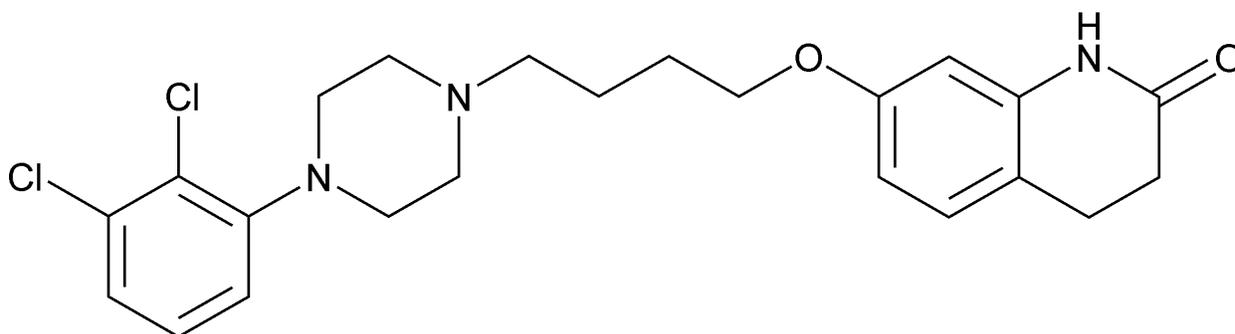
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: H. Joyce.)

Correspondence Number—C137326; C144504

Comment deadline: May 31, 2015

Aripiprazole



$C_{23}H_{27}Cl_2N_3O_2$ 448.39

2(1*H*)-Quinolinone, 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydro- ;
7-[4-[4-(2,3-Dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydrocarbostyryl [129722-12-9].

DEFINITION

Aripiprazole contains NLT 98.0% and NMT 102.0% of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** < 197K >
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Protect the solutions from light.

Diluent: Acetonitrile, methanol, water, and acetic acid (30:10:60:1)

Solution A: Acetonitrile and 0.05% trifluoroacetic acid (10:90)

Solution B: Acetonitrile and 0.05% trifluoroacetic acid (90:10)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
2	80	20
10	65	35
20	10	90
25	10	90
26	80	20
35	80	20

[Note—The gradient was established on an HPLC system with a dwell volume of approximately 650 μ L.]

System suitability solution: 1 μ g/mL each of USP Aripiprazole RS and USP Aripiprazole Related Compound F RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Aripiprazole RS in *Diluent*

Sample solution: 0.1 mg/mL of Aripiprazole in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 10-cm; 3-μm packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for aripiprazole and aripiprazole related compound F are 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 2.0 between aripiprazole and aripiprazole related compound F, *System suitability solution*

Tailing factor: NMT 1.5 for aripiprazole, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of aripiprazole (C₂₃H₂₇Cl₂N₃O₂) in the portion of Aripiprazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak area from the *Sample solution*

r_S peak area from the *Standard solution*

C_S concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)

C_U concentration of Aripiprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

-

Residue on Ignition { 281 } : NMT 0.1%

Delete the following:

-

• **Heavy Metals, Method II** { 231 }

: NMT 10 ppm •(Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

Protect the solutions from light.

Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as

directed in the Assay.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Aripiprazole taken:

$$\text{Result} = (r_i/r_U) \times (1/F) \times 100$$

r_i = peak response of each impurity from the *Sample solution*

r_U = peak response of aripiprazole from the *Sample solution*

F = relative response factor (see Table 2)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Aripiprazole related compound G ^a	0.9	0.72	0.10
Aripiprazole	1.0	—	—
Aripiprazole related compound F ^{b,c}	1.1	1.0	0.10
Aripiprazole 4,4'-dimer ^d	1.3	1.0	0.10
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.50

a 7-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy}quinolin-2(1*H*)-one.

b 4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazin 1-oxide.

c ~~For system suitability and identification purposes only.~~
 ■ If possible from the manufacturing process. ■ 1S (USP39)

d 1,1'-(Ethane-1,1-diyl)bis(2,3-dichloro-4-{4-[3,4-dihydroquinolin-2(1*H*)-one-7-yloxybutyl]piperazin-1-yl}benzene).

SPECIFIC TESTS

Change to read:

- **Loss on Drying** (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: ~~NMT 0.3%~~

■ NMT 0.5% ■ 1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.

- **USP Reference Standards** < 11 >

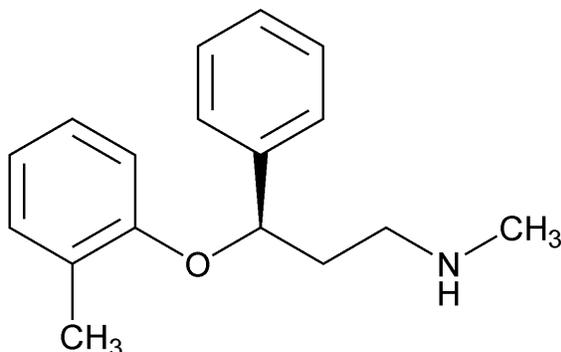
USP Aripiprazole RS USP Aripiprazole Related Compound F RS 4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazin
1-oxide.C₂₃H₂₇Cl₂N₃O₃ 464.38**BRIEFING**

Atomoxetine Hydrochloride, *USP 38* page 2313. It is proposed to clarify the monograph by replacing the dashes in the *Acceptance criteria* for atomoxetine related compound A and mandelic acid in *Table 1* with the limit for any unspecified impurity and to remove the footnotes.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: H. Joyce.)

Correspondence Number—C143633

Comment deadline: May 31, 2015**Atomoxetine Hydrochloride**

• HCl

C₁₇H₂₁NO·HCl 291.82Benzenepropanamine, *N*-methyl-*γ*-(2-methylphenoxy)-, hydrochloride, (–);(–)-*N*-Methyl-3-phenyl-3-(*o*-tolylloxy)propylamine hydrochloride [82248-59-7].**DEFINITION**

Atomoxetine Hydrochloride contains NLT 98.0% and NMT 102.0% of atomoxetine hydrochloride (C₁₇H₂₁NO·HCl), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** < 197K >
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the atomoxetine *R*-isomer from the *System suitability solution*, as obtained in the test for *Organic Impurities, Procedure 2*.
- **C. Identification Tests—General, Chloride** < 191 >: Meets the requirements of the silver

nitrate precipitate test

ASSAY

• Procedure

Buffer: 2.9 g/L of phosphoric acid in water. Adjust with 5 M potassium hydroxide solution to a pH of 2.5. To 1 L of this solution add 5.9 g of octanesulfonic acid sodium salt monohydrate.

Mobile phase: *n*-Propanol and *Buffer* (27:73). [Note—The ratio of *n*-propanol in *Buffer* can be varied between 26:74 and 29:71 to meet system suitability requirements.]

System suitability solution: 0.1 mg/mL of USP Mandelic Acid RS, 0.15 mg/mL of USP Atomoxetine Related Compound A RS, and 0.25 mg/mL of USP Atomoxetine Hydrochloride RS in *Mobile phase*

Standard solution: 0.25 mg/mL of USP Atomoxetine Hydrochloride RS in *Mobile phase*. Sonication may be used to aid in dissolution.

Sample solution: 0.25 mg/mL of Atomoxetine Hydrochloride in *Mobile phase*. Sonication may be used to aid in dissolution.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing L7

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 10 μL

Run time: 1.3 times the retention time of atomoxetine

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* in *Organic Impurities, Procedure 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between mandelic acid and atomoxetine related compound A, *System suitability solution*

Tailing factor: NMT 1.5 for atomoxetine, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of atomoxetine hydrochloride (C₁₇H₂₁NO·HCl) in the portion of Atomoxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Atomoxetine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U concentration of Atomoxetine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Delete the following:

-

- **Heavy Metals, Method II** 〈 231 〉

: NMT 10 ppm (Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

- [

Note—It is required to perform *Organic Impurities, Procedure 1* and *Organic Impurities, Procedure 2*.] ■1S (USP39)

Procedure 1

Buffer and Mobile phase: Prepare as directed in the *Assay*.

System suitability solution: 0.10 mg/mL of USP Mandelic Acid RS, 0.15 mg/mL of USP Atomoxetine Related Compound A RS, and 0.25 mg/mL of USP Atomoxetine Hydrochloride RS in *Mobile phase*

Standard solution: 0.0025 mg/mL of USP Atomoxetine Hydrochloride RS in *Mobile phase*

Sample solution: 2.5 mg/mL of Atomoxetine Hydrochloride in *Mobile phase*

Chromatographic system: Proceed as directed in the *Assay*, except for *Run time*.

Run time: 2.6 times the retention time of atomoxetine

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between mandelic acid and atomoxetine related compound A, *System suitability solution*

Tailing factor: NMT 1.5 for atomoxetine, *System suitability solution*

Relative standard deviation: NMT 5% from three injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Atomoxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each individual impurity from the *Sample solution*

r_S peak response of atomoxetine from the *Standard solution*

C_S concentration of USP Atomoxetine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U concentration of Atomoxetine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Mandelic acid ^a ■ ■ 1S (USP39)	0.20	— ■ 0.10 ■ 1S (USP39)
Atomoxetine related compound A ^a ■ ■ 1S (USP39)	0.27	— ■ 0.10 ■ 1S (USP39)
Desmethyl atomoxetine ^b ■ ^a ■ 1S (USP39)	0.73	0.3
Atomoxetine	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5
^a For system suitability purposes only. ^b (R)-N-Methyl-3-phenoxy-3-phenylpropan-1-amine. ^a (R)-N-Methyl-3-phenoxy-3-phenylpropan-1-amine. ■ 1S (USP39)		

Procedure 2

Mobile phase: Isopropyl alcohol, diethylamine, trifluoroacetic acid, and *n*-hexane (150: 1.5: 2.0: 846.5)

System suitability solution: 3.5 mg/mL of USP Atomoxetine Hydrochloride RS, 17.5 µg/mL of USP Atomoxetine *S*-Isomer RS, and 3.5 µg/mL of USP Atomoxetine Related Compound B RS, prepared by first dissolving the Reference Standards in absolute alcohol, using 25% of the final volume. Dilute with *n*-hexane to volume.

Sample solution: 3.5 mg/mL of Atomoxetine Hydrochloride prepared by first dissolving it in absolute alcohol, using 25% of the final volume. Dilute with *n*-hexane to volume.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 273 nm

Column: 4.6-mm × 25-cm; 5-µm packing L40

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 1.3 times the retention time of atomoxetine

System suitability

Sample: *System suitability solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.75 between atomoxetine *S*-isomer and atomoxetine related compound B

Tailing factor: NMT 1.8 for atomoxetine

Analysis

Sample: *Sample solution*

Calculate the percentage of atomoxetine related compound B, atomoxetine related compound C, and atomoxetine *S*-isomer in the portion of Atomoxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_{\bar{U}}$ peak response of each individual impurity from the *Sample solution*

$r_{\bar{T}}$ sum of all the peak responses of atomoxetine related compound B, atomoxetine related compound C, atomoxetine *S*-isomer, and atomoxetine from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Atomoxetine <i>S</i> -isomer ^a	0.47	0.5
Atomoxetine related compound C ^b	0.52	0.1
Atomoxetine related compound B	0.56	0.1
Atomoxetine	1.0	—
^a <i>N</i> -Methyl-3-phenyl-3-(<i>o</i> -tolylloxy)propan-1-amine. ^b <i>N</i> -Methyl-3-phenyl-3-(<i>p</i> -tolylloxy)propan-1-amine.		

SPECIFIC TESTS

- **Loss on Drying** 〈 731 〉

Analysis: Dry under vacuum at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at room temperature.

- **USP Reference Standards** 〈 11 〉

USP Atomoxetine Hydrochloride RS

USP Atomoxetine Related Compound A RS

3-(Methylamino)-1-phenylpropan-1-ol.

C₁₀H₁₅NO 165.23

USP Atomoxetine Related Compound B RS

N-Methyl-3-phenyl-3-(*m*-tolylloxy)propan-1-amine hydrochloride.

C₁₇H₂₁NO·HCl 291.82

USP Atomoxetine *S*-Isomer RS

(*S*)-*N*-Methyl-3-phenyl-3-(*o*-tolylloxy)propan-1-amine hydrochloride.

C₁₇H₂₁NO·HCl 291.82

USP Mandelic Acid RS

α-Hydroxyphenylacetic acid.

C₈H₈O₃ 152.15

BRIEFING

Atomoxetine Capsules, *USP 38* page 2311. It is proposed to clarify the monograph by replacing the dash in the *Acceptance criteria* for atomoxetine *N*-amide within *Table 1* with the limit for any individual unspecified degradation product.

Additionally, minor editorial changes have been made to update the monograph to current

USP style.

(SM4: H. Joyce.)

Correspondence Number—C155150

Comment deadline: May 31, 2015

Atomoxetine Capsules

DEFINITION

Atomoxetine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of atomoxetine (C₁₇H₂₁NO).

IDENTIFICATION

- **A. Infrared Absorption** 〈 197K 〉 or 〈 197A 〉

Standard: 6 mg/mL of USP Atomoxetine Hydrochloride RS in methanol. Dry the solution to a dry powder under an air or nitrogen purge for a minimum of 3 h.

Sample: Shake the contents of a sufficient number of Capsules, equivalent to about 60 mg of atomoxetine, with 10 mL of methanol. Centrifuge at 4000 rpm for 5 min. Evaporate the solution to a dry powder with the aid of a current of air or stream of nitrogen.

Acceptance criteria: The IR spectrum exhibits main bands at (± 2) wavenumbers (cm⁻¹) 2955, 2855, 1599–1604, 1492, 1048, 1023, and 1010.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: 5.8 g/L of monobasic potassium phosphate in water. To each liter of this solution add 3.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and *Buffer* (38:62)

System suitability solution: 0.1 mg/mL of atomoxetine (free base) from USP Atomoxetine Hydrochloride RS and 0.02 mg/mL of *o*-cresol in *Mobile phase*. Sonicate to aid in dissolution.

Standard solution: 0.1 mg/mL of atomoxetine (free base) from USP Atomoxetine Hydrochloride RS in *Mobile phase*. Sonicate to aid in dissolution.

Sample stock solution: From NLT 10 Capsules (including shells) prepared as follows. Add the intact Capsules to a suitable volumetric flask. Add *Mobile phase* to fill 65% of the final volume. Allow to stand for at least 10 min, then shake for 20 min. Dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.1 mg/mL of atomoxetine, prepared by diluting a suitable volume of *Sample stock solution* with *Mobile phase*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 7.5-cm; 3.5- μ m packing L7

Column temperature: 35^o

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

Run time: 1.7 times the retention time of atomoxetine

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for atomoxetine and *o*-cresol are 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 3.5 between atomoxetine and *o*-cresol, *System suitability solution*

Tailing factor: NMT 2.0 for atomoxetine, *System suitability solution*

Relative standard deviation: NMT 1.0% for atomoxetine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of atomoxetine ($C_{17}H_{21}NO$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of atomoxetine in the *Standard solution* (mg/mL)

C_U nominal concentration of atomoxetine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** 〈 711 〉

Medium: 0.1 N hydrochloric acid; 1000 mL, deaerated

Apparatus 2: 50 rpm, with three-prong sinker

Time: 30 min

Buffer and Mobile phase: Prepare as directed in the *Assay*.

Standard stock solution: 0.1 mg/mL of atomoxetine (free base) from USP Atomoxetine Hydrochloride RS in *Medium*. Sonicate to aid in dissolution.

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of $(L/1000)$ mg/mL, where L is the Capsule label claim in mg.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.4%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of atomoxetine ($C_{17}H_{21}NO$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_{\bar{f}}$ peak response from the *Sample solution*

$r_{\bar{s}}$ peak response from the *Standard solution*

$C_{\bar{s}}$ concentration of atomoxetine in the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of *Medium* (mL)

Tolerances: NLT 80% (Q) of the labeled amount of atomoxetine ($C_{17}H_{21}NO$) is dissolved.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

Change to read:

- **Organic Impurities**

Buffer: Dissolve 4.9 g of sodium 1-decanesulfonate and 6.9 g of monobasic potassium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 3.1.

Mobile phase: Acetonitrile and *Buffer* (41:59)

Sensitivity solution: 0.1 µg/mL of atomoxetine in *Mobile phase*

System suitability solution: 1 mg/mL of atomoxetine containing atomoxetine *N*-amide prepared as follows. Weigh equal amounts of USP Atomoxetine Hydrochloride RS and urea, and place in a volumetric flask. Add water to fill 10% of the final volume. Sonicate for 3 min. Place the flask in an 85° oven for 40 min. Allow the solution to cool to room temperature. Dilute with *Mobile phase* to volume. [Note—The oven temperature and time in the oven can be adjusted to give a suitable level of atomoxetine *N*-amide peak.]

Sample solution: 1 mg/mL of atomoxetine in *Mobile phase*, from the contents of NLT 5 Capsules prepared as follows. Transfer the Capsule contents to a suitable volumetric flask. Fill 50% of the final volume with *Mobile phase*. Swirl, and let stand for 15 min. Dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 2.3 times the retention time of atomoxetine

System suitability

Samples: *Sensitivity solution* and *System suitability solution*

[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.6 between atomoxetine and atomoxetine *N*-amide, *System suitability solution*

Relative standard deviation: NMT 5%, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of each individual impurity from the *Sample solution*

r_T sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desmethyl atomoxetine ^a	0.76	0.3
Atomoxetine	1.0	—
Atomoxetine <i>N</i> -amide ^{b,c} ■ 1S (USP39)	1.2	■ 0.2 ■ 1S (USP39)
Any individual unspecified degradation product	—	0.2
Total impurities	—	1.0
a (R)- <i>N</i> -Methyl-3-phenoxy-3-phenylpropan-1-amine. b (R)-1-Methyl-1-[3-phenyl-3-(<i>o</i> -tolylxy)propyl]urea. c For system suitability purposes only. ■ 1S (USP39)		

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP Reference Standards** { 11 }
USP Atomoxetine Hydrochloride RS

BRIEFING

Carbamazepine Extended-Release Tablets, USP 38 page 2586. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Replace the two liquid chromatographic procedures used in *Organic Impurities, Procedure 1* and *Organic Impurities, Procedure 2* with a single more specific, stability-indicating liquid chromatographic procedure. This proposed procedure was developed for the Carbamazepine family and was validated with the Waters Acquity UPLC HSS Cyano brand of L10 column. The typical retention time for carbamazepine is about 10.2 min.
2. Replace the liquid chromatographic procedure in the *Assay* with a procedure similar to the proposed test for *Organic Impurities*.
3. Add *Acceptance criteria* for a specified degradation product to the monograph. Manufacturers are encouraged to submit their approved specifications to USP if they are different from those proposed in this revision.

4. Add USP Carbamazepine Related Compound A RS and USP Carbamazepine Related Compound B RS to the *USP Reference Standards* section to support the proposed revision.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: G. Hsu, H. Joyce.)

Correspondence Number—C140170

Comment deadline: May 31, 2015

Carbamazepine Extended-Release Tablets

DEFINITION

Carbamazepine Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$).

IDENTIFICATION

- **A. Ultraviolet Absorption** (197U)

Standard solution: 10 µg/mL of USP Carbamazepine RS in methanol

Sample solution: Finely powder 1 Tablet, and quantitatively transfer the powder, with the aid of methanol, to a 100-mL volumetric flask. Add about 70 mL of methanol, and shake by mechanical means for 60 min. Sonicate for 15 min, and dilute with methanol to volume. Allow to stand for 10–15 min. Dilute a portion of the clear solution with methanol to obtain a solution containing about 10 µg/mL of carbamazepine.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

~~**Mobile phase:** Methanol, methylene chloride, and water (450:45:600)~~

~~**Internal standard solution:** 600 µg/mL of phenytoin in methanol~~

~~**Standard stock solution:** 200 µg/mL of USP Carbamazepine RS in methanol~~

~~**Standard solution:** 100 µg/mL of carbamazepine from *Standard stock solution* in *Internal standard solution*~~

~~**System suitability solution:** 50 µg/mL of carbamazepine from *Standard solution* in *Internal standard solution*~~

~~**Sample stock solution A:** Nominally 4 mg/mL of carbamazepine from finely powdered Tablets prepared as follows. Finely powder 10 Tablets. Transfer the powder to an appropriate volumetric flask with the aid of methanol. Add 70% of the flask volume of methanol. Shake by mechanical means for 60 min. Sonicate for 15 min, and dilute with methanol to volume. Allow to stand for 10–15 min, and then filter a portion of the supernatant. Use the clear filtrate.~~

~~**Sample stock solution B:** Nominally 0.2 mg/mL of carbamazepine from *Sample stock solution A* in methanol~~

Sample solution: ~~Nominally 100 µg/mL of carbamazepine from Sample stock solution B in Internal standard solution~~

Chromatographic system

~~(See Chromatography <621>, System Suitability.)~~

Mode: LC

Detector: UV 230 nm

Columns

Guard: 4.6 mm × 30 mm; 7 µm packing L7

Analytical: 3.9 mm × 30 cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: ~~System suitability solution~~

~~[Note—The relative retention times for phenytoin and carbamazepine are about 0.8 and 1.0, respectively.]~~

Suitability requirements

Resolution: ~~NLT 2.8 between phenytoin and carbamazepine~~

Relative standard deviation: ~~NMT 2.0%~~

Analysis

Samples: ~~Standard solution and Sample solution~~

Calculate the percentage of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U ~~peak response ratio of carbamazepine to the internal standard from the Sample solution~~

R_S ~~peak response ratio of carbamazepine to the internal standard from the Standard solution~~

C_S ~~concentration of USP Carbamazepine RS in the Standard solution (µg/mL)~~

C_U ~~nominal concentration of carbamazepine in the Sample solution (µg/mL)~~

Acceptance criteria: ~~90.0%–110.0%~~

■ **Solution A:** Add 0.5 mL of triethylamine and 0.5 mL of formic acid to 1000 mL of water.

Solution B: Add 0.25 mL of formic acid to 1000 mL of methanol.

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	80	20
3.0	80	20
12.0	60	40
18.0	45	55
20.0	45	55
20.1	80	20

Time (min)	Solution A (%)	Solution B (%)
23.0	80	20

System suitability solution: 0.002 mg/mL each of USP Carbamazepine RS and USP Carbamazepine Related Compound A RS in methanol

Standard solution: 0.1 mg/mL of USP Carbamazepine RS in methanol

Sample solution: Nominally 0.1 mg/mL of carbamazepine from Tablets prepared as follows. Powder NLT 10 Tablets and transfer a sufficient portion of the powder to a suitable volumetric flask. Add 60% of the final flask volume of methanol. Shake by mechanical means for about 1 h. Dilute with methanol to volume. Centrifuge to obtain a clear supernatant and use the clear supernatant. [Note—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 2.1-mm × 10-cm; 1.8-µm packing L10

Column temperature: 40°

Flow rate: 0.3 mL/min

Injection volume: 2 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.7 between carbamazepine related compound A and carbamazepine, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carbamazepine (C₁₅H₁₂N₂O) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% $\pm 1S$ (USP39)

PERFORMANCE TESTS

• Dissolution 〈 711 〉

Medium

For Tablets labeled to contain 100 mg or 200 mg: Water; 900 mL

For Tablets labeled to contain 400 mg: Water; 1800 mL

Apparatus 1: 100 rpm

Times: 3, 6, 12, and 24 h

Standard solution: USP Carbamazepine RS in *Medium*

Sample solution: Filtered portions of the solution under test, diluted with *Medium* if necessary

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 284 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$) dissolved at each time using the UV absorption.

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved
3	10%–35%
6	35%–65%
12	65%–90%
24	NLT 75%

The percentages (*Q*) of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$) dissolved at the times specified conform to *Acceptance Table 2* in 〈 711 〉.

• Uniformity of Dosage Units 〈 905 〉: Meet the requirements

IMPURITIES

Delete the following:

• Organic Impurities: Procedure 1

Mobile phase: Methanol, methylene chloride, and water (450:45:600)

System suitability solution: 60 $\mu\text{g/mL}$ of phenytoin and 20 $\mu\text{g/mL}$ of USP Carbamazepine RS in methanol

Standard solution: 4 $\mu\text{g/mL}$ of USP Carbamazepine RS in methanol

Sample solution: Use *Sample stock solution A* from the Assay.

Chromatographic system and System suitability: Proceed as directed in the Assay.

Analysis**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak response of each impurity from the *Sample solution* r_S peak response of carbamazepine from the *Standard solution* C_S concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL) C_U nominal concentration of carbamazepine in the *Sample solution* (mg/mL)**Acceptance criteria****Any individual unspecified degradation product:** NMT 0.2%

■ 1S (USP39)

Delete the following:■ ● **Organic Impurities: Procedure 2****Mobile phase:** Methanol, acetonitrile, and water (35:15:50)**System suitability solution:** 12.5 µg/mL of iminostilbene and 5.0 µg/mL of USP Carbamazepine RS in methanol**Standard solution:** 4 µg/mL of USP Carbamazepine RS in methanol**Sample solution:** Use *Sample stock solution A* from the *Assay*.**Chromatographic system:** Proceed as directed in the *Assay*.**System suitability****Sample:** *System suitability solution*

[Note—The relative retention times for carbamazepine and iminostilbene are about 0.3 and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 10.0 between carbamazepine and iminostilbene**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak response of each impurity from the *Sample solution* r_S peak response of carbamazepine from the *Standard solution* C_S concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL) C_U nominal concentration of carbamazepine in the *Sample solution* (mg/mL)**Acceptance criteria****Any individual unspecified degradation product:** NMT 0.2%**Total Impurities:** NMT 0.5% for all impurities from *Procedure 1* and *Procedure 2*.

■ 1S (USP39)

Add the following:■ ● **Organic Impurities**

Solution A, Solution B, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.002 mg/mL each of USP Carbamazepine RS, USP Carbamazepine Related Compound A RS, and USP Carbamazepine Related Compound B RS in methanol

Sample solution: Nominally 1.0 mg/mL of carbamazepine from Tablets prepared as follows. Powder NLT 10 Tablets and transfer a sufficient portion of the powder to a suitable volumetric flask. Add 60% of the final flask volume of methanol. Shake by mechanical means for about 1 h. Dilute with methanol to volume. Centrifuge to obtain a clear supernatant and use the clear supernatant. [Note—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.7 between carbamazepine related compound A and carbamazepine

Relative standard deviation: NMT 2.0%, carbamazepine and carbamazepine related compound B

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of carbamazepine related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of carbamazepine related compound B from the *Sample solution*

r_S peak response of carbamazepine related compound B from the *Standard solution*

C_S concentration of USP Carbamazepine Related Compound B RS in the *Standard solution* (mg/mL)

C_U nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each unspecified degradation product from the *Sample solution*

r_S peak response of carbamazepine from the *Standard solution*

C_S concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

C_U nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Disregard any impurity peaks less than 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
------	-------------------------	------------------------------

^a Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carbamazepine related compound A ^a	0.96	—
Carbamazepine	1.00	—
Carbamazepine related compound B	1.45	0.2
Individual unspecified degradation product	—	0.2
Total degradation products	—	0.5

^a Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

■ 1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.

Change to read:

- **USP Reference Standards** { 11 }

USP Carbamazepine RS

- USP Carbamazepine Related Compound A RS

10,11-Dihydrocarbamazepine.

C₁₅H₁₄N₂O 238.28

USP Carbamazepine Related Compound B RS

5*H*-Dibenz[*b,f*]azepine.

C₁₄H₁₁N 193.24 ■ 1S (USP39)

BRIEFING

Carbidopa and Levodopa Extended-Release Tablets, USP 38 page 2592. It is proposed to revise the monograph as follows:

1. Revise the disregard statement in the test for *Organic Impurities* to consider whether a peak is associated with carbidopa or levodopa.
2. Revise the relative response factor for levodopa related compound A based on additional supporting data.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: H. Joyce.)

Correspondence Number—C151195

Comment deadline: May 31, 2015

Carbidopa and Levodopa Extended-Release Tablets

DEFINITION

Carbidopa and Levodopa Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and of levodopa ($C_9H_{11}NO_4$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Protect the volumetric preparations from light.

Solution A: 0.24 g/L of sodium 1-decanesulfonate in water

Solution B: 11.6 g/L of monobasic sodium phosphate in water

Mobile phase: *Solution A*, *Solution B*, and water (0.13: 95: 4.87), prepared as follows. Add 0.13% of the final volume of *Solution A* to 95% of the final volume of *Solution B*. Adjust with phosphoric acid to a pH of 2.8. Dilute with water to final volume.

Standard solution: 0.1 mg/mL of USP Carbidopa RS and 0.4 mg/mL of USP Levodopa RS in solution, prepared as follows. Transfer accurately weighed portions of the Reference Standards into a suitable volumetric flask, and dissolve in 0.1 N phosphoric acid using 8% of the final volume. Sonication may be used to promote dissolution. Dilute with water to the final volume.

Sample solution: Nominally 0.1 mg/mL of carbidopa and 0.4 mg/mL of levodopa from NLT 20 finely powdered Tablets, prepared as follows. Transfer an accurately weighed portion of the powder, equivalent to 1 Tablet weight, into a suitable volumetric flask, and dissolve in 0.1 N phosphoric acid, using 10% of the final volume. Sonicate for 10 min, and then stir for 30 min. Dilute with water to volume, and stir for another 20 min. Pass the solution through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm \times 10-cm; 5- μ m packing L1

Flow rate: 2 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times for levodopa and carbidopa are 1.0 and 2.8, respectively.]

Suitability requirements

Tailing factor: NMT 1.5 for carbidopa; NMT 1.5 for levodopa

Resolution: NLT 6 between levodopa and carbidopa

Relative standard deviation: NMT 1.0% for carbidopa; NMT 1.0% for levodopa

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) or levodopa ($C_9H_{11}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of carbidopa or levodopa from the *Sample solution*

r_S peak response of carbidopa or levodopa from the *Standard solution*

C_S concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

C_U nominal concentration of carbidopa or levodopa in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of the labeled amounts of carbidopa and levodopa

PERFORMANCE TESTS

• Dissolution 〈 711 〉

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL degassed with helium

Apparatus 2: 50 rpm

Times

For Tablets that contain 25 mg of carbidopa and 100 mg of levodopa: 0.5, 1, and 4 h

For Tablets that contain 50 mg of carbidopa and 200 mg of levodopa: 0.5, 1, 2.5, and 4 h

Solution A: 0.24 g/L of sodium 1-decanesulfonate in water

Solution B: 12.7 g/L of monobasic sodium phosphate monohydrate in water

Mobile phase: *Solution A*, *Solution B*, and water (0.13: 95: 4.87), prepared as follows. Add 0.13% of the final volume of *Solution A* to 95% of the final volume of *Solution B*. Adjust with phosphoric acid to a pH of 2.8. Dilute with water to final volume.

Standard solution: 0.03 mg/mL of USP Carbidopa RS and 0.1 mg/mL of USP Levodopa RS in *Medium*. Sonication may be used to aid in dissolution.

Sample solution

For Tablets that contain 25 mg of carbidopa and 100 mg of levodopa: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size, and discard the first 1–3 mL.

For Tablets that contain 50 mg of carbidopa and 200 mg of levodopa: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size, discard the first 1–3 mL, and dilute with *Medium* (50:50).

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm \times 30-cm; 10- μ m packing L1

Flow rate: 2 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times for levodopa and carbidopa are 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between levodopa and carbidopa

Relative standard deviation: NMT 2.0% for carbidopa and NMT 2.0% for levodopa for six replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of carbidopa ($C_{10}H_{14}N_2O_4$) or of levodopa ($C_9H_{11}NO_4$) in the sample withdrawn from the vessel at each time point i :

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U peak response of carbidopa or levodopa from the *Sample solution*

r_S peak response of carbidopa or levodopa from the *Standard solution*

C_S concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amounts (Q_i) of carbidopa ($C_{10}H_{14}N_2O_4$) or of levodopa ($C_9H_{11}NO_4$) dissolved at each time point i :

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i concentration of carbidopa or levodopa in the portion of sample withdrawn at time point i (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim of carbidopa or levodopa (mg/Tablet)

V_S volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances

For Tablets that contain 25 mg of carbidopa and 100 mg of levodopa: See *Table 1*.

Table 1

Time Point (i)	Time (h)	Amount of Carbidopa Dissolved	Amount of Levodopa Dissolved
1	0.5	15%–40%	14%–39%
2	1	37%–62%	36%–61%
3	4	NLT 80%	NLT 80%

For Tablets that contain 50 mg of carbidopa and 200 mg of levodopa: See *Table 2*.

Table 2

Time Point (i)	Time (h)	Amount of Carbidopa Dissolved	Amount of Levodopa Dissolved
1	0.5	8%–33%	8%–33%
2	1	26%–51%	26%–51%
3	2.5	62%–87%	64%–89%
4	4	NLT 80%	NLT 80%

The percentages of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and of levodopa ($C_9H_{11}NO_4$) dissolved at the times specified conform to *Acceptance Table 2* in $\langle 711 \rangle$.

Test 2: If the product complies with this test, the labeling indicates that it meets *USP Dissolution Test 2*.

Medium: Simulated gastric fluid TS (prepared without enzymes); 900 mL

Apparatus 2: 50 rpm

Times: 0.5, 1, 2, and 3 h

Buffer: 6.8 g/L of monobasic potassium phosphate and 1.0 g/L of 1-hexanesulfonic acid in water. Adjust with phosphoric acid to a pH of 3.3.

Mobile phase: Filtered and degassed mixture of methanol and *Buffer* (20:80)

Standard solution: ($L/900$) mg/mL of USP Carbidopa RS and ($L/900$) mg/mL of USP Levodopa RS in *Medium*, where L is the label claim, in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times for levodopa and carbidopa are 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 2.0 between levodopa and carbidopa

Column efficiency: NLT 4000 theoretical plates for carbidopa; NLT 4000 theoretical plates for levodopa

Tailing factor: NMT 2.0 for carbidopa; NMT 2.0 for levodopa

Relative standard deviation: NMT 1.0% for carbidopa; NMT 1.0% for levodopa

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of carbidopa ($C_{10}H_{14}N_2O_4$) or of levodopa ($C_9H_{11}NO_4$) in the sample withdrawn from the vessel at each time point i :

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*

r_S = peak response of carbidopa or levodopa from the *Standard solution*

C_S = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amounts (Q_i) of carbidopa ($C_{10}H_{14}N_2O_4$) or of levodopa ($C_9H_{11}NO_4$) dissolved at each time point i :

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of carbidopa or levodopa in the portion of sample withdrawn at time point i (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim of carbidopa or levodopa (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (i)	Time (h)	Amount Dissolved
1	0.5	20%–35%
2	1	35%–60%
3	2	65%–95%
4	3	NLT 80%

The percentages of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and of levodopa ($C_9H_{11}NO_4$) dissolved at the times specified conform to *Acceptance Table 2* in $\langle 711 \rangle$.

Test 3: If the product complies with this test, the labeling indicates that it meets *USP Dissolution Test 3*.

Medium, Apparatus 2, Solution A, Solution B, Mobile phase, Standard solution, Chromatographic system, and System suitability: Proceed as directed in *Test 1*.

Times: 0.5, 1, 2.5, and 4 h

Sample solution: Pass a portion of the solution under test through a suitable filter.

Tolerances: See *Table 4*.

Table 4

Time Point (i)	Time (h)	Amount Dissolved for Tablets That Contain 25 mg of Carbidopa and 100 mg of Levodopa	Amount Dissolved for Tablets That Contain 50 mg of Carbidopa and 200 mg of Levodopa
1	0.5	15%–40%	15%–35%
2	1	25%–65%	25%–65%
3	2.5	NLT 60%	NLT 60%
4	4	NLT 80%	NLT 80%

The percentages of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and of levodopa ($C_9H_{11}NO_4$) dissolved at the times specified conform to *Acceptance Table 2* in $\langle 711 \rangle$.

Test 4: If the product complies with this test, the labeling indicates that it meets *USP Dissolution Test 4*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Times: 1, 3, and 6 h

Solution A: 0.24 g/L of sodium 1-decanesulfonate in water

Solution B: 11.6 g/L of monobasic sodium phosphate monohydrate in water

Mobile phase: *Solution A*, *Solution B*, and water (0.13: 95: 4.87), prepared as follows.

Add 0.13% of the final volume of *Solution A* to 95% of the final volume of *Solution B*.

Adjust with phosphoric acid to a pH of 2.8. Dilute with water to final volume.

Standard solution: ($L/900$) mg/mL of USP Carbidopa RS and ($L/900$) mg/mL of USP Levodopa RS in *Medium*, where L is the label claim, in mg/Tablet

Sample solution: Withdraw a 10.0-mL aliquot at each time point, and pass a portion of the solution under test through a suitable filter. Replace the 10.0-mL aliquot withdrawn for analysis with a 10.0-mL aliquot of *Medium*.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; 10- μ m packing L1

Flow rate: 2 mL/min

Injection volume: 50 μ L

Run time: NLT 3 times the retention time of levodopa

System suitability

Sample: *Standard solution*

[Note—The relative retention times for levodopa and carbidopa are 1.0 and 2.5, respectively.]

Suitability requirements

Resolution: NLT 2.0 between levodopa and carbidopa

Tailing factor: NMT 2.0 for carbidopa; NMT 2.0 for levodopa

Relative standard deviation: NMT 2.0% for carbidopa; NMT 2.0% for levodopa

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of carbidopa ($C_{10}H_{14}N_2O_4$) or of levodopa ($C_9H_{11}NO_4$) in the sample withdrawn from the vessel at each time point i :

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U peak response of carbidopa or levodopa from the *Sample solution*

r_S peak response of carbidopa or levodopa from the *Standard solution*

C_S concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amounts (Q_i) of carbidopa ($C_{10}H_{14}N_2O_4$) or of levodopa ($C_9H_{11}NO_4$) dissolved at each time point i :

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of carbidopa or levodopa in the portion of sample withdrawn at time point i (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim of carbidopa or levodopa (mg/Tablet)

V_s = volume of the *Sample solution* withdrawn from the vessel and replaced with *Medium*, 10 mL

Tolerances: See *Table 5*.

Table 5

Time Point (i)	Time (h)	Amount Dissolved for Tablets That Contain 25 mg of Carbidopa and 100 mg of Levodopa	Amount Dissolved for Tablets That Contain 50 mg of Carbidopa and 200 mg of Levodopa
1	1	35%–70%	25%–60%
2	3	NLT 65%	NLT 65%
3	6	NLT 80%	NLT 80%

The percentages of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and of levodopa ($C_9H_{11}NO_4$) dissolved at the times specified conform to *Acceptance Table 2* in $\langle 711 \rangle$.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Times: 0.5, 1, 2.5, and 4 h

Mobile phase: 13.6 g/L of monobasic potassium phosphate adjusted with phosphoric acid to a pH of 3.0

Standard solution: ($L/900$) mg/mL of USP Carbidopa RS and in ($L/900$) mg/mL of USP Levodopa RS in *Medium*, where L is the label claim, in mg/Tablet. [Note—This solution is stable for 1 day if stored at 23°–27°.]

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size, and discard the first 4–5 mL. [Note—This solution is stable for 1 day if stored at 23°–27°.]

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: LC

Detector: UV 282 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

Run time: NLT 3 times the retention time of levodopa

System suitability

Sample: *Standard solution*

[Note—The relative retention times for levodopa and carbidopa are 1.0 and 1.6, respectively.]

Suitability requirements

Resolution: NLT 2.0 between levodopa and carbidopa

Tailing factor: NMT 2.0 for carbidopa; NMT 2.0 for levodopa

Relative standard deviation: NMT 2.0% for carbidopa; NMT 2.0% for levodopa

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of carbidopa ($C_{10}H_{14}N_2O_4$) or of levodopa ($C_9H_{11}NO_4$) in the sample withdrawn from the vessel at each time point i :

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*

r_S = peak response of carbidopa or levodopa from the *Standard solution*

C_S = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) or of levodopa ($C_9H_{11}NO_4$) dissolved at each time point i :

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of carbidopa or levodopa in the portion of sample withdrawn at time point i (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim of carbidopa or levodopa (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 6*.

Table 6

Time Point (i)	Time (h)	Amount Dissolved for Tablets That Contain 25 mg of Carbidopa and 100 mg of Levodopa	Amount Dissolved for Tablets That Contain 50 mg of Carbidopa and 200 mg of Levodopa
1	0.5	25%–45%	20%–40%
2	1	40%–65%	30%–60%
3	2.5	NLT 65%	NLT 55%
4	4	NLT 80%	NLT 75%

The percentages of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and of levodopa ($C_9H_{11}NO_4$) dissolved at the times specified conform to *Acceptance Table 2* in $\langle 711 \rangle$.

- **Uniformity of Dosage Units** $\langle 905 \rangle$: Meet the requirements

IMPURITIES

Change to read:

- **Organic Impurities**

Protect all analytical solutions from light and maintain them at 2°–8° until they are injected into the chromatograph.

Buffer: 6 g/L of anhydrous monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 2.2.

Mobile phase: Alcohol and *Buffer* (5:95)

System suitability solution: 1 µg/mL of USP Levodopa Related Compound B RS and 125 µg/mL of USP Carbidopa RS in *Mobile phase*

Standard solution: 1.25 µg/mL of USP Carbidopa RS and 5 µg/mL of USP Levodopa RS in *Mobile phase*

Sensitivity solution: 0.125 µg/mL of USP Carbidopa RS and 0.5 µg/mL of USP Levodopa RS in *Mobile phase* from the *Standard solution*

Sample

■ solution: ■_{1S} (USP39)

Nominally 0.125 mg/mL of carbidopa and nominally 0.5 mg/mL of levodopa in *Mobile phase* from NLT 10 finely powdered Tablets, prepared as follows. Transfer an accurately weighed portion of the powder into a suitable volumetric flask, dissolve in *Mobile phase*, and pass through a suitable filter.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Run time: 6 times the retention time of carbidopa

Autosampler temperature: 6°

Injection volume: 20 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[Note—For the relative retention times, see *Table 7*.]

Suitability requirements

Resolution: NLT 1.5 between carbidopa and levodopa related compound B, *System suitability solution*

Relative standard deviation: NMT 3.0% for carbidopa and NMT 3.0% for levodopa for five replicate injections, *Standard solution*

Signal-to-noise ratio: NLT 10 for carbidopa in the *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dihydroxybenzaldehyde, dihydroxyphenylacetone, and any unspecified carbidopa degradant based on the label claim of carbidopa in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{\bar{U}}$ peak response of dihydroxybenzaldehyde, dihydroxyphenylacetone, or any unspecified carbidopa degradant from the *Sample solution*

r_S peak response of carbidopa from the *Standard solution*

C_S concentration of USP Carbidopa RS in the *Standard solution* (mg/mL)

C_U nominal concentration of carbidopa in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 7*)

Calculate the percentage of levodopa related compound A and any unspecified levodopa degradant based on the label claim of levodopa in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of levodopa related compound A or any unspecified levodopa degradant from the *Sample solution*

r_S peak response of levodopa from the *Standard solution*

C_S concentration of USP Levodopa RS in the *Standard solution* (mg/mL)

C_U nominal concentration of levodopa in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 7*)

Acceptance criteria: See *Table 7*. ~~Disregard any peak less than 0.05% of the carbidopa peak from the *Sample solution*.~~

- For peaks associated with carbidopa, disregard peaks less than 0.05% of carbidopa from the *Sample solution*. For peaks associated with levodopa, disregard peaks less than 0.05% of levodopa from the *Sample solution*. ■ 1S (USP39)

Table 7

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levodopa related compound A ^{a,b}	0.9	0.70 ■ 0.8 ■ 1S (USP39)	0.1
Levodopa	1.0	—	—
Methyldopa ^{c,d}	1.9	—	—
Levodopa related compound B ^a	2.1	—	—
Carbidopa	2.3	—	—
Dihydroxybenzaldehyde ^{c,e}	5.7	5.9	0.2
Dihydroxyphenylacetone ^{c,f}	6.3	1.0	1
3-O-Methylcarbidopa ^{d,g}	6.9	—	—

^a Individual impurity based on label claim of levodopa.

^b 3-(3,4,6-Trihydroxyphenyl)alanine.

^c Individual impurity based on label claim of carbidopa.

^d This impurity is listed for information only. It is monitored in the drug substance. This impurity is not to be reported and is not to be included in the *Total degradants*.

^e 3,4-Dihydroxybenzaldehyde.

^f 3,4-Dihydroxyphenylacetone.

^g (S)-2-Hydrazinyl-3-(4-hydroxy-3-methoxyphenyl)-2-methylpropanoic acid.

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any unspecified carbidopa degradant	—	1.0	0.2
Any unspecified levodopa degradant	—	1.0	0.1
Total degradants	—	—	4.0

a Individual impurity based on label claim of levodopa.
b 3-(3,4,6-Trihydroxyphenyl)alanine.
c Individual impurity based on label claim of carbidopa.
d This impurity is listed for information only. It is monitored in the drug substance. This impurity is not to be reported and is not to be included in the *Total degradants*.
e 3,4-Dihydroxybenzaldehyde.
f 3,4-Dihydroxyphenylacetone.
g (S)-2-Hydrazinyl-3-(4-hydroxy-3-methoxyphenyl)-2-methylpropanoic acid.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
- **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP Reference Standards** { 11 }
 - USP Carbidopa RS
 - USP Levodopa RS
 - USP Levodopa Related Compound B RS
 - 3-Methoxy-L-tyrosine.
C₁₀H₁₃NO₄ 211.21

BRIEFING

Carbidopa and Levodopa Orally Disintegrating Tablets, *USP 38* page 2596 and the *Revision Bulletin* posted on the USP website with an official date of Aug. 1, 2014. On the basis of comments received, it is proposed to add a requirement in the test for *Organic Impurities* to analyze the *Sample solution* within 2 h of preparation due to solution stability issues.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: H. Joyce.)

Correspondence Number—C140972

Comment deadline: May 31, 2015

Carbidopa and Levodopa Orally Disintegrating Tablets

DEFINITION

Carbidopa and Levodopa Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

• Protect the volumetric solutions from light. • (ERR 1-Apr-2014)

Buffer: 6.6 g/L of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.2

Mobile phase: Alcohol and *Buffer* (5:95)

Standard solution: 0.025 mg/mL of USP Carbidopa RS and 0.25 mg/mL of USP Levodopa RS in *Mobile phase*

Sample stock solution: Transfer NLT 10 Tablets to a 1-L volumetric flask. Add 750 mL of *Mobile phase*, sonicate for 20 min, and then stir for 20 min. Dilute with *Mobile phase* to volume.

Sample solution: Dilute the *Sample stock solution* with *Mobile phase* to obtain a nominal concentration of carbidopa of between 0.025 and 0.07 mg/mL and a nominal concentration of levodopa of 0.25 mg/mL.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5- μ m packing L1

Autosampler temperature: 6°

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times for levodopa and carbidopa are 0.42 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 2.4 for both the levodopa and carbidopa peaks

Relative standard deviation: NMT 2.0% for both carbidopa and levodopa

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*

r_S = peak response of carbidopa or levodopa from the *Standard solution*

C_S concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

C_T nominal concentration of carbidopa or levodopa in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of the labeled amounts of carbidopa and levodopa

PERFORMANCE TESTS

- **Disintegration** 〈 701 〉: NMT 60 s

- **Dissolution** 〈 711 〉

Medium: 0.1 N hydrochloric acid; 750 mL

Apparatus 2: 50 rpm

Time: 10 min

Solution A: 0.24 g/L of sodium 1-decanesulfonate in water

Mobile phase: Dissolve 11.0 g of monobasic sodium phosphate monohydrate in 1 L of water. Add 1.3 mL of *Solution A*, and adjust with phosphoric acid to a pH of 2.8.

Standard solution: ($L/800$) mg/mL each of USP Carbidopa RS and USP Levodopa RS in *Medium*, where L is the label claim in mg/Tablet of carbidopa or levodopa

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size, and discard the first 3 mL.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm \times 15.0-cm; 5- μ m packing L1

Autosampler temperature: 4 $^{\circ}$

Flow rate: 2 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times for levodopa and carbidopa are 0.4 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 2.0 for both levodopa and carbidopa

Relative standard deviation: NMT 2.0% for both levodopa and carbidopa

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_T peak response of carbidopa or levodopa from the *Sample solution*

r_S peak response of carbidopa or levodopa from the *Standard solution*

C_S concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

V = volume of the *Medium*, 750 mL

L = label claim of carbidopa or levodopa (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) is dissolved, and NLT 75% (Q) of the labeled amount of levodopa ($C_9H_{11}NO_4$) is dissolved.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

Change to read:

- **Organic Impurities**

Protect all analytical solutions from light, and maintain them at 2°–8° until they are injected into the chromatograph.

Diluent: Methanol and 0.1 N hydrochloric acid (30:70)

Mobile phase: 13.8 g/L of monobasic sodium phosphate monohydrate in water, adjusted with phosphoric acid to a pH of 2.7

System suitability solution: 0.025 mg/mL each of USP Carbidopa RS, USP Levodopa RS, USP Levodopa Related Compound A RS, USP Levodopa Related Compound B RS, and USP Methyldopa RS in *Diluent*

Standard solution: 0.025 mg/mL of USP Levodopa RS in *Diluent*

Sample solution: Transfer a weighed quantity of powder equivalent to 250 mg of levodopa from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add 80 mL of *Diluent*, sonicate for 10 min, and then stir for 30 min. Dilute with *Diluent* to volume. Centrifuge, and use the supernatant

■ inject the supernatant within 2 h. ■ 1S (USP39)

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5- μ m packing L7

Autosampler temperature: 4°

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

Run time: 6 times the retention time of carbidopa

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—For the relative retention times, see *Table 1*. If peak fronting for levodopa related compound A is observed, lowering the column temperature to 15° is recommended to eliminate this problem.]

Suitability requirements

Resolution: NLT 1.5 between levodopa related compound A and levodopa, NLT 2.0 between carbidopa and levodopa related compound B, and NLT 1.5 between methyldopa and carbidopa; *System suitability solution*

Relative standard deviation: NMT 5.0% for levodopa, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of all impurities and any unspecified degradation product other than methyldopa and 3,4-dihydroxyphenylacetone, based on the label claim of levodopa in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of levodopa related compound A or any unspecified degradation product from the *Sample solution*

r_S peak response of levodopa from the *Standard solution*

C_S concentration of USP Levodopa RS in the *Standard solution* (mg/mL)

C_U nominal concentration of levodopa in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Calculate the percentage of methyldopa and 3,4-dihydroxyphenylacetone based on the label claim of carbidopa in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of methyldopa or 3,4-dihydroxyphenylacetone from the *Sample solution*

r_S peak response of levodopa from the *Standard solution*

C_S concentration of USP Levodopa RS in the *Standard solution*

C_U nominal concentration of carbidopa in the *Sample solution*

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levodopa related compound A ^a	0.45	0.80	0.2
Levodopa	0.52	—	—
Methyldopa ^b	0.84	1.0	0.5
Carbidopa	1.0	—	—
Levodopa related compound B ^c	1.2	—	—
Carbidopa related compound A ^c	3.1	—	—
3,4-Dihydroxyphenylacetone ^{b,d}	3.9	1.0	*1.0*(RB 1-Aug-2014)

Any individual unspecified degradation product^a — 1.0 0.2 Total impurities^e — — 1.0

^a Individual impurity based on the label claim of levodopa.

^b Individual impurity based on the label claim of carbidopa.

^c Process-related impurities, included for identification only; not to be included in *Total impurities*.

^d Not to be included in *Total impurities*.

^e Excluding all process impurities and 3,4-dihydroxyphenylacetone.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
- **USP Reference Standards** { 11 }
 - USP Carbidopa RS
 - USP Levodopa RS
 - USP Levodopa Related Compound A RS
 - 3-(3,4,6-Trihydroxyphenyl)alanine.
 - $C_9H_{11}NO_5$ 213.19
 - USP Levodopa Related Compound B RS
 - 3-Methoxytyrosine.
 - $C_{10}H_{13}NO_4$ 211.21
 - USP Methyldopa RS

BRIEFING

Cefuroxime Axetil, *USP 38* page 2706 and *PF 38(4)* [July–Aug. 2012]. On the basis of comments received, the Expert Committee has canceled the monograph proposal that appeared in *PF 38(4)* and has proposed the following revisions:

1. A test for *Identification* based on HPLC retention time comparison is added to the monograph to provide a second test that is orthogonal to the currently official *Identification* test.
2. The *Definition* and the *Assay* acceptance criteria are revised to include limits based on percent purity. The limits are based on the existing acceptance criteria and are converted based on the molecular weights of cefuroxime and cefuroxime axetil. The calculation formula in the *Assay* is revised to reflect the revised limits.
3. The *Assay* and the test for *Diastereoisomer Ratio* are revised to eliminate the use of the internal standard. The *System suitability solution*, *Standard solution*, *Sample solution*, *Flow rate*, and *Injection volume* are revised to match the *European Pharmacopoeia 8.4* monograph. The column efficiency requirement in the *Assay* is deleted because the remaining criteria are adequate to evaluate system suitability. The relative standard deviation requirement specifies the relevant peaks. The calculation formulas are updated and converted to current style.
4. As part of the USP monograph modernization initiative, the monograph is revised to add a test for *Organic Impurities* based on the procedure in the *European Pharmacopoeia 8.4* monograph.
5. The Hypersil SAS brand of L13 column is suitable for the *Assay*, *Diastereoisomer Ratio*, and *Organic Impurities* procedures. The typical retention time for cefuroxime axetil diastereoisomer A is about 12 min. The Kromasil C1 and Zorbax TMS brands of L13 column are also suitable.
6. The *USP Reference Standards* section is updated to provide chemical information for USP Cefuroxime Axetil Delta-3 Isomers RS and to add a new Reference Standard that is used for peak identification.

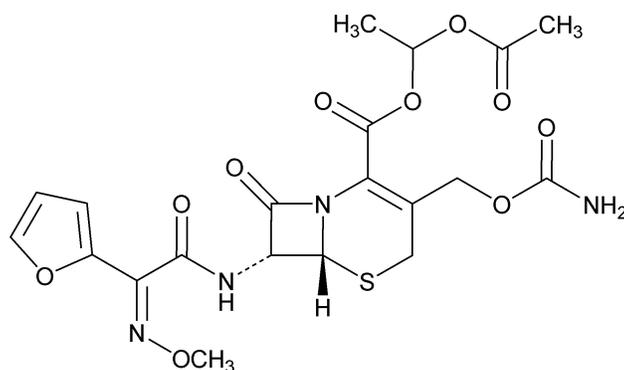
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM1: A. Wise.)

Correspondence Number—C107336; C124416; C124427

Comment deadline: May 31, 2015

Cefuroxime Axetil



$C_{20}H_{22}N_4O_{10}S$ 510.47

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[[(aminocarbonyl)oxy]methyl]-7-[[2-furanyl(methoxyimino)acetyl]amino]-8-oxo-, 1-(acetyloxy)ethyl ester, [6R-[6 α ,7 β (Z)]]-; (RS)-1-Hydroxyethyl (6R,7R)-7-[2-(2-furyl)glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, 7²-(Z)-(O-methyloxime), 1-acetate 3-carbamate [64544-07-6].

DEFINITION

Change to read:

Cefuroxime Axetil is a mixture of the diastereoisomers of cefuroxime axetil ($C_{20}H_{22}N_4O_{10}S$). It contains the equivalent of ~~NLT 745 μ g/mg and NMT 875 μ g/mg~~

■ NLT 90.0% and NMT 105.0% ■ 1S (USP39)

of cefuroxime ($C_{16}H_{16}N_4O_8S$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K): Meets the requirements

Add the following:

- **B.** The retention times of cefuroxime axetil diastereoisomers A and B of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

ASSAY

Change to read:

- **Procedure**

Solution A: ~~23 g/L of monobasic ammonium phosphate~~

Mobile phase: ~~Methanol and Solution A (38:62)~~

Internal standard solution: ~~5.4 mg/mL of acetanilide in methanol~~

System suitability stock solution: ~~0.16 mg/mL of USP Cefuroxime Axetil Delta-3 Isomers RS in methanol~~

Standard stock solution: ~~1.2 mg/mL of USP Cefuroxime Axetil RS in methanol~~

System suitability solution: ~~0.24 mg/mL of cefuroxime axetil from the Standard stock~~

~~solution, 0.54 mg/mL of acetanilide from the *Internal standard solution* and 12.16 µg/mL of cefuroxime delta-3 isomers from *System suitability stock solution* in *Solution A*.~~

Standard solution: ~~0.24 mg/mL of cefuroxime axetil from the *Standard stock solution*, 0.54 mg/mL of acetanilide from the *Internal standard solution* in *Solution A* containing 7.6% of the final volume of methanol. Use this solution promptly or refrigerate and use on the day prepared.~~

Sample stock solution: ~~1.2 mg/mL of Cefuroxime Axetil in methanol~~

Sample solution: ~~0.24 mg/mL of cefuroxime axetil from the *Sample stock solution*, 0.54 mg/mL of acetanilide from the *Internal standard solution* in *Solution A* containing 7.6% of the final volume of methanol. Use this solution promptly or refrigerate and use on the day prepared.~~

Chromatographic system

~~(See *Chromatography* <621>, *System Suitability*.)~~

Mode: ~~LC~~

Detector: ~~UV 278 nm~~

Column: ~~4.6 mm x 25 cm, 5 µm packing L13~~

Flow rate: ~~1.5 mL/min~~

Injection volume: ~~10 µL~~

System suitability

Samples: ~~*System suitability solution* and *Standard solution*~~

~~[Note—The relative retention times are about 0.4 for acetanilide, 0.8 for cefuroxime axetil diastereoisomer B, 0.9 for cefuroxime axetil diastereoisomer A, and 1.0 for cefuroxime axetil delta-3 isomers.]~~

Suitability requirements

Resolution: ~~NLT 1.5 between cefuroxime axetil diastereoisomer A and B; NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*~~

Column efficiency: ~~NLT 3000 theoretical plates for the cefuroxime axetil diastereoisomer A peak, *Standard solution*~~

Relative standard deviation: ~~NMT 2.0%, *Standard solution*~~

Analysis

Samples: ~~*Standard solution* and *Sample solution*~~

Calculate the quantity, in µg/mg, of cefuroxime ($C_{16}H_{16}N_4O_8S$) in the portion of Cefuroxime Axetil taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P$$

R_U = peak response ratio of cefuroxime axetil diastereoisomers A and B to the internal standard from the *Sample solution*

R_S = peak response ratio of cefuroxime axetil diastereoisomers A and B to the internal standard from the *Standard solution*

C_S = concentration of USP Cefuroxime Axetil RS in the *Standard solution* (mg/mL)

C_U = concentration of Cefuroxime Axetil in the *Sample solution*, corrected for water (mg/mL)

P = potency of cefuroxime, on the anhydrous basis, in USP Cefuroxime Axetil RS (µg/mg)

Acceptance criteria: ~~745–875 µg/mg on the anhydrous basis~~

■ **Solution A:** 23 g/L of monobasic ammonium phosphate

Mobile phase: Methanol and *Solution A* (38:62)

System suitability stock solution: 0.1 mg/mL of USP Cefuroxime Axetil Delta-3 Isomers RS in methanol

System suitability solution: 10 µg/mL of cefuroxime axetil delta-3 isomers from *System suitability stock solution* and 0.2 mg/mL of USP Cefuroxime Axetil RS in *Mobile phase*

Standard solution: 0.2 mg/mL of USP Cefuroxime Axetil RS in *Mobile phase*. Protect the solution from light, refrigerate, and use on the day prepared.

Sample solution: 0.2 mg/mL of Cefuroxime Axetil in *Mobile phase*. Protect the solution from light, refrigerate, and use on the day prepared.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; 5-µm packing L13

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between cefuroxime axetil diastereoisomers A and B; NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*

Relative standard deviation: NMT 2.0% for the sum of cefuroxime axetil diastereoisomers A and B, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cefuroxime ($C_{16}H_{16}N_4O_8S$) in the portion of Cefuroxime Axetil taken:

$$\text{Result} = (r_U/r_T) \times (C_S/C_U) \times P \times F \times 100$$

r_U

= sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Sample solution*

r_T

= sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Standard solution*

C_S

= concentration of USP Cefuroxime Axetil RS in the *Standard solution* (mg/mL)

C_U

= concentration of Cefuroxime Axetil in the *Sample solution* (mg/mL)

P

= potency of cefuroxime, on the anhydrous basis, in USP Cefuroxime Axetil RS ($\mu\text{g}/\text{mg}$)

F

= conversion factor, 0.001 mg/ μg

Acceptance criteria: 90.0%–105.0% on the anhydrous basis ■ 1S (USP39)

IMPURITIES

Add the following:

■ • Organic Impurities

Solution A, Mobile phase, System suitability solution, Sample solution, and

Chromatographic system: Proceed as directed in the *Assay*.

Peak identification solution: 30 $\mu\text{g}/\text{mL}$ of USP Cefuroxime Axetil *E*-Isomers RS in methanol

Reference solution: 2 $\mu\text{g}/\text{mL}$ of USP Cefuroxime Axetil RS in *Mobile phase*. Protect the solution from light, refrigerate, and use on the day prepared.

System suitability

Samples: *System suitability solution* and *Peak identification solution*

[Note—See *Table 1* for relative retention times. Use the *Peak identification solution* to identify the locations of the cefuroxime axetil *E*-isomers.]

Suitability requirements

Resolution: NLT 1.5 between cefuroxime axetil diastereoisomers A and B; NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*

Analysis

Samples: *Sample solution* and *Reference solution*

Calculate the percentage of each impurity in the portion of Cefuroxime Axetil taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of each individual impurity from the *Sample solution*

r_T sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Sample solution*

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05 times the sum of the responses of the two major peaks from the *Reference solution*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methoxyiminofuranyl acetic acid ^a	0.24	0.15
^a (Z)-2-(Furan-2-yl)-2-(methoxyimino)acetic acid.		

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cefuroxime ^b	0.30	0.5
Cefuroxime lactone ^c	0.42	0.15
Cefuroxime axetil diastereoisomer B	0.9	—
Cefuroxime axetil diastereoisomer A	1.0	—
Cefuroxime axetil delta-3 isomers ^{d,e}	1.2	1.0
Cefuroxime axetil <i>E</i> -isomers ^{d,f}	1.7	0.8
	2.1	
	2.5	
Cefuroxime axetil dimer ^{g,h}	3.4	0.5
	3.8	
Any other individual impurity	—	0.1
Total impurities	—	2.5
a (Z)-2-(Furan-2-yl)-2-(methoxyimino)acetic acid.		

^b (6*R*,7*R*)-3-[(Carbamoyloxy)methyl]-7-[(Z)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

^c (Z)-*N*-((5*aR*,6*R*)-1,7-Dioxo-1,3,4,5*a*,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl)-2-(furan-2-yl)-2-(methoxyimino)acetamide.

^d The system may resolve two isomers. The limit is for the sum of the two isomers.

^e (1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(Z)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate.

^f (1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*E*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

^g The system may resolve three isomers. The limit is for the sum of the three isomers.

^h (6*R*,6'*R*,7*R*,7'*R*,*Z*)-Oxybis(ethane-1,1-diyl)bis{3-[(carbamoyloxy)methyl]-7-[(Z)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate}.

■ 1S (USP39)

SPECIFIC TESTS

- **Crystallinity** { 695 } : Particles that do not show birefringence or exhibit extinction positions are amorphous, and particles that show birefringence and exhibit extinction positions are crystalline.
- **Water Determination, Method I** { 921 } : NMT 1.5%

Change to read:

- **Diastereoisomer Ratio**

~~Solution A, Mobile phase, Internal standard solution, System suitability stock solution, System suitability solution, Standard solution, Sample stock solution, Sample solution, Chromatographic system and System suitability~~

■ **Solution A, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: ■1S (USP39)**

Proceed as directed in the Assay.

Analysis

Sample: *Sample solution*

Calculate the ratio of cefuroxime axetil diastereoisomer A to the sum of the cefuroxime axetil diastereoisomers A and B:

$$\text{Result} = \frac{r_A}{r_A + r_B}$$

■ $\text{Result} = r_A/r_T$ ■1S (USP39)

r_A = peak response of cefuroxime axetil diastereoisomer A

~~r_B = peak response of cefuroxime axetil diastereoisomer B~~

■ r_T = sum of the peak responses of cefuroxime axetil diastereoisomers A and B ■1S (USP39)

Acceptance criteria: 0.48–0.55

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label it to indicate whether it is amorphous or crystalline.

Change to read:

- **USP Reference Standards** { 11 }

USP Cefuroxime Axetil RS

USP Cefuroxime Axetil Delta-3 Isomers RS

■ (1*R*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate.
C₂₀H₂₂N₄O₁₀S 510.47

USP Cefuroxime Axetil *E*-Isomers RS

(1*R*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*E*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.
C₂₀H₂₂N₄O₁₀S 510.47 ■1S (USP39)

BRIEFING

Cefuroxime Axetil Tablets, *USP 38* page 2708 and *PF 38(4)* [July–Aug. 2012]. On the basis of comments received, the Expert Committee canceled the monograph proposal that appeared in *PF 38(4)* and proposed the following revisions:

1. The Assay is revised to use the *System suitability solution*, *Standard solution*, and *Sample solution* from the Assay procedure in the *British Pharmacopoeia 2014* monograph. This modernizes the procedure because it eliminates the use of the

internal standard. The *Column efficiency* requirement in the *Assay* is deleted because the remaining criteria are adequate to evaluate system suitability. The *Relative standard deviation* requirement is revised to clarify the relevant peaks. The calculation formula is updated and converted to current style.

2. The *Identification* test is updated to reflect the changes to the *Assay*.
3. The *Dissolution* test is updated to include a calculation formula.
4. As part of USP's monograph modernization initiative, this monograph is revised to add a test for *Organic Impurities* based on the *Related Substances* procedure in the *British Pharmacopoeia 2014* monograph.
5. The Hypersil SAS brand of L13 column is suitable for the *Assay* and *Organic Impurities* procedures. The typical retention time for cefuroxime axetil diastereoisomer A is about 10 min.
6. The test for *Water Determination* is deleted because water content is formulation specific.
7. Storage conditions, based on the approved storage conditions from an FDA-approved manufacturer, are added to the *Packaging and Storage* section.
8. The *USP Reference Standards* section is updated to provide chemical information for USP Cefuroxime Axetil Delta-3 Isomers RS and to add a new Reference Standard that is used for peak identification.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM1: A. Wise.)

Correspondence Number—C107701; C109614; C124503; C124417; C124512

Comment deadline: May 31, 2015

Cefuroxime Axetil Tablets

DEFINITION

Cefuroxime Axetil Tablets contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of cefuroxime ($C_{16}H_{16}N_4O_8S$).

IDENTIFICATION

Change to read:

- **A.** ~~The retention times of the major peaks for cefuroxime axetil diastereoisomers A and B in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.~~
- The retention times of the peaks of cefuroxime axetil diastereoisomers A and B of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

ASSAY

Change to read:

- **Procedure**

Solution A: 23 g/L of monobasic ammonium phosphate in water

Mobile phase: ~~Methanol and Solution A (38:62)~~

Internal standard solution: ~~5.4 mg/mL of acetanilide in methanol~~

Standard stock solution: ~~1.2 mg/mL of USP Cefuroxime Axetil RS in methanol~~

System suitability stock solution: ~~0.16 mg/mL of USP Cefuroxime Axetil Delta-3 Isomers RS in methanol~~

System suitability solution: ~~0.24 mg/mL of cefuroxime axetil from Standard stock solution, 0.54 mg/mL of acetanilide from Internal standard solution and 12.16 µg/mL of cefuroxime axetil delta-3 isomers from System suitability stock solution in Solution A~~

Standard solution: ~~0.24 mg/mL of cefuroxime axetil from Standard stock solution and 0.54 mg/mL of acetanilide from Internal standard solution in Solution A. Use this solution promptly, or refrigerate and use on the day prepared.~~

Sample stock solution: ~~Nominally 2 mg/mL of cefuroxime from finely powdered Tablets (NLT 10) prepared as follows. Transfer a suitable portion of the powder with the aid of methanol to a suitable volumetric flask. Add methanol to fill the volumetric flask to half its capacity, and shake by mechanical means for about 10 min. Dilute with methanol to volume. Pass through a suitable filter.~~

Sample solution: ~~Nominally 0.2 mg/mL of cefuroxime axetil from Sample stock solution and 0.54 mg/mL of acetanilide from Internal standard solution in Solution A containing 7.6% of the final volume of methanol. Use this solution promptly, or refrigerate and use on the day prepared.~~

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6 mm × 25 cm; 5 µm packing L13

Flow rate: 1.2 mL/min

Injection size: 10 µL

System suitability

Samples: *System suitability solution and Standard solution*

[Note—The relative retention times are about 0.4 for acetanilide, 0.8 for cefuroxime axetil diastereoisomer B, 0.9 for cefuroxime axetil diastereoisomer A, and 1.0 for cefuroxime axetil delta-3 isomers.]

Suitability requirements

Resolution: NLT 1.5 between cefuroxime axetil diastereoisomer A and B; NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*

Column efficiency: NLT 3000 theoretical plates for the cefuroxime axetil diastereoisomer A peak, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of cefuroxime ($C_{16}H_{16}N_4O_8S$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P \times F \times 100$$

R_U peak response ratio of cefuroxime axetil diastereoisomers A and B to the internal standard from the *Sample solution*

~~R_s = peak response ratio of cefuroxime axetil diastereoisomers A and B to the internal standard from the *Standard solution*~~

~~C_s = concentration of USP Cefuroxime Axetil RS in the *Standard solution* (mg/mL)~~

~~C_p = nominal concentration of cefuroxime axetil in the *Sample solution* (mg/mL)~~

~~P = potency of cefuroxime, on the anhydrous basis, in USP Cefuroxime Axetil RS ($\mu\text{g}/\text{mg}$)~~

~~F = conversion factor, 0.001 mg/ μg~~

■ Solution A:

23 g/L of monobasic ammonium phosphate in water

Mobile phase: Methanol and *Solution A* (38:62)

Buffer: 23 g/L of monobasic ammonium phosphate in water, adjusted with phosphoric acid to a pH of 2.4

System suitability stock solution: 0.1 mg/mL of USP Cefuroxime Axetil Delta-3 Isomers RS in methanol

System suitability solution: 10 $\mu\text{g}/\text{mL}$ of cefuroxime axetil delta-3 isomers from *System suitability stock solution* and 0.3 mg/mL of USP Cefuroxime Axetil RS in *Mobile phase*

Standard solution: 0.3 mg/mL of cefuroxime axetil in *Mobile phase*. Protect the solution from light, refrigerate, and use on the day prepared.

Sample stock solution: Nominally 5 mg/mL of cefuroxime from finely powdered Tablets (NLT 5), prepared as follows. Transfer a suitable portion of the powder to a volumetric flask. Disperse in *Buffer*, using 5% of the final volume. Sonicate if necessary. Add methanol to fill the volumetric flask to about half its capacity and shake by mechanical means for about 10 min. Dilute with methanol to volume, and filter.

Sample solution: Nominally 0.25 mg/mL of cefuroxime from the *Sample stock solution* in *Mobile phase*. Protect the solution from light, refrigerate, and use on the day prepared.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L13

Flow rate: 1.2 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 4* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between cefuroxime axetil diastereoisomers A and B; NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*

Relative standard deviation: NMT 2.0% for the sum of cefuroxime axetil diastereoisomers A and B, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cefuroxime ($\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (C_S/C_U) \times P \times F \times 100$$

 r_U

= sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Sample solution*

 r_T

= sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Standard solution*

 C_S

= concentration of USP Cefuroxime Axetil RS in the *Standard solution* (mg/mL)

 C_U

= nominal concentration of cefuroxime in the *Sample solution* (mg/mL)

 P

= potency of cefuroxime, on the anhydrous basis, in USP Cefuroxime Axetil RS ($\mu\text{g}/\text{mg}$)

 F

= conversion factor, 0.001 mg/ μg

■ 1S (USP39)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

● **Dissolution** 〈 711 〉

Test 1

Medium: 0.07 N hydrochloric acid; 900 mL

Apparatus 2: 55 rpm

Times: 15 and 45 min

Standard solution: 10–20 $\mu\text{g}/\text{mL}$ of cefuroxime from USP Cefuroxime Axetil RS in *Medium*

Sample solution: Pass portions of the solution under test through a suitable filter and dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 278 nm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

- Determine the percentage (Q) of the labeled amount of cefuroxime ($\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times P \times F \times 100$$

A_U

= absorbance of the *Sample solution*

A_S

= absorbance of the *Standard solution*

C_S

= concentration of USP Cefuroxime Axetil RS in the *Standard solution* (mg/mL)

V

= volume of *Medium*, 900 mL

L

= label claim (mg/Tablet)

P

= potency of cefuroxime, on the anhydrous basis, in USP Cefuroxime Axetil RS ($\mu\text{g}/\text{mg}$)

F

= conversion factor, 0.001 mg/ μg

■ 1S (USP39) ■

Acceptance criteria

For Tablets labeled to contain nominally 500 mg of cefuroxime: See *Table 1*.

Table 1

Time (min)	Amount Dissolved (%)
15	NLT 50
45	NLT 70

For all other Tablets: See *Table 2*.

Table 2

Time (min)	Amount Dissolved (%)
15	NLT 60
45	NLT 75

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium, Times, and Analysis: Proceed as directed in *Test 1*.

Apparatus 2: 100 rpm

Acceptance criteria: See *Table 3*.

Table 3

Time (min)	Amount Dissolved (%)
15	NLT 60
45	NLT 75

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

Add the following:

• **Organic Impurities**

Solution A, Mobile phase, Buffer, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the Assay.

Peak identification solution: 30 µg/mL of USP Cefuroxime Axetil E-Isomers RS in methanol
Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; 5-µm packing L13

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Peak identification solution*

[Note—See *Table 4* for the relative retention times. The *Peak identification solution* is used to identify the locations of the cefuroxime axetil E-isomers.]

Suitability requirements

Resolution: NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of each individual impurity from the *Sample solution*

r_T sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Sample solution*

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methoxyiminofuranyl acetic acid ^{a,b}	0.34	—

^a Process impurities are controlled in the drug substance and are not to be reported here. They are not included in total impurities.

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cefuroxime ^c	0.41	0.70
Cefuroxime lactone ^d	0.52	0.15
Cefuroxime axetil diastereoisomer B	0.90	—
Cefuroxime axetil diastereoisomer A	1.0	—
Cefuroxime axetil delta-3 isomers ^{e,f}	1.1	1.0
Cefuroxime axetil <i>E</i> -isomers ^{e,g}	1.6	1.0
	1.9	
Cefuroxime axetil dimer ^{a,h,i}	2.6	—
	3.1	
	3.6	
Any other individual impurity	—	0.2
Total impurities	—	3.0

^a Process impurities are controlled in the drug substance and are not to be reported here. They are not included in total impurities.

^b (Z)-2-(Furan-2-yl)-2-(methoxyimino)acetic acid.

^c (6*R*,7*R*)-3-[(Carbamoyloxy)methyl]-7-[(Z)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

^d (Z)-*N*-((5*aR*,6*R*)-1,7-Dioxo-1,3,4,5*a*,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl)-2-(furan-2-yl)-2-(methoxyimino)acetamide.

^e The system may resolve two isomers. The limit is for the sum of the two isomers.

^f (1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(Z)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate.

^g (1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*E*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

^h The system may resolve three isomers.

ⁱ (6*R*,6'*R*,7*R*,7'*R*,*Z*)-Oxybis(ethane-1,1-diyl) bis{3-[(carbamoyloxy)methyl]-7-[(Z)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate}.

■ 1S (USP39)

SPECIFIC TESTS

Delete the following:

● ~~Water Determination, Method I (921): NMT 6.0%~~ ■ 1S (USP39)

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in well-closed containers.

■ Store at controlled room temperature. ■ 1S (USP39)

- **Labeling:** The labeling indicates whether the Tablets contain amorphous or crystalline Cefuroxime Axetil. If Tablets contain a mixture of amorphous and crystalline Cefuroxime Axetil, label to indicate the percentage of each contained therein. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

- **USP Reference Standards** { 11 }

USP Cefuroxime Axetil RS

USP Cefuroxime Axetil Delta-3 Isomers RS

■ (1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate.
C₂₀H₂₂N₄O₁₀S 510.47

USP Cefuroxime Axetil *E*-Isomers RS

(1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*E*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.
C₂₀H₂₂N₄O₁₀S 510.47

■ 1S (USP39)

BRIEFING

Cetylpyridinium Chloride, *USP 38* page 2737. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Replace the titration procedure in the *Assay* with a stability-indicating liquid chromatographic method using the Primesep D brand of L78 column. The retention time for cetylpyridinium in the analysis is about 3.5 min.
2. Revise the acceptance criteria in the *Definition* and *Acceptance criteria* in the *Assay* from NLT 99.0% and NMT 102.0% to NLT 98.0% and NMT 102.0%, which is typical for chromatographic procedures.
3. Replace *Identification* test *B*, which uses UV absorption, with a retention time agreement based upon the proposed method in the *Assay*.
4. Remove the organoleptic procedure for pyridine in the *Organic Impurities* test because it is now addressed as a residual solvent (see *General Notices and Requirements*).
5. Add a liquid chromatographic method to the *Organic Impurities* test based on the method used in the proposed new *Assay* procedure.
6. Delete the *Melting Range or Temperature* test. The proposed selective test for *Organic Impurities* is sufficient to monitor the purity of the drug substance.

Additionally, minor editorial changes have been made to update the monograph to current

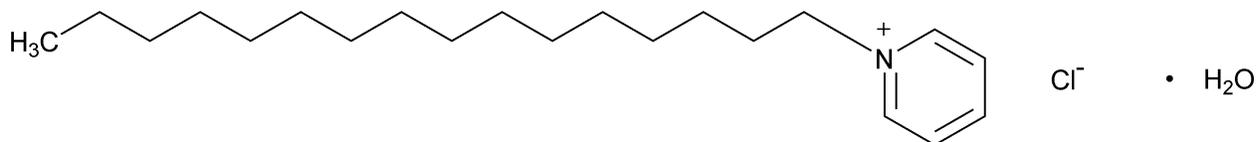
USP style.

(SM1: D.A. Porter.)

Correspondence Number—C123398

Comment deadline: May 31, 2015

Cetylpyridinium Chloride



$C_{21}H_{38}ClN \cdot H_2O$ 358.00

$C_{21}H_{38}ClN$ 339.99

Pyridinium, 1-hexadecyl-, chloride, monohydrate;
1-Hexadecylpyridinium chloride monohydrate [6004-24-6].
Anhydrous [123-03-5].

DEFINITION

Change to read:

Cetylpyridinium Chloride contains ~~NLT 99.0%~~

■ NLT 98.0% ■ 1S (USP39)

and NMT 102.0% of cetylpyridinium chloride ($C_{21}H_{38}ClN$), calculated on the anhydrous basis.

IDENTIFICATION

● A. Infrared Absorption < 197K >

Delete the following:

● B. Ultraviolet Absorption < 197U >

Sample solution: 40 µg/mL in water

Acceptance criteria: Meets the requirements ■ 1S (USP39)

Add the following:

- B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

● C. Identification Tests—General, Chloride < 191 >

Sample solution: 2 mg/mL in water

Acceptance criteria: A 10-mL portion of the *Sample solution* meets the requirements, except that when silver nitrate TS is added, turbidity is produced rather than a curdy white precipitate.

ASSAY

Change to read:

- **Procedure**

Sample solution: Dissolve 200 mg of Cetylpyridinium Chloride in 75 mL of water. Add 10 mL of chloroform, 0.4 mL of bromophenol blue solution (1 in 2000), and 5 mL of a freshly prepared solution of sodium bicarbonate (4.2 in 1000).

Analysis: Titrate the *Sample solution* with 0.02 M sodium tetraphenylboron VS until the blue color disappears from the chloroform layer. Add the last portions of the sodium tetraphenylboron solution dropwise, agitating vigorously after each addition. Each mL of 0.02 M sodium tetraphenylboron is equivalent to 6.800 mg of cetylpyridinium chloride ($C_{21}H_{38}ClN$).

Acceptance criteria: 99.0%–102.0% on the anhydrous basis

- Use 0.1% trifluoroacetic acid-rinsed glassware and silanized vials for all solutions containing cetylpyridinium chloride, as cetylpyridinium may react with the surface.

Solution A: Trifluoroacetic acid and water (1:999)

Solution B: Acetonitrile and trifluoroacetic acid (999:1)

Mobile phase: *Solution A* and *Solution B* (62.5: 37.5)

Standard solution: 0.25 mg/mL of USP Cetylpyridinium Chloride RS in *Solution A*

Sample solution: 0.25 mg/mL of Cetylpyridinium Chloride in *Solution A*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 258 nm

Column: 2.1-mm × 10-cm; 5- μ m packing L78

Column temperature: 40°

Flow rate: 0.6 mL/min

Injection volume: 2 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cetylpyridinium chloride ($C_{21}H_{38}ClN$) in the portion of Cetylpyridinium Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Cetylpyridinium Chloride RS in the *Standard solution* (mg/mL)

C_U

= concentration of Cetylpyridinium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis ■ 1S (USP39)

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.2% on the anhydrous basis

Delete the following:

•

• **Heavy Metals, Method II** 〈 231 〉

: NMT 20 ppm • (Official 1-Dec-2015)

Change to read:

- **Organic Impurities, Pyridine**

Sample solution: Dissolve 1 g in 10 mL of sodium hydroxide solution (1 in 10) without heating.

Acceptance criteria: The odor of pyridine is not immediately perceptible.

- Use 0.1% trifluoroacetic acid-rinsed glassware and silanized vials for all solutions containing cetylpyridinium chloride, as cetylpyridinium may react with the surface.

Solution A, Solution B, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 2.5 µg/mL of USP Cetylpyridinium Chloride RS in *Solution A*

Sample solution: 2.5 mg/mL of Cetylpyridinium Chloride in *Solution A*

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each unspecified impurity in the portion of Cetylpyridinium Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of each unspecified impurity from the *Sample solution*

r_S

= peak response of cetylpyridinium from the *Standard solution*

C_S

= concentration of USP Cetylpyridinium Chloride RS in the *Standard solution* (mg/mL)

C_U

= concentration of Cetylpyridinium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.04%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cetylpyridinium chloride	1.0	—
Any individual unspecified impurity	—	0.1
Total impurities	—	1.0

■ 1S (USP39)

SPECIFIC TESTS

Delete the following:

- ~~Melting Range or Temperature, Class I (741): 80°–84°, the preliminary drying treatment being omitted~~ ■ 1S (USP39)

- **Acidity**

Analysis: Dissolve 500 mg of sample in 50 mL of water, add phenolphthalein TS, and titrate with 0.020 N sodium hydroxide.

Acceptance criteria: NMT 2.5 mL is required for neutralization.

- **Water Determination, Method I (921):** 4.5%–5.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

- **USP Reference Standards (11)**

USP Cetylpyridinium Chloride RS

BRIEFING

Clemastine Fumarate Tablets, USP 38 page 2858. As part of the USP monograph modernization initiative, the following changes are proposed:

1. Add a new stability-indicating HPLC procedure in the test for *Organic Impurities*. The HPLC procedure is based on analyses performed with the Zorbax Eclipse XDB C8 brand of L7 column. The typical retention time for clemastine is about 0.8 min.
2. Replace the *Identification* by TLC procedure with the retention time agreement in the *Assay*.
3. An orthogonal *Identification* test *B* is added based on UV spectrum as proposed in the test for *Organic Impurities*.
4. Remove the UV procedure in *Uniformity of Dosage Units* to allow the flexibility for the test.
5. The *Packaging and Storage* section is revised to be consistent with the information

from the drug product package insert.

6. Add USP 4-Chlorobenzophenone RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: D. Min, D. Vicchio.)

Correspondence Number—C104088

Comment deadline: May 31, 2015

Clemastine Fumarate Tablets

DEFINITION

Clemastine Fumarate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clemastine fumarate ($C_{21}H_{26}ClNO \cdot C_4H_4O_4$).

IDENTIFICATION

Delete the following:

■ • **Thin Layer Chromatography**

Diluent: Chloroform and methanol (50:50)

Standard solution: 2.5 mg/mL of USP Clemastine Fumarate RS in *Diluent*

Sample solution: Place a portion of powdered Tablets, equivalent to 2.5 mg of clemastine fumarate, in a glass-stoppered flask. Add 10 mL of *Diluent*, and shake for 20 min. Filter, wash the residue with two 5 mL portions of *Diluent*, and evaporate the combined filtrate and washings to dryness under vacuum. Dissolve the residue in 1 mL of *Diluent*.

Chromatographic System

Chromatography ~~(621)~~, *Thin-Layer Chromatography*

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

Solution A: 17 mg/mL of bismuth subnitrate in glacial acetic acid and water (1:4)

Solution B: 0.4 g/mL of potassium iodide in water

Spray reagent: Mix 5.0 mL of *Solution A*, 5.0 mL of *Solution B*, and 20 mL of glacial acetic acid in a 100 mL volumetric flask, and dilute with water to volume.

Developing solvent system: Chloroform, methanol, and ammonium hydroxide (90:10:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter. Dry the plate at room temperature with the aid of a current of air. Locate the spots on the plate by spraying first with *Spray reagent*, then with 3% hydrogen peroxide.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. ■ 1S (USP39)

Add the following:

- • **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the

Standard solution, as obtained in the Assay. ■1S (USP39)

Add the following:

- **B.** The UV spectrum of the clemastine peak of the *Diluted sample solution* corresponds to that of the *Standard solution*, as obtained in the proposed test for *Organic Impurities*.
■1S (USP39)

ASSAY

Change to read:

● **Procedure**

Solution A: 9.47 g/L of anhydrous dibasic sodium phosphate in water

Solution B: 9.08 g/L of monobasic potassium phosphate in water

Solution C: *Solution A* and *Solution B* (612:388)

Buffer: *Solution C* and water (25:75)

Mobile phase: Methanol and *Buffer* (83:17)

Diluent: Methanol and water (50:50)

Standard solution: 0.14 mg/mL of USP Clemastine Fumarate RS in *Diluent*

Sample solution: Transfer a quantity of NLT 20 finely powdered Tablets, equivalent to 14 mg of clemastine fumarate, to a 200-mL conical flask. Pipet 100 mL of *Diluent* into the flask, shake for 30 min, centrifuge, and filter the supernatant.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm;

■10-μm■1S (USP39)

packing L7

Flow rate: 4 mL/min

Injection volume: 100 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clemastine fumarate ($C_{21}H_{26}ClNO \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Clemastine Fumarate RS in the *Standard solution* (mg/mL)

C_U nominal concentration of clemastine fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution 〈 711 〉

Buffer: Dissolve 20.0 g of citric acid monohydrate in 1000 mL of water, add 22.0 mL of sodium hydroxide solution (3 in 10) and 8.8 mL of hydrochloric acid, and dilute with water to 2000 mL. Adjust, if necessary, with sodium hydroxide solution (1 in 2) to a pH of 4.0.

Medium: *Buffer*; 500 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Clemastine Fumarate RS in *Medium* with a similar concentration to the *Sample solution*

Sample solution: Centrifuge 60 mL of the solution under test for 20 min at 4000 rpm.

Instrumental conditions

Mode: UV

Analytical wavelength: About 420 nm

Blank: *Medium*

Analysis

Samples: *Standard solution, Sample solution, and Blank*

Transfer 50.0 mL of the *Samples* to individual 125-mL separatory funnels, and treat each of the solutions as follows. Add 10 mL of methyl orange solution (2 in 10,000), mix, add 20.0 mL of chloroform, shake simultaneously by mechanical means for 10 min, remove the chloroform layer, and centrifuge the chloroform layer for 10 min at 4000 rpm. Use the *Blank* to set the instrument.

Calculate the percentage of the labeled amount of clemastine fumarate ($C_{21}H_{26}ClNO \cdot C_4H_4O_4$) dissolved.

Tolerances: NLT 75% (Q) of the labeled amount of clemastine fumarate ($C_{21}H_{26}ClNO \cdot C_4H_4O_4$) is dissolved.

Change to read:

• Uniformity of Dosage Units 〈 905 〉 : • ~~Procedure for content uniformity~~

~~**Solution A:** 0.1 mg/mL of bromocresol purple in 0.33 N acetic acid~~

~~**Solution B:** Methanol and 0.33 N acetic acid (1:9)~~

~~**Standard stock solution:** Transfer 27 mg of USP Clemastine Fumarate RS to a 100-mL volumetric flask. Add 10 mL of methanol, and dilute with 0.33 N acetic acid to volume.~~

~~**Standard solution:** 27 µg/mL of USP Clemastine Fumarate RS in *Solution B*, from *Standard stock solution*.~~

~~**Sample solution:** Mix 1 finely powdered Tablet with a volume of *Solution B* sufficient to obtain 27 µg/mL of clemastine fumarate solution. Shake for 30 min, filter, and discard the first few mL of the filtrate.~~

~~Instrumental conditions~~

~~**Mode:** UV~~

~~**Analytical wavelength:** about 406 nm~~

~~**Blank:** *Solution B*~~

~~Analysis:~~

~~**Samples:** *Standard solution, Sample solution, and Blank*~~

~~Transfer 15.0 mL of the *Samples* to individual 125-mL separatory funnels. Add 25 mL of *Solution A* and 50.0 mL of chloroform to each, and shake by mechanical means for 15 min. Allow the layers to separate, and filter the chloroform layers. Concomitantly determine the absorbances of the filtered solutions of the *Sample solution* and the *Standard solution*, using the *Blank* to set the instrument.~~

~~Calculate the percentage of the labeled amount of clemastine fumarate ($C_{21}H_{26}ClNO \cdot C_4H_4O_4$) in the Tablet taken:~~

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

~~A_U absorbance of the *Sample solution*~~

~~A_S absorbance of the *Standard solution*~~

~~C_S concentration of USP Clemastine Fumarate RS in the *Standard solution* ($\mu\text{g/mL}$)~~

~~C_U nominal concentration of clemastine fumarate in the *Sample solution* ($\mu\text{g/mL}$)~~

~~**Acceptance criteria:** Meet the requirements~~

■ Meet the requirements ■ 1S (USP39)

IMPURITIES

Add the following:

■ • Organic Impurities

Buffer: 4.1 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.0.

Solution A: Methanol, acetonitrile, and *Buffer* (35:35:30)

Solution B: Methanol, acetonitrile, and *Buffer* (40: 37.5: 22.5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
3.1	0	100
18	0	100
18.1	100	0
25	100	0

Standard stock solution 1: 0.14 mg/mL of USP Clemastine Fumarate RS in *Solution A*. Sonicate for NLT 5 min or until dissolved.

Standard stock solution 2: 0.14 mg/mL of USP 4-Chlorobenzophenone RS in methanol. Sonicate for NLT 5 min or until dissolved.

System suitability solution: 2.8 $\mu\text{g/mL}$ each of USP Clemastine Fumarate RS and USP 4-Chlorobenzophenone RS in *Solution A* from *Standard stock solution 1* and *Standard stock solution 2*

Sensitivity solution: 0.14 $\mu\text{g/mL}$ of USP Clemastine Fumarate RS in *Solution A* from *Standard stock solution 1*

Standard solution: 2.8 µg/mL of USP Clemastine Fumarate RS in *Solution A* from *Standard stock solution 1*

Sample solution: Nominally 0.28 mg/mL of clemastine fumarate from Tablets in *Solution A* prepared as follows. Transfer a quantity of NLT 20 finely powdered Tablets, equivalent to 14 mg of clemastine fumarate, to a 50-mL volumetric flask. Add 25 mL of *Solution A*, shake the flask for NLT 30 min, and sonicate for NLT 15 min. Dilute with *Solution A* to volume. Pass an aliquot through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate.

Diluted sample solution: Nominally 2.8 µg/mL of clemastine fumarate in *Solution A* from *Sample solution*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 220 nm. For *Identification test B*, use a diode array detector in the range of 200–300 nm.

Column: 4.6-mm × 5-cm; 1.8-µm packing L7

Flow rate: 1.2 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

[Note—The relative retention times for clemastine and 4-chlorobenzophenone are 1.0 and 1.7, respectively.]

Suitability requirements

Resolution: NLT 1.5 between clemastine and 4-chlorobenzophenone, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution*, *Diluted sample solution*, and *Sample solution*

[Note—The *Diluted sample solution* is used for *Identification test B*.]

Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each unspecified degradation product from the *Sample solution*

r_S peak response of clemastine from the *Standard solution*

C_S concentration of USP Clemastine Fumarate RS in the *Standard solution* (mg/mL)

C_U nominal concentration of clemastine fumarate in the *Sample solution* (mg/mL)

Acceptance criteria

Any unspecified degradation product: NMT 0.5%

Total impurities: NMT 2.0%

■ 1S (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in well-closed containers.
- Store at controlled room temperature. ■ 1S (USP39)

Change to read:

- **USP Reference Standards** { 11 }
 - USP 4-Chlorobenzophenone RS
 - 4-Chlorobenzophenone.
 - C₁₃H₉ClO 216.66 ■ 1S (USP39)

USP Clemastine Fumarate RS

BRIEFING

Clomipramine Hydrochloride Compounded Oral Suspension, Veterinary. Because there is currently no existing *USP* monograph for this dosage form, a new compounded preparation monograph is proposed based on a validated stability-indicating method used to assess stability. The liquid chromatographic procedure in the *Assay* is based on analyses validated using the Luna C18(2) brand of L1 column. The typical retention time for clomipramine hydrochloride is about 8.4 min.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C154508

Comment deadline: May 31, 2015

Add the following:

■ **Clomipramine Hydrochloride Compounded Oral Suspension, Veterinary**

DEFINITION

Clomipramine Hydrochloride Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of clomipramine hydrochloride (C₁₉H₂₃ClN₂·HCl). Prepare Clomipramine Hydrochloride Compounded Oral Suspension, Veterinary 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* { 795 }).

Clomipramine Hydrochloride tablets ^a equivalent to	100 mg of clomipramine hydrochloride
Vehicle: a 1:1 mixture of Ora-Plus ^b and Ora-Sweet SF ^b , a sufficient quantity to make	100 mL
^a Clomicalm 20-mg tablets, Novartis Animal Health, Greensboro, NC.	

^b Perrigo, Allegan, MI.

Place the *Clomipramine Hydrochloride* tablets in a suitable container and comminute to a fine powder. Wet the powder with a small amount of *Vehicle* and triturate to make a smooth paste.

Add the *Vehicle* to make the mortar contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the remainder of the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY

• Procedure

Solution A: Methanol and acetonitrile (50:50)

Solution B: 25 mM monobasic potassium phosphate adjusted with phosphoric acid to a pH of 3.2

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	40	60
4	50	50
10	50	50
10.5	40	60
20	40	60

Standard solution: 0.1 mg/mL of clomipramine hydrochloride prepared from USP Clomipramine Hydrochloride RS and methanol. Vortex for about 30 s until dissolved.

Sample solution: Transfer 1.0 mL of Oral Suspension, Veterinary to a 10-mL volumetric flask, and rinse the pipette with about 2 mL of methanol. Vortex for 30 s and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV-Vis 254 nm

Column: 2.0-mm × 10-cm; 2.5-μm packing L1

Column temperature: 50°

Flow rate: 0.275 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

[Note—The retention time for clomipramine hydrochloride is about 8.4 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clomipramine hydrochloride (C₁₉H₂₃ClN₂·HCl) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clomipramine hydrochloride from the *Sample solution*

r_S = peak response of clomipramine hydrochloride from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Clomipramine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_{\bar{F}}$ nominal concentration of clomipramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** 〈 791 〉: 3.8–4.8

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant containers. Store in a refrigerator (2°–8°) or at controlled room temperature.
- **Beyond-Use Date:** NMT 90 days after the day on which it was compounded when stored in a refrigerator (2°–8°) or at controlled room temperature
- **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*. Label it to state that it is for veterinary use.
- **USP Reference Standards** 〈 11 〉
USP Clomipramine Hydrochloride RS

■ 1S (USP39)

BRIEFING

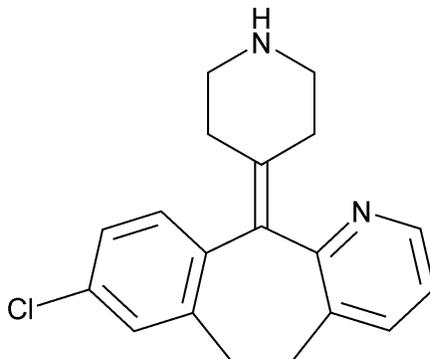
Desloratadine, PF 39(2) [Mar.–Apr. 2013]. The previous proposal for this new monograph, which appeared as an *In-Process Revision* in PF 39(2) [Mar.–Apr. 2013], is canceled and now being repropose with changes that reflect comments received.

1. To harmonize this monograph with the one in the *European Pharmacopoeia 8.0*, a validated liquid chromatographic procedure is proposed for the *Assay* and *Organic Impurities, Procedure 1*. The method is based on analyses performed using the YMC J'sphere ODS-M80 brand of L1 column. The typical retention time for desloratadine is about 21 min.
2. Because of different impurity profiles, a flexible monograph approach is proposed for impurities. Thus, the *Organic Impurities* procedure published in PF 39(2) is renamed *Organic Impurities, Procedure 2*. The method is based on analyses performed using the Hypersil BDS brand of L7 column. The typical retention time for desloratadine is about 16 min.
3. A labeling section was added to distinguish between the two impurity tests.
4. Add USP Desloratadine Related Compound B RS used in the *Assay* and test for *Organic Impurities, Procedure 1* to the *USP Reference Standards* section.
5. The *Loss on Drying* test is being replaced with the more specific *Water Determination, Method Ic* test with a limit of NMT 0.5%.
6. The *Residue on Ignition* limit is widened to NMT 0.2% to match the wider approved limits.
7. The reference to *Heavy Metals* 〈 231 〉 was removed.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: D. Vicchio.)

Correspondence Number—C95399; C141572; C132946; C132948

*Comment deadline: May 31, 2015***Add the following:****■ Desloratadine**C₁₉H₁₉ClN₂ 310.82

Benzo[5,6]cyclohepta[1,2-*b*]pyridine, 8-chloro-6,11-dihydro-11-(4-piperidinylidene)-, 5*H*-; 8-Chloro-6,11-dihydro-11-(piperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine [100643-71-8].

DEFINITION

Desloratadine contains NLT 98.0% and NMT 102.0% of desloratadine (C₁₉H₁₉ClN₂), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **Procedure**

Buffer: Dissolve 0.865 g of sodium dodecyl sulfate in water, add 0.5 mL of trifluoroacetic acid, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (43:57)

System suitability solution: 0.08 mg/mL of USP Desloratadine RS and 0.2 µg/mL of USP Desloratadine Related Compound B RS in *Mobile phase*

Standard solution: 0.08 mg/mL of USP Desloratadine RS in *Mobile phase*

Sample solution: 0.08 mg/mL of Desloratadine in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 4-µm packing L1

Column temperature 35°

Flow rate: 1 mL/min

Injection volume: 100 µL

Run time: NLT 2 times the retention time of desloratadine

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between desloratadine related compound B and desloratadine, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of desloratadine (C₁₉H₁₉ClN₂) in the portion of Desloratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)

C_U concentration of Desloratadine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.2%

- **Organic Impurities, Procedure 1**

Use *Organic Impurities, Procedure 1*, when the impurity profile includes desloratadine related compound B and fluorodesloratadine.

Buffer, Mobile phase, System suitability solution, Sample solution, and

Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.08 µg/mL of USP Desloratadine RS in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between desloratadine related compound B and desloratadine, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Desloratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{i\bar{}}$ peak response of each impurity from the *Sample solution*

$r_{s\bar{}}$ peak response of desloratadine from the *Standard solution*

$C_{s\bar{}}$ concentration of USP Desloratadine RS in the *Standard solution* ($\mu\text{g/mL}$)

$C_{i\bar{}}$ concentration of Desloratadine in the *Sample solution* ($\mu\text{g/mL}$)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fluorodesloratadine ^a	0.8	0.6	0.2
Desloratadine related compound B	0.9	0.6	0.3
Desloratadine	1.0	—	—
Any other individual unspecified impurity	—	1.00	0.10
Total impurities	—	—	0.4

^a 8-Chloro-11-fluoro-11-(piperidin-4-yl)-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

• **Organic Impurities, Procedure 2**

Use *Organic Impurities, Procedure 2*, when the impurity profile includes dechloro desloratadine, desloratadine related compound A, and dehydrodesloratadine.

Buffer: 1.36 g/L of monobasic potassium phosphate in water. Add 10 mL of triethylamine per L of the solution, and adjust with dilute phosphoric acid (1 in 10) to a pH of 2.0.

Solution A: Acetonitrile, methanol, and *Buffer* (10:10:80)

Solution B: Acetonitrile, tetrahydrofuran, and *Buffer* (70:5:30)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
15	90	10
20	70	30
25	60	40
30	50	50
38	50	50
40	100	0
45	100	0

System suitability solution: 0.15 $\mu\text{g/mL}$ of USP Desloratadine Related Compound A RS and 100 $\mu\text{g/mL}$ of USP Desloratadine RS in *Solution A*

Standard solution: 0.5 $\mu\text{g/mL}$ of USP Desloratadine RS in *Solution A*

Sample solution: 500 $\mu\text{g/mL}$ of Desloratadine in *Solution A*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Flow rate: 1 mL/min

Injection volume: 60 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between desloratadine and desloratadine related compound A, *System suitability solution*

Tailing factor: NMT 3.0 for desloratadine, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Desloratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of desloratadine from the *Standard solution*

C_S = concentration of USP Desloratadine RS in the *Standard solution* (μg/mL)

C_U = concentration of Desloratadine in the *Sample solution* (μg/mL)

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dechloro desloratadine ^a	0.38	0.90	0.15
Desloratadine	1.0	—	—
Desloratadine related compound A	1.30	0.86	0.15
Dehydro desloratadine ^b	1.59	1.00	0.15
Loratadine ^c	2.25	0.79	0.20
Any other individual unspecified impurity	—	1.00	0.10
Total impurities	—	—	0.40

^a 6,11-Dihydro-11-(piperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

^b 8-Chloro-11-(piperidin-4-ylidene)benzo[5,6]cyclohepta[1,2-*b*]pyridine.

^c 8-Chloro-6,11-dihydro-11-(1-ethoxycarbonylpiperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

SPECIFIC TESTS

- **Water Determination, Method Ic** 〈 921 〉: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.
- **Labeling:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.
- **USP Reference Standards** 〈 11 〉

USP Desloratadine RS

USP Desloratadine Related Compound A RS

8-Bromo-6,11-dihydro-11-(piperidin-4-ylidene)-5H-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

C₁₉H₁₉BrN₂ 355.27

USP Desloratadine Related Compound B RS

8-Chloro-11-(1,2,3,6-tetrahydropyridin-4-yl)-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

C₁₉H₁₉ClN₂ 310.82

■ 1S (USP39)

BRIEFING

Desloratadine Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is proposed. The liquid chromatographic procedure in the *Assay* is based on analyses performed with the Altima CN brand of L10 column. The retention time for desloratadine is about 4.7 min. The liquid chromatographic procedure in the test for *Organic Impurities* was validated with the YMC basic C8 brand of L7 column. The retention time for desloratadine is about 12.5 min.

(SM4: S. Ramakrishna, D. Vicchio.)

Correspondence Number—C129432

Comment deadline: May 31, 2015

Add the following:

■ **Desloratadine Tablets**

DEFINITION

Desloratadine Tablets contain NLT 93.0% and NMT 105.0% of the labeled amount of desloratadine (C₁₉H₁₉ClN₂).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the desloratadine peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.

ASSAY● **Procedure**

Use amber, low-actinic glassware.

Buffer: Dissolve 4.35 g of dibasic potassium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Methanol and *Buffer* (20:80)

Diluent: Methanol and water (90:10)

Standard solution: 0.02 mg/mL of USP Desloratadine RS in *Diluent*

Sample stock solution: Nominally 0.2 mg/mL of desloratadine, prepared as follows.

Transfer NLT 20 Tablets into a suitable volumetric flask, add water to fill 10% of the flask volume, and allow the Tablets to disperse. Add methanol, about 50% of the flask volume, and stir for NLT 60 min. Allow the solution to cool to room temperature and dilute with methanol to volume. Centrifuge a portion of this solution and use the supernatant.

Sample solution: Nominally 0.02 mg/mL of desloratadine in *Diluent*, from *Sample stock solution*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detectors

Assay: UV 241 nm

Identification B: Diode array; UV 230–400 nm

Column: 4.6-mm × 15-cm; 5-μm packing L10

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: NLT 4.2 times the retention time of desloratadine

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of desloratadine (C₁₉H₁₉ClN₂) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)

C_U nominal concentration of desloratadine in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–105.0%

PERFORMANCE TESTS

- **Dissolution** 〈 711 〉

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: 0.01 mg/mL of USP Desloratadine RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size. Discard the first 5 mL of the filtrate.

Instrumental conditions

Mode: UV

Analytical wavelength: 282 nm

Cell: 1.0 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U absorbance of the *Sample solution*

A_S absorbance of the *Standard solution*

C_S concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$) is dissolved.

- **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

IMPURITIES

- **Organic Impurities**

Solution A: Dissolve 4.35 g of dibasic potassium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 3.2.

Solution B: Acetonitrile

Solution C: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	70	15	15
12	70	15	15
30	40	30	30
45	40	30	30
47	70	15	15
55	70	15	15

Diluent: Methanol and water (90:10)

Standard solution: 0.002 mg/mL each of USP Desloratadine RS and USP Desloratadine Related Compound F RS in *Diluent*

Sensitivity solution: 0.1 µg/mL of USP Desloratadine RS in *Diluent*

Sample solution: Nominally 0.2 mg/mL of desloratadine, prepared as follows. Transfer NLT 20 Tablets into a suitable volumetric flask, add 10% of the flask volume of water, and allow the Tablets to disperse. Add methanol, about 50% of the flask volume, and stir for at least 60 min. Allow the solution to cool to room temperature, and dilute with methanol to volume. Centrifuge a portion of this solution and use the supernatant.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 241 nm

Column: 4.6-mm × 15-cm; 3-µm packing L7

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Column efficiency: NLT 1500 theoretical plates for desloratadine, *Standard solution*

Relative standard deviation: NMT 5.0% for desloratadine, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution*, *Sensitivity solution*, and *Sample solution*

Calculate the percentage of desloratadine related compound F in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_{\bar{F}}$ peak response of desloratadine related compound F from the *Sample solution*

$r_{\bar{S}}$ peak response of desloratadine related compound F from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Desloratadine Related Compound F RS in the *Standard solution* (mg/mL)

$C_{\bar{U}}$ nominal concentration of desloratadine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_{\bar{F}}$ peak response of each unspecified impurity from the *Sample solution*

$r_{\bar{S}}$ peak response of desloratadine from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)

$C_{\bar{U}}$ nominal concentration of desloratadine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dechloro desloratadine ^a	0.37	— ^b
Desloratadine	1.00	—
Dehydro desloratadine ^c	1.4	— ^b
Desloratadine related compound F	1.8	0.2
Loratadine ^d	2.7	— ^b
Any unspecified degradation product	—	0.2
Total impurities	—	0.4

^a 6,11-Dihydro-11-(piperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

^b Process impurity controlled in the drug substance monograph. Provided for information only; the content is not calculated and not reported.

^c 8-Chloro-11-(piperidin-4-ylidene)benzo[5,6]cyclohepta[1,2-*b*]pyridine.

^d 8-Chloro-6,11-dihydro-11-(1-ethoxycarbonylpiperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine (loratadine).

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers and store at controlled room temperature.

- **USP Reference Standards** { 11 }

USP Desloratadine RS

USP Desloratadine Related Compound F RS

8-Chloro-6,11-dihydro-11-(*N*-formyl-4-piperidinyldiene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

C₂₀H₁₉ClN₂O 338.83

■ 1S (USP39)

BRIEFING

Desloratadine Orally Disintegrating Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is proposed.

1. The liquid chromatographic procedure in the *Assay* is based on analyses performed with the ACE C18 brand of L1 column manufactured by Advanced Chromatography Technology. The retention time for desloratadine is about 6 min.
2. The liquid chromatographic procedure in the test for *Dissolution* is based on analyses performed with the ACE C18 brand of L1 column manufactured by Advanced Chromatography Technology. The retention time for desloratadine is about 7 min.
3. The liquid chromatographic procedure in the test for *Organic Impurities* was validated with the Hypersil BDS C8 brand of L7 column manufactured by Thermo

Scientific. The retention time for desloratadine is about 23 min.

(SM4: D. Vicchio, R. Ravichandran.)

Correspondence Number—C106488

Comment deadline: May 31, 2015

Add the following:

■ **Desloratadine Orally Disintegrating Tablets**

DEFINITION

Desloratadine Orally Disintegrating Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$).

IDENTIFICATION

• **A. Ultraviolet Absorption** (197U)

Standard solution and **Sample solution:** Proceed as directed in the *Assay*.

Instrumental conditions

Mode: UV

Wavelength range: 230–330 nm

[Note—Alternatively, a diode array detector may be used in the *Assay* to obtain the spectra.]

Acceptance criteria: The UV spectrum of the *Sample solution* corresponds to that of the *Standard solution*.

• **B.** The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Buffer: 6.8 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile, methanol, and *Buffer* (28:7:65)

Diluent: Methanol and 0.1N hydrochloric acid (40:60)

Standard solution: 0.05 mg/mL of USP Desloratadine RS in *Diluent*. Sonication may be used to aid dissolution.

Sample stock solution: Nominally 0.25 mg/mL of desloratadine, prepared as follows.

Transfer 10 Tablets to a suitable volumetric flask, add water to 15% of the flask volume, and shake until the Tablets disintegrate completely. Add 75% of the flask volume of *Diluent* and sonicate for 30 min with intermittent shaking, and dilute with *Diluent* to volume. Centrifuge a portion of this solution. Use the supernatant.

Sample solution: Nominally 0.05 mg/mL of desloratadine from the *Sample stock solution* in *Diluent*; centrifugate

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 258 nm

Column: 4.6-mm × 25-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)

C_U nominal concentration of desloratadine in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **Disintegration** $\langle 701 \rangle$: NMT 30 s

- **Dissolution** $\langle 711 \rangle$

Medium: 0.1 N hydrochloric acid (degassed); 900 mL

Apparatus 2: 50 rpm

Time: 10 min

Buffer: 6.8 g/L of monobasic potassium phosphate

Solution A: Acetonitrile and methanol (80:20)

Mobile phase: *Solution A* and *Buffer* (40:60)

Standard stock solution: 0.28 mg/mL of USP Desloratadine RS in methanol. Sonication may be used to aid dissolution.

Standard solution: ($L/900$) mg/mL of USP Desloratadine RS from the *Standard stock solution* in *Medium*, where L is the label claim (mg/Tablet)

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: LC

Detector: UV 258 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 40 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$) is dissolved.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

- **Organic Impurities**

Buffer: Add 10 mL/L of triethylamine to a 1.36 g/L solution of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.5.

Solution A: Methanol, acetonitrile, and *Buffer* (15:5:80)

Solution B: Acetonitrile, tetrahydrofuran, and *Buffer* (70:5:30)

Solution C: Dilute 8.5 mL of hydrochloric acid with methanol to 1 L.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
40	50	50
50	50	50
52	100	0
65	100	0

Diluent: *Solution C* and *Buffer* (30:70)

System suitability stock solution: 0.05 mg/mL each of USP Desloratadine Related Compound A RS and USP Desloratadine Related Compound F RS in methanol

System suitability solution: 0.5 mg/mL of USP Desloratadine RS, 1.0 µg/mL each of USP Desloratadine Related Compound A RS and USP Desloratadine Related Compound F RS, prepared as follows. Transfer 50 mg of USP Desloratadine RS into a 100-mL volumetric flask, add 70 mL of *Diluent*, and sonicate to dissolve. Add 2 mL of the *System suitability stock solution* and dilute with *Diluent* to volume.

Standard solution: 0.0025 µg/mL of USP Desloratadine RS and 1.0 µg/mL of USP Desloratadine Related Compound F RS in *Diluent*

Sample solution: Nominally 0.500 µg/mL of desloratadine from NLT 40 Tablets, prepared as follows. Transfer an amount of powder to a suitable volumetric flask to obtain the nominal concentration of desloratadine. Add 70% of the flask volume of *Mobile phase* and sonicate

for 20 min with intermittent shaking. Dilute with *Diluent* to volume. Centrifuge a portion of the solution and use the supernatant.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 40 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—Relative retention times are given in *Table 2*.]

Suitability requirements

Resolution: NLT 2.0 between desloratadine and desloratadine related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for desloratadine and desloratadine related compound F, *Standard solution*

Relative standard deviation: NMT 10.0% desloratadine related compound F, *Standard solution*

Signal-to-noise ratio: NLT 10 for desloratadine peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the impurities using the relative retention times given in *Table 2*.

Calculate the percentage of desloratadine related compound F in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of desloratadine related compound F from the *Sample solution*

r_S peak response of desloratadine related compound F from the *Standard solution*

C_S concentration of USP Desloratadine Related Compound F RS in the *Standard solution* (μg/mL)

C_U nominal concentration of desloratadine in the *Sample solution* (μg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any unspecified degradation product from the *Sample solution*

r_S peak response of desloratadine from the *Standard solution*

C_S concentration of USP Desloratadine RS in the *Standard solution* (μg/mL)

C_U nominal concentration of desloratadine in the *Sample solution* (μg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Compound	Relative Retention Time	Acceptance Criteria, NMT (%)
Dechloro desloratadine ^{a,b}	0.42	—
Desloratadine	1.00	—
Desloratadine related compound A ^b	1.09	—
Dehydro desloratadine ^{b,c}	1.33	—
Desloratadine related compound F	1.37	0.2
Loratadine ^{b,d}	1.89	—
Any other individual unspecified degradation product	—	0.20
Total degradation products	—	0.5

^a 6,11-Dihydro-11-(piperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

^b This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.

^c 8-Chloro-11-(piperidin-4-ylidene)benzo[5,6]cyclohepta[1,2-*b*]pyridine.

^d 8-Chloro-6,11-dihydro-11-(1-ethoxycarbonylpiperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.

- **USP Reference Standards** { 11 }

USP Desloratadine RS

USP Desloratadine Related Compound A RS

8-Bromo-6,11-dihydro-11-(piperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

C₁₉H₁₉BrN₂ 355.27

USP Desloratadine Related Compound F RS

8-Chloro-6,11-dihydro-11-(*N*-formyl-4-piperidinylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

C₁₉H₁₉ClN₂O 338.83

■ 1S (USP39)

BRIEFING

Dexchlorpheniramine Maleate Tablets, USP 38 page 3046 and PF 40(1) [Jan.–Feb. 2014]. The U.S. Pharmacopeial Convention (USP) is engaged in the ongoing challenge of obtaining information needed to create and sustain up-to-date drug product quality monographs for drugs marketed under the FDA over-the-counter (OTC) drug monograph system (21 CFR Part 330). A revision to modernize the *Assay* and *Identification* tests is being proposed.

1. Replace the *Identification—Organic Nitrogenous Bases* procedure, which uses carbon

disulfide and requires extensive sample preparation, with retention time matching in *Identification* test A using the procedure from the *Assay*.

2. It is proposed to add *Identification* test C as an orthogonal identification test based on spectral comparison of the dexchlorpheniramine peak using photo-diode array detection (190–400 nm) with the *Assay* procedure.
3. Replace the nonstability-indicating UV procedure in the *Assay* with the liquid chromatography procedure in *Drug Product Assay Tests—Organic Chemical Medicines* { 321 }, *Procedure 1*. *Procedure 1* was validated with the Waters Acquity UPLC HSS T3 brand of L1 column. The retention time for dexchlorpheniramine is about 6.1 min.
4. Additional Reference Standards have been added to the *USP Reference Standards* section to support the revision of the *Assay*.

General chapter { 321 }, published in the *Pharmacopeial Forum* [PF 41(1)], is a procedure that was developed and validated for OTC medicines. Further information can be found in the *Stimuli* article entitled “Medicines Marketed under the Food and Drug Administration Over-the-Counter Drug Monograph System Regulations: Strategy for Developing Compendial Quality Standards”, also published in PF 41(1). This article describes in detail the rationale for the development of this chapter and other related chapters, as well as the associated effect on drug product monographs. USP plans to continue with this initiative to revise or establish standards for OTC drug products.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: A. Potts.)

Correspondence Number—C151308

Comment deadline: May 31, 2015

Dexchlorpheniramine Maleate Tablets

DEFINITION

Dexchlorpheniramine Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of dexchlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$).

IDENTIFICATION

Delete the following:

- • ~~A. Identification—Organic Nitrogenous Bases { 181 }:~~ Meet the requirements ■ 1S (USP39)

Add the following:

- • **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

- **B.**

Analysis: Shake a quantity of finely powdered Tablets, equivalent to 150 mg of dexchlorpheniramine maleate, with 100 mL of 1 N acetic acid for 10 min, and filter through a sintered-glass funnel into a suitable vessel. Adjust the filtrate with sodium hydroxide

solution (1 in 10) to a pH of 11, and extract the solution with six 100-mL portions of solvent hexane, filtering each hexane extract using suitable means to separate the hexane layer from the aqueous layer. Concentrate the combined extracts on a steam bath to a small volume, transfer to a smaller, more suitable vessel, and evaporate just to the point where hexane vapors are no longer perceptible. Transfer the oily residue, with the aid of four 3-mL portions of dimethylformamide, to a suitable glass-stoppered graduated cylinder, dilute with dimethylformamide to 15.0 mL, mix, and centrifuge if necessary.

Acceptance criteria: The optical rotation of the solution so obtained in a 100-mm tube after correcting for the blank is between $+0.24^{\circ}$ and $+0.35^{\circ}$ (distinction from chlorpheniramine maleate).

Add the following:

- C. The UV-Vis spectrum (190–400 nm) of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

ASSAY

Delete the following:

■● **Procedure**

Diluent: Dilute hydrochloric acid (1 in 120)

Standard stock solution: 0.4 mg/mL of USP Dexchlorpheniramine Maleate RS in water

Standard solution: 40 µg/mL of USP Dexchlorpheniramine Maleate RS, prepared as follows: Transfer 10.0 mL of the *Standard stock solution* to a separator, adjust with 1 N sodium hydroxide to a pH of 11, and cool. Extract with two 50 mL portions of solvent hexane, shaking each portion for 2 min before separating the phases, and combining the hexane extracts in a second separator. Extract the hexane solution with two 40 mL portions of *Diluent*, combine the *Diluent* extracts in a 100 mL volumetric flask, and add *Diluent* to volume. Filter the solution into a glass-stoppered conical flask, discarding the first few mL of the filtrate.

Sample solution: Nominally 40 µg/mL of dexchlorpheniramine maleate, prepared as follows: Transfer an equivalent to 8 mg of dexchlorpheniramine maleate, from NLT 20 finely powdered Tablets, to a 250 mL separator. Mix with 50 mL of water for 10 min, adjust with sodium hydroxide solution (1 in 10) to a pH of 11, and cool to room temperature. Extract the mixture with two 75 mL portions of solvent hexane, and combine the extracts in a second separator. Extract the solvent hexane solution with three 50 mL portions of *Diluent*, combining the *Diluent* extracts in a 200 mL volumetric flask. Add *Diluent* to volume.

Instrumental conditions

Analytical wavelength: Maximum absorbance at about 264 nm

Cell: 1 cm

Blank: *Diluent*

Analysis

Samples: *Standard solution* and *Sample solution*

Concomitantly determine the absorbance of the *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of dexchlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

~~A_U~~ absorbance of the *Sample solution*

~~A_S~~ absorbance of the *Standard solution*

~~C_S~~ concentration of USP Dexchlorpheniramine Maleate RS in the *Standard solution* ($\mu\text{g/mL}$)

~~C_U~~ nominal concentration of dexchlorpheniramine maleate in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–110.0% \pm 1S (USP39)

Add the following:

- Drug Product Assay Tests—Organic Chemical Medicines, Procedure 1 (321):** 90.0%–110.0% \pm 1S (USP39)

PERFORMANCE TESTS

• Dissolution (711)

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 45 min

Solution A: Sodium hydroxide solution (1 in 2)

Internal standard solution: 90 $\mu\text{g/mL}$ of dexbrompheniramine maleate in water

Standard stock solution: 12.5 $\mu\text{g/mL}$ of USP Dexchlorpheniramine Maleate RS in water

Standard solution: Pipet 5 mL of the *Standard stock solution* into a 50-mL centrifuge tube, and add 10.0 mL of water and 1.0 mL of *Internal standard solution*. Adjust with *Solution A* to a pH of 11 ± 0.1 , and add 3.0 mL of chromatographic hexane. Insert the stopper in the tube, shake by mechanical means for 3 min, centrifuge, and use the clear supernatant hexane layer.

Sample solution: Pipet 15 mL of a portion of the solution under test into a 50-mL centrifuge tube, and add 1.0 mL of *Internal standard solution*. Adjust with *Solution A* to a pH of 11 ± 0.1 , and add 3.0 mL of chromatographic hexane. Insert the stopper in the tube, shake by mechanical means for 3 min, centrifuge, and use the clear supernatant hexane layer.

Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm \times 1.8-m; contains a packing consisting of 1.2% phase G16 and 0.5% potassium hydroxide on support S1AB

Temperatures

Injection port: 250 $^{\circ}$

Detector: 250 $^{\circ}$

Column: 205 $^{\circ}$

Flow rate: 60 mL/min

Carrier gas: Helium

Injection volume: 2 μL

System suitability**Sample:** *Standard solution*

[Note—The relative retention times for dexchlorpheniramine and dexbrompheniramine are about 0.7 and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 1.9 between dexchlorpheniramine and dexbrompheniramine**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution and Sample solution*

Calculate the quantity of dexchlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) dissolved by comparing the peak response ratios.

Tolerances: NLT 75% (*Q*) of the labeled amount of dexchlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) is dissolved.

- **Uniformity of Dosage Units** $\langle 905 \rangle$: Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.

Change to read:

- **USP Reference Standards** $\langle 11 \rangle$

- USP Caffeine RS

- 1S (*USP39*)

- USP Dexchlorpheniramine Maleate RS

- USP Dextromethorphan Hydrobromide RS

- USP Diphenhydramine Hydrochloride RS

- USP Doxylamine Succinate RS

- USP Pseudoephedrine Hydrochloride RS

BRIEFING

Dihydroxyaluminum Sodium Carbonate, *USP 38* page 3125. In preparation for the omission of the flame tests from general chapter *Identification Tests—General* $\langle 191 \rangle$, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to replace the use of the flame test under *Identification* with the cross reference to the test for *Sodium Content*, which employs atomic absorption spectroscopy.

The chemical formula in the *Definition* and in the *Assay* is updated to be consistent with the *USAN* dictionary

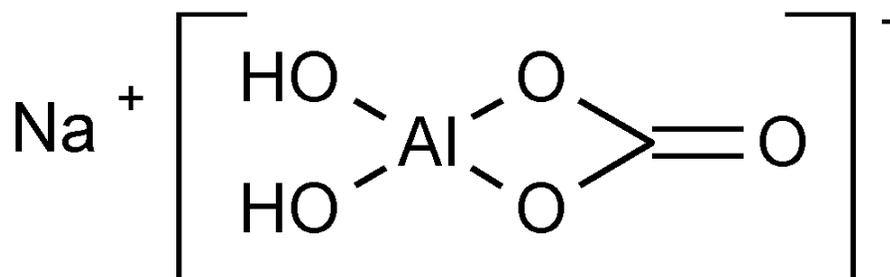
The *Identification* test is subdivided into three separate tests for clarity.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: R. S. Prasad.)

Correspondence Number—C154663

Comment deadline: May 31, 2015

Dihydroxyaluminum Sodium Carbonate

$\text{NaAl}(\text{OH})_2\text{CO}_3$ 143.99

Aluminum, [carbonato(1-)-O]dihydroxy-, monosodium salt;
Sodium (T-4)-[carbonato(2-)-O,O']dihydroxyaluminate(1-);
Sodium (carbonato)dihydroxyaluminate(1-) [539-68-4]; [16482-55-6].

DEFINITION**Change to read:**

Dihydroxyaluminum Sodium Carbonate contains NLT 98.3% and NMT 107.9% of dihydroxyaluminum sodium carbonate ($\text{CH}_2\text{AlNaO}_5$)

■ $[\text{NaAl}(\text{OH})_2\text{CO}_3]$, ■ 1S (USP39)

calculated on the dried basis.

IDENTIFICATION**Delete the following:**

■ • ~~A.~~

~~**Sample solution:** Combine 1 g with 20 mL of 3 N hydrochloric acid.~~

~~**Acceptance criteria:** The sample dissolves with effervescence and meets the requirements of *Identification Tests—General, Aluminum* <191> and the flame test in *Identification Tests—General, Sodium* <191>.~~ ■ 1S (USP39)

Add the following:

■ • A.

Sample solution: Combine 1 g with 20 mL of 3 N hydrochloric acid.

Acceptance criteria: The sample dissolves with effervescence. ■ 1S (USP39)

Add the following:

■ • B. **Identification Tests—General, Aluminum** <191>

Sample: Sample solution prepared in *Identification test A*

Acceptance criteria: Meets the requirements ■ 1S (USP39)

Add the following:

- **C.** The *Sample solution*, prepared and tested as directed in the test for *Sodium Content*, exhibits a significant absorption at the sodium emission line at 589.0 nm. ■1S (USP39)

ASSAY**Change to read:**● **Procedure**

Edetate disodium titrant: Dissolve 18.6 g of edetate disodium in water to make 500 mL, and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.

Sample: 300 mg undried

Analysis: Transfer the *Sample* to a 250-mL beaker, add 10 mL of 2 N sulfuric acid, cover the beaker, heat to 80° for 5 min, and boil for 1 min. Add 30.0 mL of 0.1 M edetate disodium VS, again boil for 1 min, cool, and then add 10 mL of acetic acid–ammonium acetate buffer TS, 50 mL of acetone, and 2 mL of dithizone TS. Using a pH meter, adjust with the addition of ammonium hydroxide or dilute sulfuric acid to a pH of 4.5. Titrate with 0.05 M zinc sulfate VS, maintaining the pH of 4.5 by the addition of ammonium hydroxide as necessary, to an orange-pink color. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M *Edetate disodium titrant* is equivalent to 14.40 mg of dihydroxyaluminum sodium carbonate ($\text{CH}_2\text{AlNaO}_5$).

■ $[\text{NaAl}(\text{OH})_2\text{CO}_3]$. ■1S (USP39)

Acceptance criteria: 98.3%–107.9% on the dried basis

IMPURITIES● **Mercury, Method IIa** (261)

Sample solution: 2.0 g in 35 mL of 1 N sulfuric acid

Acceptance criteria: NMT 1 ppm

● **Isopropyl Alcohol**

Isopropyl alcohol-free dihydroxyaluminum sodium carbonate: Use a portion of Dihydroxyaluminum Sodium Carbonate that has been previously tested as directed in this section and found to be free of isopropyl alcohol.

Sodium chloride solution: 0.2 g/mL in water

Standard stock solution: 20 mg/mL of isopropyl alcohol in *Sodium chloride solution*

Standard solution A: 0.4 mg/mL of isopropyl alcohol in *Sodium chloride solution* from *Standard stock solution*

Standard solution B: 0.8 mg/mL of isopropyl alcohol in *Sodium chloride solution* from *Standard stock solution*

Standard solution C: 1.0 mg/mL of isopropyl alcohol in *Sodium chloride solution* from *Standard stock solution*

Standard solution D: 1.2 mg/mL of isopropyl alcohol in *Sodium chloride solution* from *Standard stock solution*

Headspace containers: Use suitable 20-mL containers capable of being tightly closed with an inert septum and a metallic crimp cap.

Standard preparations: To four separate 20-mL *Headspace containers*, add 1.0 g of *Isopropyl alcohol-free dihydroxyaluminum sodium carbonate*. To the containers add, respectively, 10.0 mL of the appropriate *Standard solution*. These containers contain about 4, 8, 10, and 12 mg of isopropyl alcohol, respectively. [Note—Keep the containers cool until sealed.] Seal the containers, place in a water bath maintained at 70°, and allow to stand for 1 h.

Sample preparation: Transfer 1.0 g of the *Dihydroxyaluminum Sodium Carbonate* to a *Headspace container*, and add 10.0 mL of *Sodium chloride solution*. [Note—Keep the container cool until sealed.] Seal the container, place in a water bath maintained at 70°, and allow to stand for 1 h.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.9-m × 3-mm; packed with support S3

Temperatures

Column: 180°

Injection port: 200°

Detector: 250°

Injection volume: 1 mL of gaseous phase

System suitability

Samples: *Standard preparations* containing 10 mg per container

Suitability requirements

Relative standard deviation: NMT 4% for replicate injections

Analysis

Samples: *Standard preparations* and *Sample preparation*

[Note—Use peak areas where peak responses are indicated.]

Using a gas-tight syringe, separately inject equal volumes of the gaseous headspace of the *Standard preparations* and the *Sample preparation* into the gas chromatograph. Record the chromatograms, and measure the peak responses. Determine, based on a retention time comparison, if isopropyl alcohol is detected in the *Sample preparation*. Plot the responses of the *Standard preparations* versus the content, in mg, of isopropyl alcohol in each container, draw the straight line best fitting the plotted points, and calculate the correlation coefficient for the line. A suitable system is one that yields a line having a correlation coefficient of NLT 0.99. From the graph, determine the total amount, T_U , in mg, of isopropyl alcohol in the *Sample preparation*.

Calculate the percentage of isopropyl alcohol in the *Dihydroxyaluminum Sodium Carbonate* taken:

$$\text{Result} = 0.1 \times (T_U/W_U)$$

W_U weight of the *Dihydroxyaluminum Sodium Carbonate* taken (g)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS● **Sodium Content**

Potassium chloride solution: 38 mg/mL of potassium chloride in water

Sodium chloride stock solution: 25.42 µg/mL of sodium chloride in water (10.0 µg/mL of sodium) from sodium chloride previously dried at 105° for 2 h

Standard solution A: 0.5 µg/mL of sodium from *Sodium chloride stock solution* prepared as follows. On the day of use, transfer 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* to a 100-mL volumetric flask. Add 5.0 mL of *Sodium chloride stock solution* and dilute with water to volume.

Standard solution B: 1.0 µg/mL of sodium from *Sodium chloride stock solution* prepared as follows. On the day of use, transfer 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* to a 100-mL volumetric flask. Add 10.0 mL *Sodium chloride stock solution* and dilute with water to volume.

Sample solution: Transfer 250 mg of Dihydroxyaluminum Sodium Carbonate, previously dried, to a 200-mL volumetric flask. Add 40 mL of 1 N hydrochloric acid, and boil for 1 min. Cool, and dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask containing 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution*, and dilute with water to volume.

Blank solution: Pipet 4 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* into a 100-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* { 851 } .)

Mode: Atomic absorption spectroscopy

Analytical wavelength: Sodium emission line at 589.0 nm

Lamp: Sodium hollow-cathode

Flame: Air-acetylene

Blank: *Blank solution*

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Sample solution*, and *Blank solution*

Plot the absorbances of the *Standard solutions* versus the concentrations, in µg/mL of sodium, and draw a straight line between the plotted points. From the graph so obtained, determine the concentration, *C*, in µg/mL of sodium in the *Sample solution*.

Calculate the percentage of sodium in the portion of Dihydroxyaluminum Sodium Carbonate taken:

$$\text{Result} = 4000 \times (C/W)$$

C = concentration of sodium in the *Sample solution* (µg/mL)

W = weight of Dihydroxyaluminum Sodium Carbonate taken (mg)

Acceptance criteria: 15.2%–16.8%

● **Acid-Neutralizing Capacity** { 301 }

Sample: 425 mg of undried material

Analysis: Proceed as directed using the *Sample*. Each mg of dihydroxyaluminum sodium carbonate [NaAl(OH)₂CO₃] has an expected acid-neutralizing capacity of 0.0278 mEq.

Acceptance criteria: NLT 75.0% of the expected mEq value, calculated in relation to the

results of the Assay

- **pH** 〈 791 〉: 9.9–10.2 in a suspension (1 in 25)

- **Loss on Drying** 〈 731 〉

Sample: Dry at 130° to constant weight.

Acceptance criteria: NMT 14.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

BRIEFING

Diphenhydramine Hydrochloride and Ibuprofen Capsules. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is proposed.

1. The liquid chromatographic procedure in the *Assay* is based on analyses performed with the Inertsil Ph-3 brand of L11 column. The typical retention times for diphenhydramine and ibuprofen are about 3.5 and 10.4 min, respectively.
2. The liquid chromatographic procedure in the *Dissolution* is based on analyses performed with the Inertsil C8 brand of L7 column. The typical retention times for ibuprofen and diphenhydramine are about 5.2 and 9.7 min, respectively.
3. The liquid chromatographic procedure in the *Organic Impurities* test is based on analyses performed with the Inertsil Ph-3 brand of L11 column. The typical retention times for diphenhydramine and ibuprofen are about 5 and 20 min, respectively.

(SM2: H. Cai.)

Correspondence Number—C126560

Comment deadline: May 31, 2015

Add the following:

■ **Diphenhydramine Hydrochloride and Ibuprofen Capsules**

DEFINITION

Diphenhydramine Hydrochloride and Ibuprofen Capsules contain NLT 95.0% and NMT 105.0% of the labeled amounts of diphenhydramine hydrochloride (C₁₇H₂₁NO·HCl) and ibuprofen (C₁₃H₁₈O₂).

IDENTIFICATION

- **A.** The retention times of the diphenhydramine and ibuprofen peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the diphenhydramine and ibuprofen peaks of the *Sample solution* and those of the *Standard solution* exhibit maxima at the same wavelengths of 265 and 273 nm, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to

a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (38:62)

Standard solution: 0.05 mg/mL of USP Diphenhydramine Hydrochloride RS and 0.4 mg/mL of USP Ibuprofen RS in *Mobile phase*

Sample stock solution: Nominally 0.25 mg/mL of diphenhydramine hydrochloride and 2.0 mg/mL of ibuprofen, prepared as follows. Transfer NLT 5 Capsules (including shells) to a suitable volumetric flask, add 4% of the final volume of water, and sonicate for 20 min. Dissolve and dilute with *Mobile phase* to volume. Pass a portion through a suitable filter of 0.45- μ m pore size.

Sample solution: Nominally 0.05 mg/mL of diphenhydramine hydrochloride and 0.4 mg/mL of ibuprofen in *Mobile phase* from *Sample stock solution*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detectors

Assay: UV 220 nm

Identification test A: Diode array, UV 200–400 nm

Column: 4.6-mm \times 10-cm; 3- μ m packing L11

Column temperature: 25 $^{\circ}$

Flow rate: 1.0 mL/min

Injection volume: 5 μ L

Run time: NLT 4 times the retention time of diphenhydramine

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for both diphenhydramine and ibuprofen

Relative standard deviation: NMT 2.0% for both diphenhydramine and ibuprofen

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) and ibuprofen ($C_{13}H_{18}O_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of diphenhydramine or ibuprofen from the *Sample solution*

r_S peak response of diphenhydramine or ibuprofen from the *Standard solution*

C_S concentration of USP Diphenhydramine Hydrochloride RS or USP Ibuprofen RS in the *Standard solution* (mg/mL)

C_U nominal concentration of diphenhydramine hydrochloride or ibuprofen in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• Dissolution { 711 }

Medium: Phosphate buffer, pH 7.2 (27.22 mg/mL of monobasic potassium phosphate in water and adjust with 100 mg/mL of sodium hydroxide to a pH of 7.2); 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 100 mg/mL of sodium hydroxide to a pH of 6.6.

Mobile phase: Methanol and *Buffer* (65:35)

Standard stock solution: 0.27 mg/mL of USP Diphenhydramine Hydrochloride RS and 2.2 mg/mL of USP Ibuprofen RS, prepared as follows. Transfer known amounts of USP Diphenhydramine Hydrochloride RS and USP Ibuprofen RS to a suitable volumetric flask. Add 5% of the final volume of methanol and sonicate to dissolve. Dilute with *Medium* to volume.

Standard solution: 0.027 mg/mL of USP Diphenhydramine Hydrochloride RS and 0.22 mg/mL of USP Ibuprofen RS in *Medium* from *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size and discard the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Column temperature: 35 $^{\circ}$

Flow rate: 1.0 mL/min

Injection volume: 5 μ L

Run time: NLT 2.3 times the retention time of ibuprofen

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for both diphenhydramine and ibuprofen

Relative standard deviation: NMT 2.0% for both diphenhydramine and ibuprofen

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) and ibuprofen ($C_{13}H_{18}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of diphenhydramine or ibuprofen from the *Sample solution*

r_S = peak response of diphenhydramine or ibuprofen from the *Standard solution*

C_S = concentration of USP Diphenhydramine Hydrochloride RS and USP Ibuprofen RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim of diphenhydramine or ibuprofen (mg/Capsule)

Tolerances: NLT 75% (Q) of the labeled amounts of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) and ibuprofen ($C_{13}H_{18}O_2$) is dissolved.

- **Uniformity of Dosage Units** $\langle 905 \rangle$: Meet the requirements

IMPURITIES• **Organic Impurities**

Buffer: Proceed as directed in the *Assay*.

Mobile phase: Acetonitrile and *Buffer* (32:68)

Diluent: Acetonitrile and *Buffer* (40:60)

System suitability solution: 0.0005 mg/mL of USP Diphenhydramine Related Compound A RS and 0.25 mg/mL of USP Diphenhydramine Hydrochloride RS in *Diluent*. Sonicate if necessary to dissolve.

Standard solution: 0.00125 mg/mL of USP Diphenhydramine Hydrochloride RS and 0.01 mg/mL of USP Ibuprofen RS in *Diluent*. Sonicate to dissolve.

Sample solution: Nominally 0.25 mg/mL of diphenhydramine hydrochloride and 2 mg/mL of ibuprofen, prepared as follows. Transfer a suitable amount of Capsule contents from NLT 10 Capsules to a dry volumetric flask. Add about 60% of the final volume of *Diluent* and dissolve the contents completely by using a vortex. Dilute with *Diluent* to volume. Pass a portion through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 10-cm; 3- μ m packing L11

Column temperature: 25 $^{\circ}$

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

Run time: NLT 20 times the retention time of diphenhydramine

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between diphenhydramine related compound A and diphenhydramine, *System suitability solution*

Relative standard deviation: NMT 5.0% for both diphenhydramine and ibuprofen, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of diphenhydramine from the *Standard solution*

C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor of each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Diphenhydramine related compound A	0.9	1.0	0.2
Diphenhydramine	1.0	1.0	—
4-Methyldiphenhydramine ^a	1.3	1.0	0.2
4-Bromodiphenhydramine ^b	1.7	0.9	0.2
Benzhydro ^c	2.3	1.6	0.2
Ibuprofen	4.1	—	—
Any other individual impurity	—	1.0	0.20
Total impurities	—	—	1.0
^a <i>N,N</i> -Dimethyl-2-[phenyl(<i>p</i> -tolyl)methoxy]ethanamine.			

^b 2-[(4-Bromophenyl)(phenyl)methoxy]-*N,N*-dimethylethanamine.

^c Diphenylmethanol.

SPECIFIC TESTS

- **Microbial Enumeration Tests** $\langle 61 \rangle$ and **Tests for Specified Microorganisms** $\langle 62 \rangle$: NMT 5×10^2 cfu/g for the total aerobic microbial count; NMT 10^2 cfu/g for total combined yeasts and molds count. It meets the requirements for absence of *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers and store at 20° – 25° . Protect from light and excessive heat above 40° .
- **USP Reference Standards** $\langle 11 \rangle$
 USP Diphenhydramine Hydrochloride RS
 USP Diphenhydramine Related Compound A RS
 2-(Diphenylmethoxy)-*N*-methylethanamine hydrochloride.
 $C_{16}H_{19}NO \cdot HCl$ 277.79
 USP Ibuprofen RS

■ 1S (USP39)

BRIEFING

Docosate Potassium, USP 38 page 3184. As part of USP monograph modernization efforts the monograph is revised as follows:

1. *Identification* test A is updated to include a reference to general chapter *Infrared Absorption* $\langle 197 \rangle$.
2. In preparation for the omission of the flame tests from *Identification Tests—General* $\langle 191 \rangle$, proposed in PF 41(2) [Mar.–Apr. 2015], the reference to $\langle 191 \rangle$ in *Identification* test B is deleted and a complete description of the flame test is

included in the monograph. Manufacturers are encouraged to submit a replacement wet chemistry or an instrumental procedure for the Expert Committee's consideration.

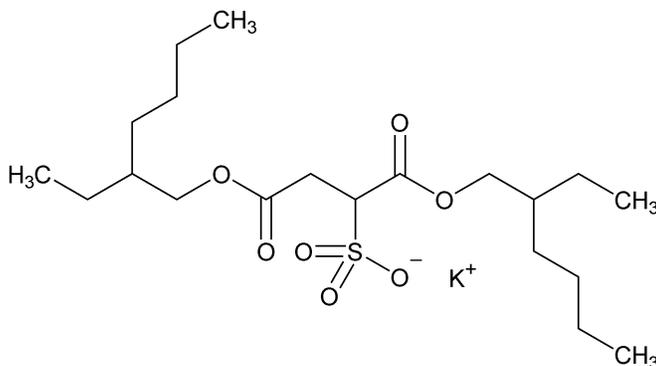
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: R.S. Prasad.)

Correspondence Number—C154664

Comment deadline: May 31, 2015

Docusate Potassium



$C_{20}H_{37}KO_7S$ 460.67

Butanedioic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, potassium salt;
Potassium 1,4-bis(2-ethylhexyl) sulfosuccinate [7491-09-0].

DEFINITION

Docusate Potassium contains NLT 95.0% and NMT 100.5% of docusate potassium ($C_{20}H_{37}KO_7S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.**

■ **Infrared Absorption** $\langle 197 \rangle$ ■ 1S (*USP39*)

Sample: Place a small piece of Docusate Potassium on a salt plate, add 1 drop of acetone, and promptly cover with another salt plate. Rub the plates together to dissolve the specimen, slide the plates apart, and allow the acetone to evaporate.

Acceptance criteria: The IR-absorption spectrum of the film exhibits maxima only at the same wavelengths as that of a similar preparation of USP Docusate Potassium RS.

■ Meets the requirements ■ 1S (*USP39*)

Delete the following:

■ • **B. Identification Tests—General, Potassium** $\langle 191 \rangle$: Meets the requirements of the flame

test ■1S (USP39)

Add the following:

- **B.** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.] ■1S (USP39)

ASSAY

● **Procedure**

Solution A: 2.500 g/L of tetra-*n*-butylammonium iodide in water

Solution B: A solution containing 100 g/L of anhydrous sodium sulfate and 10 g/L of sodium carbonate in water

Sample: 100 mg

Analysis: Dissolve the *Sample* in 50 mL of chloroform in a glass-stoppered, 250-mL conical flask. Add 50 mL of *Solution B* and 500 µL of bromophenol blue TS. Titrate with *Solution A* until 1 mL from the endpoint, and shake the stoppered flask vigorously for 2 min. Continue the titration in 2-drop increments, shaking vigorously for 10 s after each addition, and then allow the flask to stand for 10 s. Continue the titration until the chloroform layer just assumes a blue color. Each mL of *Solution A* is equivalent to 3.118 mg of docusate potassium (C₂₀H₃₇KO₇S).

Acceptance criteria: 95.0%–100.5% on the dried basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: 18.0%–20.0% on the dried basis

Delete the following:

-

● **Heavy Metals, Method I** 〈 231 〉

Sample: 2.0 g

Analysis: Transfer the *Sample* to a platinum crucible, and ignite until free from carbon. Cool, moisten the residue with 1 mL of hydrochloric acid, and evaporate on a steam bath to dryness. Add 2 mL of 6 N acetic acid, digest on a steam bath for 5 min, filter into one of a pair of matched 50-mL, color-comparison tubes, and wash the residue with sufficient water to make 25 mL.

Acceptance criteria: NMT 10 ppm (Official 1-Dec-2015)

● **Limit of Bis(2-ethylhexyl) Maleate**

Mobile phase: Alcohol and water (78:22), filtered and degassed

Standard solution: 80 µg/mL of USP Bis(2-ethylhexyl) Maleate RS in alcohol

Sample solution: 20 mg/mL of Docusate Potassium in alcohol. [Note—If necessary, warm the mixture by using the steam bath to achieve a complete dissolution.]

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 210 nm
Column: 4.6-mm × 3-cm; 3.5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 3 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of bis(2-ethylhexyl) maleate in the portion of Docusate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of bis(2-ethylhexyl) maleate from the *Sample solution*

r_S = peak response of bis(2-ethylhexyl) maleate from the *Standard solution*

C_S = concentration of USP Bis(2-ethylhexyl) Maleate RS in the *Standard solution* (mg/mL)

C_U = concentration of Docusate Potassium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.4%

SPECIFIC TESTS

- **Loss on Drying** { 731 }

Analysis: Dry in a glass container at 105° for 4 h.

Acceptance criteria: NMT 3.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

- **USP Reference Standards** { 11 }

USP Bis(2-ethylhexyl) Maleate RS

C₂₀H₃₆O₄ 340.51

USP Docusate Potassium RS

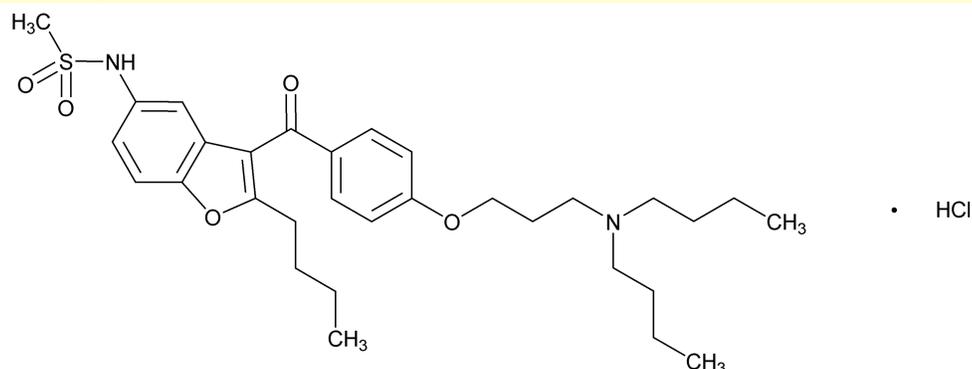
BRIEFING

Dronedarone Hydrochloride. Because there is no existing *USP* monograph for this drug substance, a new monograph is proposed based on validated methods of analysis. The liquid chromatographic procedures for the *Assay* and *Organic Impurities* are based on analyses performed with the Zorbax Stable Bond-CN brand of L10 column. The typical retention time of dronedarone is about 7 min under the *Assay* conditions and about 34 min under the *Organic Impurities* test conditions.

(SM2: S. Ramakrishna.)

Correspondence Number—C131624

Comment deadline: May 31, 2015

Add the following:**■ Dronedarone Hydrochloride**

$C_{31}H_{44}N_2O_5S \cdot HCl$ 593.2

N-{2-Butyl-3-[4-(3-dibutylaminopropoxy)benzoyl]benzofuran-5-yl}methanesulfonamide, hydrochloride [141625-93-6].

DEFINITION

Dronedarone Hydrochloride contains NLT 98.0% and NMT 101.5% of dronedarone hydrochloride, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K): Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General, Chloride** (191)
Sample solution: Dissolve 100 mg of Dronedarone Hydrochloride in 8 mL of methanol and dilute with water to 10.0 mL. Use 2.0 mL for the analysis.
Acceptance criteria: Meets the requirements

ASSAY

- **Procedure**
Buffer: Combine 2.0 mL of triethylamine with 1 L of water and adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (50:50)
System suitability stock solution: 1 mg/mL each of USP Dronedarone Hydrochloride RS and USP Dronedarone Related Compound A RS in methanol
System suitability solution: 0.01 mg/mL each of USP Dronedarone Hydrochloride RS and USP Dronedarone Related Compound A RS in *Mobile phase* from *System suitability stock solution*
Standard solution: 0.1 mg/mL of USP Dronedarone Hydrochloride RS in *Mobile phase*
Sample solution: 0.1 mg/mL of Dronedarone Hydrochloride in *Mobile phase*
Chromatographic system
Mode: LC
Detector: UV 288 nm
Column: 4.6-mm × 25-cm; 5-μm packing L10

Flow rate: 0.8 mL/min

Injection volume: 20 μ L

Run time: NLT 2.15 times the retention time of dronedarone

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for dronedarone related compound A and dronedarone are 0.71 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8 between dronedarone and dronedarone related compound A, *System suitability solution*

Tailing factor: 0.8–2.2 for dronedarone, *System suitability solution*

Relative standard deviation: NMT 0.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dronedarone hydrochloride ($C_{31}H_{44}N_2O_5 \cdot HCl$) in the portion of Dronedarone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dronedarone from the *Sample solution*

r_S = peak response of dronedarone from the *Standard solution*

C_S = concentration of USP Dronedarone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Dronedarone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the anhydrous and solvent-free basis

IMPURITIES

- **Residue on Ignition** $\langle 281 \rangle$: NMT 0.1%

- **Organic Impurities**

Solution A: Combine 2 mL of triethylamine with 950 mL of water. Adjust with phosphoric acid to a pH of 4.0 and then dilute with water to 1 L.

Solution B: Acetonitrile

Mobile phase: See *Table 1*. [Note—Collect data for 58 min.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
15	60	40
25	60	40
40	50	50
45	40	60
58	40	60
60	70	30
70	70	30

Diluent: Acetonitrile and *Solution A*, 50:50

Standard stock solution: 0.2 mg/mL of USP Dronedarone Hydrochloride RS and 0.4 mg/mL of USP Dronedarone Related Compound A RS, respectively, in methanol

Standard solution: 2 µg/mL of USP Dronedarone Hydrochloride RS and 4 µg/mL of USP Dronedarone Related Compound A RS, respectively, in *Diluent* from the *Standard stock solution*

Sensitivity solution: 1 µg/mL of USP Dronedarone Hydrochloride RS and 2 µg/mL of USP Dronedarone Related Compound A RS in *Diluent* from the *Standard solution*

Sample solution: 2 mg/mL of Dronedarone Hydrochloride in *Diluent*. Sonicate for 5 min to dissolve completely. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

Mode: LC

Detector: UV 246 nm

Column: 4.6-mm × 25-cm; 5-µm packing L10

Flow rate: 0.8 mL/min

Injection volume: 25 µL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[Note—The relative retention times for dronedarone related compound A and dronedarone are 0.58 and 1.00, respectively.]

Suitability requirements

Resolution: NLT 25 between dronedarone and dronedarone related compound A, *Standard solution*

Signal-to-noise ratio: NLT 10 for dronedarone and NLT 20 for dronedarone related compound A, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dronedarone related compound A in the portion of Dronedarone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of dronedarone related compound A from the *Sample solution*

r_S peak response of dronedarone related compound A from the *Standard solution*

C_S concentration of USP Dronedarone Related Compound A RS in the *Standard solution* (mg/mL)

C_U concentration of Dronedarone Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Dronedarone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any unspecified impurity from the *Sample solution*

r_S peak response of dronedarone from the *Standard solution*

C_S concentration of USP Dronedarone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U concentration of Dronedarone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: Disregard peaks below 0.05%.

Dronedarone related compound A: NMT 0.15%

Any unspecified impurity: NMT 0.10%

Total impurities: NMT 0.3%

SPECIFIC TESTS

- **Water Determination, Method Ia** 〈 921 〉: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Protect from light and store at room temperature.

- **USP Reference Standards** 〈 11 〉

USP Dronedarone Hydrochloride RS

USP Dronedarone Related Compound A RS

N-(2-Butyl-3-{4-[3-(butylamino)propoxy]benzoyl}benzofuran-5-yl)methanesulfonamide.

C₂₇H₃₆N₂O₅S 500.65

■ 1S (USP39)

BRIEFING

Dronedarone Tablets. Because there is no existing *USP* monograph for this dosage form, a new monograph is proposed based on validated methods of analysis. The liquid chromatographic procedures for the *Assay* and *Organic Impurities* are based on analyses performed with the Zorbax Stable Bond-CN brand of L10 column. The typical retention time of dronedarone is about 7 min under the specified conditions for the *Assay* and *Organic Impurities*.

(SM2: S. Ramakrishna.)

Correspondence Number—C131625

Comment deadline: May 31, 2015

Add the following:

■ Dronedarone Tablets

DEFINITION

Dronedarone Tablets contain an amount of dronedarone hydrochloride equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of dronedarone free base (C₃₁H₄₄N₂O₅S).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the dronedarone peak in the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: Combine 2.0 mL of triethylamine with 1 L of water and adjust with phosphoric acid

to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (50:50)

System suitability stock solution: 0.2 mg/mL each of USP Dronedarone Hydrochloride RS and USP Dronedarone Related Compound A RS in methanol

System suitability solution: 0.01 mg/mL each of USP Dronedarone Hydrochloride RS and USP Dronedarone Related Compound A RS in *Mobile phase* from the *System suitability stock solution*

Standard stock solution: 2.13 mg/mL of USP Dronedarone Hydrochloride RS in methanol

Standard solution: 0.11 mg/mL of USP Dronedarone Hydrochloride RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: Nominally equivalent to 4 mg/mL of dronedarone in methanol prepared as follows. Dissolve and dilute in methanol to volume, an amount equivalent to 400 mg of dronedarone from NLT 20 finely powdered Tablets, taken in a 100-mL volumetric flask. Sonicate for about 5 min and allow to settle at room temperature.

Sample solution: Nominally equivalent to 0.1 mg/mL of dronedarone in *Mobile phase* from the *Sample stock solution*. Pass through a suitable filter of 0.45- μ m pore size and discard the first 3 mL of the filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detectors

Assay: UV 288 nm

Identification test B: UV diode array

Column: 4.6-mm \times 25-cm; 5- μ m packing L10

Flow rate: 0.8 mL/min

Injection volume: 20 μ L

Run time: NLT 2.15 times the retention time of dronedarone

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for dronedarone related compound A and dronedarone are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8 between dronedarone and dronedarone related compound A

Tailing factor: 0.8–2.1 for dronedarone

Relative standard deviation: NMT 1.5%, for dronedarone

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dronedarone free base ($C_{31}H_{44}N_2O_5S$) in the portion of Tablets taken:

$$(r_U/r_S) \times (C_S/C_U) \times (M_{r2}/M_{r1}) \times 100$$

r_U = peak response of dronedarone from the *Sample solution*

r_S = peak response of dronedarone from the *Standard solution*

C_S = concentration of USP Dronedarone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of dronedarone in the *Sample solution* (mg/mL)

M_{r2} = molecular weight of dronedarone hydrochloride, 593.22

$M_{r\bar{f}}$ molecular weight of dronedarone free base, 556.76

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• Dissolution 〈 711 〉

Medium: 13.61 g/L of monobasic potassium phosphate in water. Adjust with 0.1 M hydrochloric acid or 0.1 M sodium hydroxide as needed to a pH of 4.5; 1000 mL

Apparatus 2: 75 rpm, with sinker ring

Times: 30 and 90 min

Standard solution: 0.43 mg/mL of USP Dronedarone Hydrochloride RS prepared as follows.

Dissolve a suitable amount of USP Dronedarone Hydrochloride RS in 2% of the total volume of methanol and dilute with *Medium* to volume.

Sample solution: Pass a portion of sample under test through a suitable filter.

Instrumental conditions

Analytical wavelength: UV 288 nm

Cell: 1 mm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dronedarone dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times (M_{r2}/M_{r1}) \times 100$$

A_U = absorbance from the *Sample solution*

A_S = absorbance from the *Standard solution*

C_S = concentration of USP Dronedarone Hydrochloride RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 1000 mL

L = label claim (mg/Tablet)

M_{r2} molecular weight of dronedarone hydrochloride, 593.22

M_{r1} molecular weight of dronedarone free base, 556.76

Tolerances

30 min: 20.0%–60.0% (Q) of the labeled amount of dronedarone free base ($C_{31}H_{44}N_2O_5S$) is dissolved.

90 min: NLT 80% (Q) of the labeled amount of dronedarone free base ($C_{31}H_{44}N_2O_5S$) is dissolved.

• Uniformity of Dosage Units 〈 905 〉: Meet the requirements

IMPURITIES

• Organic Impurities

Buffer, Mobile phase, and System suitability stock solution: Proceed as directed in the *Assay*.

System suitability solution: 0.01 mg/mL each of USP Dronedarone Hydrochloride RS and USP Dronedarone Related Compound A RS prepared as follows. To a suitable amount of *System suitability stock solution*, add 20% of the total volume of methanol and dilute with

Mobile phase to volume.

Standard stock solution: 0.4 mg/mL of USP Dronedarone Hydrochloride RS in methanol

Standard solution: 0.002 mg/mL of USP Dronedarone Hydrochloride RS prepared as follows. To a suitable amount of *Standard stock solution*, add 25% of the total volume of methanol and dilute with *Mobile phase* to volume.

Sensitivity solution: 0.0005 mg/mL of USP Dronedarone Hydrochloride RS prepared as follows. To a suitable amount of the *Standard solution*, add 20% of the total volume of methanol and dilute with *Mobile phase* to volume.

Sample stock solution: Nominally equivalent to 4 mg/mL of dronedarone in methanol prepared as follows. Dissolve and dilute in methanol to volume, an amount equivalent to 400 mg of dronedarone from NLT 20 finely powdered Tablets, taken in a 100-mL volumetric flask. Sonicate for about 5 min and allow to settle at room temperature.

Sample solution: Nominally equivalent to 1 mg/mL of dronedarone in *Mobile phase* from *Sample stock solution*. Pass through a suitable filter of 0.45- μ m pore size and discard the first 3 mL of the filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 246 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L10

Flow rate: 0.8 mL/min

Injection volume: 20 μ L

Run time: NLT 3.6 times the retention time of dronedarone

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[Note—The relative retention times for dronedarone related compound A and dronedarone are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8 between dronedarone and dronedarone related compound A, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$(r_U/r_S) \times (C_S/C_U) \times (M_{r2}/M_{r1}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of dronedarone from the *Standard solution*

C_S = concentration of USP Dronedarone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of dronedarone in the *Sample solution* (mg/mL)

M_{r2} = molecular weight of dronedarone hydrochloride, 593.22

M_{r1} = molecular weight of dronedarone free base, 556.76

Acceptance criteria: Disregard peaks less than 0.05%.

Any unspecified impurity: NMT 0.20%

Total impurities: NMT 0.4%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store at controlled room temperature.
 - **USP Reference Standards** { 11 }
 - USP Dronedarone Hydrochloride RS
 - USP Dronedarone Related Compound A RS
 - N*-(2-Butyl-3-{4-[3-(butylamino)propoxy]benzoyl}benzofuran-5-yl)methanesulfonamide.
 - C₂₇H₃₆N₂O₅S 500.65
- 1S (USP39)

BRIEFING

Duloxetine Hydrochloride, USP 38 page 3243. It is proposed to revise the monograph as follows:

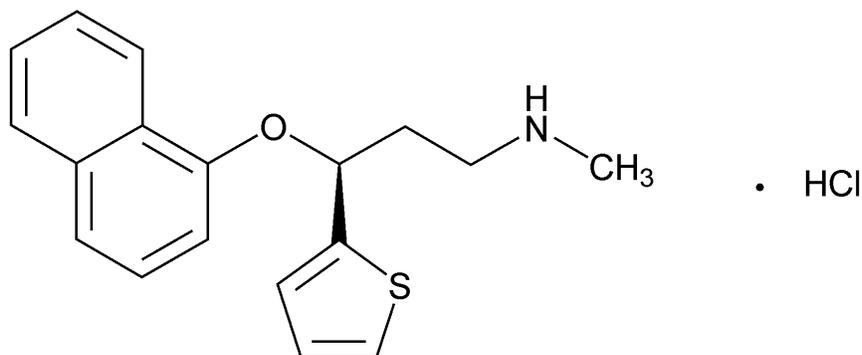
1. Revise the *System suitability solution* description within the *Assay* to clarify that duloxetine related compound F is present in USP Duloxetine Hydrochloride RS. USP has received questions about the source of duloxetine related compound F in this solution.
2. Replace the dashes and footnotes for several impurities in *Table 1* with the limit for any individual unspecified impurity.
3. Correct the chemical name for duloxetine β-naphthol-1-yl isomer.

Additionally, minor editorial changes have been made to update the monograph to current USP style

(SM4: H. Joyce.)

Correspondence Number—C125065; C150679

Comment deadline: May 31, 2015

Duloxetine Hydrochloride

C₁₈H₁₉NOS·HCl 333.88

2-Thiophenepropanamine, *N*-methyl-γ-(1-naphthalenyloxy)-, hydrochloride, (*S*)-;
 (+)-(*S*)-*N*-Methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride [136434-34-9].

DEFINITION

Duloxetine Hydrochloride contains NLT 97.0% and NMT 102.0% of duloxetine hydrochloride ($C_{18}H_{19}NOS \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈 197K 〉
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the duloxetine *S*-isomer from the *System suitability solution* in the test for *Limit of Duloxetine Related Compound A*.
- **C. Identification Tests—General, Chloride** 〈 191 〉
Sample solution: 5 mg/mL in methanol
Acceptance criteria: Meets the requirements

ASSAY

Change to read:

- **Procedure**

Protect solutions of duloxetine from light.

Buffer: 2.9 g/L of phosphoric acid in water. Adjust with sodium hydroxide solution to a pH of 2.5. To each L of this solution add 10.3 g of sodium 1-hexanesulfonate monohydrate, and dissolve.

Mobile phase: Acetonitrile, *n*-propanol, and *Buffer* (13:17:70)

Diluent: Acetonitrile and water (25:75)

System suitability solution: 0.2 mg/mL of USP Duloxetine Hydrochloride RS

■(contains duloxetine related compound F) ■1S (USP39)

in *Mobile phase*. Heat the solution to at least 40° for a minimum of 1 h. [~~Note—The resulting solution contains duloxetine impurity B, duloxetine impurity C, duloxetine impurity D, duloxetine impurity E, and duloxetine related compound F.~~

■The resulting solution contains duloxetine alcohol, duloxetine 4-naphthyl isomer, α -naphthol, duloxetine β -naphthol-1-yl isomer, and duloxetine related compound F. ■1S (USP39)

]

Standard solution: 0.1 mg/mL of USP Duloxetine Hydrochloride RS in *Diluent*

Sample solution: 0.1 mg/mL of Duloxetine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 3.5- μ m packing L7

Column temperature: 40 ± 3°

Flow rate: 1 mL/min

Injection volume: 10 μ L

Run time: 2 times the retention time of duloxetine

System suitability

Sample: *System suitability solution*

[~~Note—See *Table 1* for the relative retention times.~~]

Suitability requirements

Resolution: NLT 1.5 between duloxetine and duloxetine related compound F

Tailing factor: NMT 1.5 for duloxetine

Relative standard deviation: NMT 1.0% for duloxetine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of duloxetine hydrochloride ($C_{18}H_{19}NOS \cdot HCl$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Duloxetine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U concentration of Duloxetine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

Delete the following:

-

- **Heavy Metals, Method II** (231)

: NMT 10 ppm (Official 1-Dec-2015)

- **Residue On Ignition** (281) : NMT 0.2%

Change to read:

- **Organic Impurities**

Protect solutions of duloxetine from light.

Buffer, Mobile phase, Diluent, and System suitability solution: Proceed as directed in the *Assay*.

Sensitivity solution: 0.2 µg/mL of USP Duloxetine Hydrochloride RS in *Diluent*

Sample solution: 0.2 mg/mL of Duloxetine Hydrochloride in *Diluent*

Chromatographic system: Proceed as directed in the *Assay*, except for *Run time*.

Run time: 2.4 times the retention time of duloxetine

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between duloxetine impurity C and duloxetine impurity D;

■ duloxetine 4-naphthyl isomer and α-naphthol; ■ 1S (USP39)

NLT 1.5 between duloxetine and duloxetine related compound F, *System suitability solution*

Tailing factor: NMT 1.5 for duloxetine, *System suitability solution*

Relative standard deviation: NMT 1.0% for duloxetine, *System suitability solution*

Signal-to-noise ratio: NLT 20 for the duloxetine peak, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of any individual impurity in the portion of Duloxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_T sum of the responses of all the peaks from the *Sample solution*

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
Duloxetine impurity B ^{a,g}	0.15	0.36	—
Duloxetine impurity C ^{b,g}	0.43	1.0	—
Duloxetine impurity D ^{c,g}	0.48	1.8	—
Duloxetine impurity E ^{d,g}	0.74	1.0	—
Duloxetine	1.0	—	—
Duloxetine related compound F ^e	1.1	1.0	0.5
Duloxetine impurity G ^{f,g}	1.4	0.51	—
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.6

a 3-(Methylamino)-1-(thiophen-2-yl)propan-1-ol.
b 4-[3-(Methylamino)-1-(thiophen-2-yl)propyl]naphthalen-1-ol.
c Naphthalen-1-ol.
d 1-[3-(Methylamino)-1-(thiophen-2-yl)propyl]naphthalen-2-ol.
e (S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-3-yl)propan-1-amine.
f 1-Fluoronaphthalene.
g Controlled at *Any individual unspecified impurity* level.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
Duloxetine alcohol ^a	0.15	0.36	0.1
Duloxetine 4-naphthyl isomer ^b	0.43	1.0	0.1
α -Naphthol ^c	0.48	1.8	0.1
Duloxetine β -naphthol-1-yl isomer ^d	0.74	1.0	0.1
Duloxetine	1.0	—	—
Duloxetine related compound F ^e	1.1	1.0	0.5
Fluoronaphthalene ^f	1.4	0.51	0.1
Any individual unspecified impurity	—	1.0	0.1

a 3-(Methylamino)-1-(thiophen-2-yl)propan-1-ol.

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
Total impurities	—	—	0.6
a 3-(Methylamino)-1-(thiophen-2-yl)propan-1-ol.			
b 4-[3-(Methylamino)-1-(thiophen-2-yl)propyl]naphthalen-1-ol.			
c Naphthalen-1-ol.			
d 2-[3-(Methylamino)-1-(thiophen-2-yl)propyl]naphthalen-1-ol.			
e (S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-3-yl)propan-1-amine.			
f 1-Fluoronaphthalene.			

■ 1S (USP39)

● **Limit of Duloxetine Related Compound A**

Mobile phase: Hexane and isopropyl alcohol (83:17). To 1 L of this mixture add 2 mL of diethylamine.

System suitability solution: 0.1 mg/mL each of USP Duloxetine Hydrochloride RS and USP Duloxetine Related Compound A RS in *Mobile phase*. Sonication may be used to aid in dissolution.

Sensitivity solution: 0.1 µg/mL of USP Duloxetine Hydrochloride RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Duloxetine Hydrochloride in *Mobile phase*. Sonication may be used to aid in dissolution.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L40

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 2 times the retention time of duloxetine

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[Note—The relative retention times for duloxetine and duloxetine related compound A are 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 3.5 between duloxetine and duloxetine related compound A, *System suitability solution*

Tailing factor: 0.8–1.5 each for duloxetine and duloxetine related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0% for duloxetine, *System suitability solution*

Signal-to-noise ratio: NLT 3, *Sensitivity solution*

Analysis**Sample:** *Sample solution*

Calculate the percentage of duloxetine related compound A in the portion of Duloxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of duloxetine related compound A from the *Sample solution*

r_T sum of the peak responses of duloxetine and duloxetine related compound A from the *Sample solution*

Acceptance criteria: NMT 0.5%**SPECIFIC TESTS**

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 3 h.**Acceptance criteria:** NMT 0.5%**ADDITIONAL REQUIREMENTS**

- **Packaging and Storage:** Protect from light. Store at room temperature.

- **USP Reference Standards** 〈 11 〉

USP Duloxetine Hydrochloride RS USP Duloxetine Related Compound A RS

(*R*)-*N*-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propan-1-amine hydrochloride.

C₁₈H₁₉NOS·HCl 333.88

BRIEFING

Edetate Disodium, *USP 38* page 3263. As part of USP monograph modernization efforts, the following changes are proposed:

1. *Identification* test *A* is revised to remove the redundant requirement to use an undried sample. Since the label for USP Edetate Disodium RS states "Do not dry", the general chapter *Spectrophotometric Identification Tests* 〈 197 〉 indicates that the sample should also be undried.
2. In preparation for the omission of the flame tests from general chapter *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to replace the use of the flame test in *Identification* test *C* with the pyroantimonate precipitation test currently described in this general chapter. This test is also consistent with test (a) in the 2.3.1 *Identification reactions of ions and functional groups* in *European Pharmacopoeia*, and is employed in the *European Pharmacopoeia* monograph for *Edetate Disodium*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: R.S. Prasad.)

Correspondence Number—C154665

Comment deadline: May 31, 2015**Edetate Disodium** $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ 372.24 $C_{10}H_{14}N_2Na_2O_8$ 336.21Glycine, *N,N'*-1,2-ethanediybis[*N*-(carboxymethyl)-, disodium salt, dihydrate;

Disodium (ethylenedinitrilo)tetraacetate dihydrate [6381-92-6].

Anhydrous [139-33-3].

DEFINITION

Edetate Disodium contains NLT 99.0% and NMT 101.0% of edetate disodium ($C_{10}H_{14}N_2Na_2O_8$), calculated on the dried basis.

IDENTIFICATION***Change to read:***

- **A. Infrared Absorption** 〈 197K〉

~~**Sample:** Undried~~~~**Acceptance criteria:** Meets the requirements~~

- **1S** (*USP39*)

- **B.**

Sample: 50 mg**Analysis:** To 5 mL of water in a test tube add 2 drops of ammonium thiocyanate TS and 2 drops of ferric chloride TS. To the deep red solution add the *Sample*.**Acceptance criteria:** The red color is discharged, leaving a yellowish solution.***Change to read:***

- **C. Identification Tests—General, Sodium** 〈 191〉: It meets the requirements of the flame

- pyroantimonate precipitation ■ **1S** (*USP39*)

test.

ASSAY

- **Procedure**

Sample solution: Dissolve 5 g of Edetate Disodium in about 100 mL of water contained in a 250-mL volumetric flask. Add water to volume.**Analysis:** Place 200 mg of chelometric standard calcium carbonate, previously dried at 110 ° for 2 h and cooled in a desiccator, into a 400-mL beaker. Add 10 mL of water, and swirl to form a slurry. Cover the beaker with a watch glass, and without removing the latter, add 2 mL of 3 N hydrochloric acid from a pipet. Swirl the contents of the beaker, and dissolve the calcium carbonate. With water, wash down the sides of the beaker, the outer surface of the pipet, and the watch glass, and dilute with water to 100 mL. While stirring the solution, preferably with a magnetic stirrer, add 30 mL of the *Sample solution* from a

50-mL buret. Add 15 mL of 1 N sodium hydroxide and 0.30 g of hydroxy naphthol blue, and continue the titration with the *Sample solution* to a blue endpoint.

Calculate the weight of edetate disodium ($C_{10}H_{14}N_2Na_2O_8$) in the portion of Edetate Disodium taken:

$$\text{Result} = (V_T/V_U) \times W \times (M_{r1}/M_{r2})$$

V_T = total volume of the *Sample solution* (mL)

V_U = volume of the *Sample solution* consumed in the titration (mL)

W = weight of calcium carbonate (mg)

M_{r1} = molecular weight of edetate disodium, 336.21

M_{r2} = molecular weight of calcium carbonate, 100.09

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

Delete the following:

-

• Heavy Metals, Method II (231)

: NMT 50 ppm (Official 1-Dec-2015)

- **Calcium**

Sample solution: 1 g of Edetate Disodium in 20 mL of water

Analysis: To the *Sample solution* add 2 drops of methyl red TS, and neutralize with 6 N ammonium hydroxide. Add 3 N hydrochloric acid dropwise until the solution is just acidic, and then add 1 mL of ammonium oxalate TS.

Acceptance criteria: No precipitate is formed.

- **Limit of Nitritotriacetic Acid**

Mobile phase: Add 10 mL of 1.0 M tetrabutylammonium hydroxide in methanol to 200 mL of water, and adjust with 1 M phosphoric acid to a pH of 7.5 ± 0.1 . Transfer the solution so obtained to a 1000-mL volumetric flask. Add 90 mL of methanol, and dilute with water to volume. Pass through a filter of 0.5- μ m or finer pore size, and degas.

Cupric nitrate solution: 10 mg/mL of cupric nitrate [$Cu(NO_3)_2$]

Standard stock solution: Transfer 100 mg of nitritotriacetic acid to a 10-mL volumetric flask. Add 0.5 mL of ammonium hydroxide, mix, and dilute with water to volume.

Standard solution: Transfer 1.0 g of Edetate Disodium to a 100-mL volumetric flask. Add 100 μ L of *Standard stock solution*, and dilute with *Cupric nitrate solution* to volume. If necessary, sonicate to dissolve.

System suitability solution: Transfer 10 mg of Edetate Disodium to a 100-mL volumetric flask. Add 100 μ L of *Standard stock solution*, and dilute with *Cupric nitrate solution* to volume. If necessary, sonicate to dissolve.

Sample solution: 10 mg/mL of Edetate Disodium in *Cupric nitrate solution*. If necessary, sonicate to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; packing L7

Flow rate: 2 mL/min

Injection volume: 50 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for nitrilotriacetic acid, copper, and edetate are about 0.35, 0.65, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3 between nitrilotriacetic acid and copper, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: NMT 0.1%; the response of the nitrilotriacetic acid peak of the *Sample solution* does not exceed the difference between the nitrilotriacetic acid peak responses from the *Standard solution* and the *Sample solution*.

SPECIFIC TESTS

• **pH** 〈 791 〉

Sample solution: 50 mg/mL

Acceptance criteria: 4.0–6.0

• **Loss on Drying** 〈 731 〉

Analysis: Dry at 150° for 6 h.

Acceptance criteria: 8.7%–11.4%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

• **USP Reference Standards** 〈 11 〉

USP Edetate Disodium RS

BRIEFING

Esmolol Hydrochloride, *USP 38* page 3368 and *PF 40(3)* [May–June 2014]. On the basis of comments received, it is proposed to revise the *pH* section to include the preparation of the *Sample solution*.

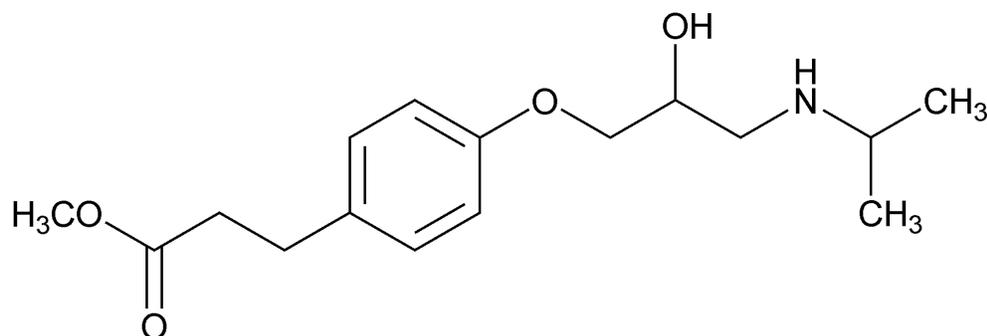
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: S. Ramakrishna.)

Correspondence Number—C155412

Comment deadline: May 31, 2015

Esmolol Hydrochloride



• HCl

$C_{16}H_{25}NO_4 \cdot HCl$ 331.83

Benzenepropanoic acid, 4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-, methyl ester, hydrochloride, (\pm);

(\pm)-Methyl *p*-[2-hydroxy-3-(isopropylamino)propoxy]hydrocinnamate hydrochloride [81161-17-3].

DEFINITION

Esmolol Hydrochloride contains NLT 98.0% and NMT 102.0% of esmolol hydrochloride ($C_{16}H_{25}NO_4 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** \langle 197K \rangle
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

Buffer: Dissolve 3.0 g of potassium dihydrogen phosphate in 650 mL of water.

Mobile phase: Acetonitrile, methanol, and *Buffer* (150:200:650)

System suitability stock solution: 1 mg/mL of USP Esmolol Hydrochloride RS prepared as follows. Transfer a suitable quantity of USP Esmolol Hydrochloride RS to a suitable volumetric flask, and dissolve in and dilute with 1 N hydrochloric acid to volume. Allow the contents to stand for at least 30 min. [Note—This results in the partial degradation of the esmolol resulting in the production of esmolol free acid (see *System suitability* for the relative retention times).]

System suitability solution: 0.2 mg/mL of USP Esmolol Hydrochloride RS in water from *System suitability stock solution*

Standard solution: 200 μ g/mL of USP Esmolol Hydrochloride RS in water

Sample solution: 200 μ g/mL of Esmolol Hydrochloride in water

Chromatographic system

(See *Chromatography* \langle 621 \rangle , *System Suitability*.)

Mode: LC

Detector: UV 222 nm

Column: 3.9-mm \times 30-cm; 10- μ m packing L1

Flow rate: 2 mL/min

Injection volume: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for esmolol free acid and esmolol are 0.41 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between esmolol free acid and esmolol, *System suitability solution*

Tailing factor: NMT 2.0 for the esmolol peak, *System suitability solution*

Relative standard deviation: NMT 2.0%

■ 0.73%, ■ 1S (USP38)

Standard solution

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of esmolol hydrochloride ($C_{16}H_{25}NO_4 \cdot HCl$) in the portion of the Esmolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of esmolol from the *Sample solution*

r_S = peak response of esmolol from the *Standard solution*

C_S = concentration of USP Esmolol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Esmolol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Delete the following:

•

• **Heavy Metals** { 231 }

Standard solution: Into a 50-mL color-comparison tube pipet 2 mL of *Standard Lead Solution* [20 μ g of lead (Pb)], and dilute with water to 25 mL. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid to a pH between 3.0 and 4.0, dilute with water to 40 mL, and mix.

Sample solution: Into a 50-mL color-comparison tube dissolve 1 g of the sample in water, and dilute with water to 25 mL. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid to a pH between 3.0 and 4.0, dilute with water to 40 mL, and mix.

Analysis

Samples: *Standard solution* and *Sample solution*

To each of the tubes add 10 mL of hydrogen sulfide TS, and mix. Allow to stand for 2 min. View downward into the tube over a white background.

Acceptance criteria: The color of the *Sample solution* is not darker than the color of the *Standard solution* (NMT 20 ppm). • (Official 1-Dec-2015)

- **Residue on Ignition** (281): NMT 0.1%
- **Organic Impurities**

Buffer and System suitability solution: Proceed as directed in the *Assay*.

Solution A: Methanol

Solution B: Proceed as directed for *Mobile phase* in the *Assay*.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	0	100
20	0	100
25	25	75
35	25	75
36	0	100
40	0	100

Sample solution: 1 mg/mL of Esmolol Hydrochloride in water

Chromatographic system: Proceed as directed in the *Assay*, except include a column temperature of 30°.

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 4.0 between esmolol free acid and esmolol

Tailing factor: NMT 2.0 for the esmolol peak

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual impurity in the portion of Esmolol Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of each individual impurity from the *Sample solution*

r_T sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Esmolol free acid ^a	0.43	0.4
Esmolol isopropylamide analog ^b (if present)	0.65	0.25
<i>N</i> -Ethyl esmolol ^c (if present)	0.84	0.15
Esmolol	1.0	—
Esmolol dimer ^d	6.5	0.5

Any other individual unspecified impurity	—	0.10
Total impurities	—	1.0
a	3-{4-[2-Hydroxy-3-(isopropylamino)propoxy]phenyl}propanoic acid.	
b	3-{4-[2-Hydroxy-3-(isopropylamino)propoxy]phenyl}- <i>N</i> -isopropylpropionamide.	
c	Methyl 3-{4-[3-(ethylamino)-2-hydroxypropoxy]phenyl}propionate.	
d	Methyl 3-{4-[2-hydroxy-3-(3-{4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl}- <i>N</i> -isopropylpropanamido)propoxy]phenyl}propanoate.	

SPECIFIC TESTS

Change to read:

- pH \langle 791 \rangle

■ **Sample solution:** 100 mg/mL of Esmolol Hydrochloride in water ■ 1S (USP39)

Acceptance criteria: 3.0–5.0

- **Water Determination, Method Ia** \langle 921 \rangle : NMT 1.0%

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Protect from freezing, and store at controlled

■ 1S (USP38)

room temperature.

- **USP Reference Standards** \langle 11 \rangle

USP Esmolol Hydrochloride RS

Benzenepropanoic acid, 4-[2-hydroxy-3-[(1-methylethyl)-amino]propoxy]-, methyl ester, hydrochloride, (\pm)-.

C₁₆H₂₅NO₄·HCl 331.83

BRIEFING

Ethambutol Hydrochloride Compounded Oral Suspension. Because there is currently no existing *USP* monograph for this dosage form, a new compounded preparation monograph is proposed based on a validated stability-indicating method used to assess stability. The liquid chromatographic procedure in the *Assay* is based on analyses validated using the Zorbax StableBond-CN brand of L10 column. The typical retention time for ethambutol hydrochloride is about 3.7 min.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C154508

Comment deadline: May 31, 2015

Add the following:

■ **Ethambutol Hydrochloride Compounded Oral Suspension**

DEFINITION

DEFINITION

Ethambutol Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$).

Prepare Ethambutol Hydrochloride Compounded Oral Suspension 100 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈 795 〉).

Ethambutol Hydrochloride tablets ^a equivalent to	3 g of ethambutol hydrochloride
Vehicle: a 1:1 mixture of Ora-Plus ^b and Ora-Sweet SF ^b , a sufficient quantity to make	30 mL
^a Ethambutol Hydrochloride 100-mg tablet(s), Lupin Pharmaceuticals, Baltimore, MD.	

^b Perrigo Laboratories, Allegan, MI.

Place the required number of tablets in a suitable container and comminute to a fine powder. Wet the powder with a small amount of *Vehicle* and triturate to make a smooth paste. Add the *Vehicle* to make the contents pourable. Transfer the contents stepwise and quantitatively to a calibrated container using the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY

- Procedure**

Solution A: 0.1% Triethylamine in water, adjusted with phosphoric acid to a pH of 7.0.

Pass through a nylon filter of 0.45- μ m pore size and degas.

Mobile phase: Acetonitrile and *Solution A* (50:50)

Diluent: 1.4 g/L of sodium phosphate in water adjusted with phosphoric acid to a pH of 6.8

Standard stock solution: 10 mg/mL of USP Ethambutol Hydrochloride RS in *Diluent*.

Sonicate to mix well. Store at 2°–8°.

Standard solution: Transfer 0.25 mL of the *Standard stock solution* to a 5-mL volumetric flask, dilute with *Diluent* to volume, and mix well. Transfer an aliquot to a centrifuge tube, and centrifuge for 5 min at 14,000 rpm at 2°–8°. Transfer the supernatant to an amber vial and store at 2°–8°.

Sample solution: Transfer 2 mL of Oral Suspension to a 20-mL volumetric flask, dilute with *Diluent* to volume, and sonicate to mix well. Transfer 0.25 mL of the resultant solution to a 5-mL volumetric flask, dilute with *Diluent* to volume, and mix well. Transfer an aliquot to a centrifuge tube, and centrifuge for 5 min at 14,000 rpm at 2°–8°. Transfer the supernatant to an amber vial and store at 2°–8°.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV-Vis 200 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L10

Temperatures

Autosampler: 5°

Column: 30°

Flow rate: 1.3 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

[Note—The retention time for ethambutol hydrochloride is about 3.7 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of ethambutol hydrochloride from the *Sample solution*

r_S peak response of ethambutol hydrochloride from the *Standard solution*

C_S concentration of USP Ethambutol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of ethambutol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** 〈 791 〉: 3.5–5.6

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant plastic containers. Store in a refrigerator (2°–8°) or at controlled room temperature.
 - **Beyond-Use Date:** NMT 90 days after the date on which it was compounded when stored in a refrigerator (2°–8°) or at controlled room temperature
 - **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
 - **USP Reference Standards** 〈 11 〉
USP Ethambutol Hydrochloride RS
- 1S (USP39)

BRIEFING

Etidronate Disodium, *USP 38* page 3420. In preparation for the omission of the flame tests from general chapter *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to replace the use of the flame test in *Identification* test *B* with the pyroantimonate precipitation test currently described in this general chapter. This test is also consistent with test (a) in 2.3.1 *Identification reactions of ions and functional groups* in the *European Pharmacopoeia*, and is employed in the *European Pharmacopoeia* monograph for *Etidronate Disodium*.

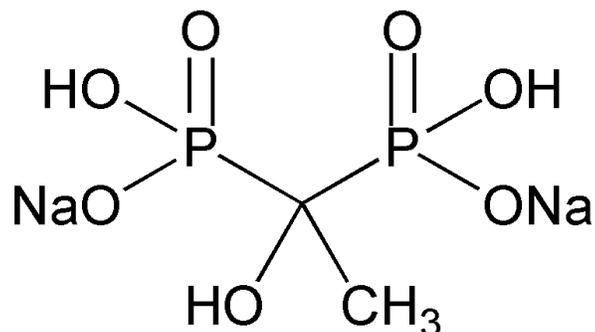
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM3: R.S. Prasad.)

Correspondence Number—C154667

Comment deadline: May 31, 2015

Etidronate Disodium



$C_2H_6Na_2O_7P_2$ 249.99

Phosphonic acid, (1-hydroxyethylidene)bis-, disodium salt;
Disodium dihydrogen (1-hydroxyethylidene)diphosphonate [7414-83-7].

DEFINITION

Etidronate Disodium contains NLT 97.0% and NMT 101.0% of etidronate disodium ($C_2H_6Na_2O_7P_2$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈 197 〉: The spectra of trifluorovinyl chloride polymer and mineral oil dispersions of it, separately prepared from a test specimen recrystallized from water and dried at 105° for 1 h, exhibit maxima in the regions of 4000–1350 cm^{-1} and 1350–450 cm^{-1} , respectively, only at the same wavelengths as those of similar preparations of USP Etidronate Disodium RS.

Change to read:

- **B. Identification Tests—General, Sodium** 〈 191 〉: Meets the requirements for the flame
■ pyroantimonate precipitation ■_{1S} (USP39)

test

ASSAY

Change to read:

- **Procedure**

Mobile phase: 35–40 mM ammonium nitrate solution in water. Adjust with dilute ammonium hydroxide to a pH of 7.0.

Standard solution: 0.73–0.75 mg/mL of USP Etidronic Acid Monohydrate RS in 1 N sodium

hydroxide solution and *Mobile phase* (1:150)

Sample solution: 0.84–0.86 mg/mL of Etidronate Disodium in *Mobile phase*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 4.6-mm × 150-mm; packing L23

Temperatures

Detector: 32°

Column: 32°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 1.5% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of etidronate disodium (C₂H₆Na₂O₇P₂) in the portion of Etidronate Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Etidronic Acid Monohydrate RS in the *Standard solution* (mg/mL)

C_U = concentration of Etidronate Disodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of etidronate disodium, ~~250.00~~

■ 249.99 ■ 1S (USP39)

M_{r2} = molecular weight of etidronic acid monohydrate, 224.05

Acceptance criteria: 97.0%–101.0% on the anhydrous basis

IMPURITIES

Delete the following:

•

• **Heavy metals, Method II** { 231 }

Standard solution: 0.5 g of USP Etidronate Disodium RS

Standard solution: 2.5 mL of *Standard Lead Solution*

Analysis: Transfer the *Sample solution* and the *Standard solution* to separate quartz crucibles, add 0.5 g of magnesium oxide to each crucible, and mix. Evaporate the *Standard solution* to dryness at 110° for 1 h, and ignite each crucible over a flame to a light gray color. Ignite at 800° for 1 h, cool, and dissolve the residues by the dropwise addition of hydrochloric acid, and add 3 mL of water. Adjust with ammonia TS to a pH of 8.5, then adjust with acetic acid to a pH of 4. Make a final pH adjustment to 3.4 ± 0.05,

using dilute hydrochloric acid. Filter into 50-mL color comparison tubes, and dilute with water to 40 mL.

Acceptance criteria: NMT 50 ppm (Official 1-Dec-2015)

SPECIFIC TESTS

• Limit of Phosphite

Solution A: 0.65 mg/mL of anhydrous sodium carbonate and 0.40 mg/mL of sodium bicarbonate in water

Solution B: 4.68 mg/mL of anhydrous sodium carbonate and 2.89 mg/mL of sodium bicarbonate in water

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
6.0	100	0
6.1	0	100
8.0	0	100
8.1	100	0
15	100	0

Suppressor regenerant solution: 12.5 mM sulfuric acid. [Note—This solution is needed only if the chemical suppression option is used.]

Standard solution: Equivalent to 0.016 mg/mL of dibasic sodium phosphite on the anhydrous basis from USP Etidronate Disodium Related Compound A RS and 0.015 mg/mL of dibasic sodium phosphate in *Solution A*. [Note—USP Etidronate Disodium Related Compound A RS is sodium phosphite dibasic pentahydrate.]

Sample solution: 5.0 mg/mL of Etidronate Disodium in *Solution A*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Conductivity

Columns

Guard: 4-mm × 50-mm; packing L61. [Note—Use either a 4-mm anion self-regenerating suppressor or a suitable chemical suppressor.]

Analytical: 4-mm × 25-cm; packing L61

Flow rate: 1.0 mL/min. [Note—When a chemical suppressor is used, the flow rate is 3–5 mL/min for the *Suppressor regenerant solution*.]

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[Note—The elution order is phosphite, followed by phosphate.]

Suitability requirements

Resolution: NLT 2.5 between phosphite and phosphate

Relative standard deviation: NMT 10% for each peak, for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of phosphite, determined as monobasic sodium phosphite, in the portion of Etidronate Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of phosphite from the *Sample solution*

r_S = peak response of phosphite from the *Standard solution*

C_S = concentration of USP Etidronate Disodium Related Compound A RS on the anhydrous basis in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

M_{r1} = molecular weight of monobasic sodium phosphite, 103.98

M_{r2} = molecular weight of dibasic sodium phosphite, 125.96

Acceptance criteria: NMT 1.0% of phosphite, determined as monobasic sodium phosphite

- **pH** 〈 791 〉

Sample solution: 10 mg/mL

Acceptance criteria: 4.2–5.2

- **Water Determination, Method Ic** 〈 921 〉

Sample: 100 mg of finely powdered Etidronate Disodium in 10 mL of a mixture of acetic acid and formamide (1:1)

Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers

- **USP Reference Standards** 〈 11 〉

USP Etidronate Disodium RS

USP Etidronate Disodium Related Compound A RS

Sodium phosphite dibasic pentahydrate.

$\text{Na}_2\text{HPO}_3 \cdot 5\text{H}_2\text{O}$ 216.04

USP Etidronic Acid Monohydrate RS

$\text{C}_2\text{H}_8\text{O}_7\text{P}_2 \cdot \text{H}_2\text{O}$ 224.04

BRIEFING

Fluorometholone, *USP 38* page 3547 and *PF 40(1)* [Jan.–Feb. 2014]. The previously published proposal is canceled and replaced with the following proposal based on the comments received:

1. The *Identification test B* and *Identification test C* are replaced with an *Identification test* based on HPLC retention time agreement from the *Assay*.
2. A new HPLC-based *Organic Impurities* procedure is added based on analyses performed using the Symmetry C18 brand of L1 column. The current *Assay* procedure is replaced with a similar procedure to that used in the test for *Organic Impurities*. The typical retention time for fluorometholone is about 29 min.
3. The test and *Acceptance criteria* for *Specific Rotation* are revised to eliminate the use of pyridine, which is a safety hazard.
4. A new Reference Standard is added to the *USP Reference Standards* section to support the revision.

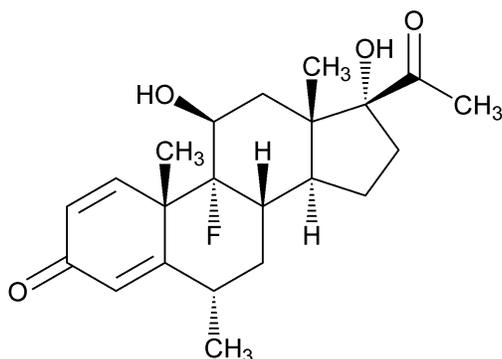
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: D. Vicchio, H. Cai.)

Correspondence Number—C145312

Comment deadline: May 31, 2015

Fluorometholone



$C_{22}H_{29}FO_4$ 376.46

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-6-methyl-, (6 α ,11 β)-;
9-Fluoro-11 β ,17-dihydroxy-6 α -methylpregna-1,4-diene-3,20-dione [426-13-1].

DEFINITION

Fluorometholone contains NLT 97.0% and NMT 103.0% of fluorometholone ($C_{22}H_{29}FO_4$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)

Delete the following:

- **B. Ultraviolet Absorption** (197U)

Analytical wavelength: 239 nm

Sample solution: 10 $\mu\text{g}/\text{mL}$ in methanol

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%. ■1S (USP39)

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

Delete the following:

- **C. Thin-Layer Chromatography**

Standard solution: 500 $\mu\text{g}/\text{mL}$ of USP Fluorometholone RS in methanol

Sample solution: 500 µg/mL of Fluorometholone in methanol

Chromatographic system

(See *Chromatography* ~~(621)~~ *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 100 µL

Developing solvent system: Methylene chloride and acetone (4:1)

Visualization: Short-wavelength UV

Analysis

Samples: *Standard solution and Sample solution*

Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. ■1S (USP39)

ASSAY

Change to read:

● **Procedure**

Mobile phase: Prepare a suitable filtered solution of methanol and water (60:40) such that the retention time of fluorometholone is about 3 min.

Standard solution: 100 µg/mL of USP Fluorometholone RS in *Mobile phase*

Sample solution: 100 µg/mL of Fluorometholone in *Mobile phase*

Chromatographic system

(See *Chromatography* ~~(621)~~, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6 mm × 25 cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of fluorometholone ($C_{22}H_{29}FO_4$) in the portion of Fluorometholone taken:

$$\text{Result} = r_U/r_S \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

~~r_S peak response from the *Standard solution*~~

~~C_S concentration of USP Fluorometholone RS in the *Standard solution* ($\mu\text{g/mL}$)~~

~~C_U concentration of Fluorometholone in the *Sample solution* ($\mu\text{g/mL}$)~~

Acceptance criteria: ~~97.0%–103.0% on the dried basis~~

■ **Mobile phase:** Mix 425 g (538 mL) of methanol and 400 g (400 mL) of water. Adjust with phosphoric acid to a pH of 2.4.

Standard stock solution: 0.25 mg/mL of USP Fluorometholone RS prepared as follows.

Transfer a suitable amount of USP Fluorometholone RS to a suitable volumetric flask and dissolve in 2% of the final volume of tetrahydrofuran. Dilute with *Mobile phase* to volume.

Standard solution: 50 $\mu\text{g/mL}$ of USP Fluorometholone RS in *Mobile phase* from *Standard stock solution*

Sample stock solution: 0.25 mg/mL of Fluorometholone prepared as follows. Transfer a suitable amount of Fluorometholone to a suitable volumetric flask and dissolve in 2% of the final volume of tetrahydrofuran. Dilute with *Mobile phase* to volume.

Sample solution: 50 $\mu\text{g/mL}$ of Fluorometholone in *Mobile phase* from *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L1

Flow rate: 0.6 mL/min

Injection volume: 20 μL

Run time: NLT 1.6 times the retention time of fluorometholone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.3

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fluorometholone ($\text{C}_{22}\text{H}_{29}\text{FO}_4$) in the portion of Fluorometholone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of fluorometholone from the *Sample solution*

r_S

= peak response of fluorometholone from the *Standard solution*

C_S

= concentration of USP Fluorometholone RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= concentration of Fluorometholone in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 97.0%–103.0% on the dried basis \blacksquare_{1S} (USP39)

IMPURITIES

- **Residue on Ignition** $\langle 281 \rangle$: NMT 0.2%

Add the following:

• Organic Impurities

Mobile phase: Proceed as directed in the *Assay*.

System suitability stock solution: 0.5 mg/mL each of USP Fluorometholone RS and USP Fluorometholone Related Compound A RS prepared as follows. Transfer a suitable amount of USP Fluorometholone RS and USP Fluorometholone Related Compound A RS to a suitable volumetric flask and dissolve in 4% of the final volume of tetrahydrofuran. Dilute with *Mobile phase* to volume.

System suitability solution: 0.005 mg/mL each of USP Fluorometholone RS and USP Fluorometholone Related Compound A RS in *Mobile phase* from *System suitability stock solution*

Standard stock solution: 0.5 mg/mL of USP Fluorometholone RS prepared as follows. Transfer a suitable amount of USP Fluorometholone RS to a suitable volumetric flask and dissolve in 4% of the final volume of tetrahydrofuran. Dilute with *Mobile phase* to volume.

Standard solution: 0.5 $\mu\text{g/mL}$ of USP Fluorometholone RS in *Mobile phase* from *Standard stock solution*

Sample solution: 0.5 mg/mL of Fluorometholone prepared as follows. Transfer a suitable amount of Fluorometholone to a suitable volumetric flask and dissolve in 4% of the final volume of tetrahydrofuran. Dilute with *Mobile phase* to volume.

Chromatographic system: Proceed as directed in the *Assay* except for the *Run time*.

Run time: NLT 2.1 times the retention time of fluorometholone

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3 between fluorometholone and fluorometholone related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Fluorometholone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_S peak response of fluorometholone from the *Standard solution*

C_S concentration of USP Fluorometholone RS in the *Standard solution* (mg/mL)

C_T concentration of Fluorometholone in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peaks below 0.02%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fluorometholone	1.0	—
Fluorometholone related compound A	1.2	0.5
Individual unspecified impurities	—	0.10
Total impurities	—	1.0

■ 1S (USP39)

SPECIFIC TESTS

Change to read:

- **Optical Rotation, Specific Rotation** 〈 781S 〉

Sample solution: 10 mg/mL, in pyridine

■ 10 mg/mL in dimethyl sulfoxide ■ 1S (USP39)

Acceptance criteria: $+52^{\circ}$ to $+60^{\circ}$

■ $+62^{\circ}$ to $+70^{\circ}$ ■ 1S (USP39)

- **Loss on Drying** 〈 731 〉

Analysis: Dry under vacuum at 60° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Fluorometholone RS

■ USP Fluorometholone Related Compound A RS

11 β ,17 α -Dihydroxy-6 α -methylpregna-1,4-diene-3,20-dione.

$C_{22}H_{30}O_4$ 358.47 ■ 1S (USP39)

BRIEFING

Glyburide and Metformin Hydrochloride Tablets, USP 38 page 3689. On the basis of comments received, it is proposed to make the following changes for clarity:

1. Add a *Note* in the *Assay for Metformin Hydrochloride* to allow changes in mobile phase composition to optimize the separation.
2. Delete the requirements for *Capacity factor* and *Column efficiency* from *System suitability* in the *Assay for Glyburide*, because the remaining requirements are adequate to evaluate the system suitability.
3. Delete the requirements for *Column efficiency* from *System suitability* in the *Dissolution* test for *Glyburide*, because the remaining requirements are adequate to evaluate the system suitability.
4. Update the calculation formula under *Glyburide* in *Impurities* to be consistent with *USP* style by moving the relative response factors from the numerator into the denominator, and to recalculate the *F* value for glyburide related compound A.
5. Under the *Dissolution* test, revise the concentrations of the *Standard solution* and *Sample solution* for consistency.
6. Under the *Dissolution* test for *Metformin hydrochloride*, indicate that the *Standard solution* may be diluted with *Medium*, if necessary, and revise the calculation to include dilution factor *D*.
7. The molecular formula and molecular weight for *USP Glyburide Related Compound A* *RS* are added in the *USP Reference Standards* section for clarity and uniformity.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: R.S. Prasad.)

Correspondence Number—C152098

Comment deadline: May 31, 2015

Glyburide and Metformin Hydrochloride Tablets

DEFINITION

Glyburide and Metformin Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of glyburide ($C_{23}H_{28}ClN_3O_5S$) and metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$).

IDENTIFICATION

- **A. Glyburide:** The retention time of the glyburide peak of the *Sample solution* corresponds to that of the major peak of the *Standard solution*, as obtained in the *Assay for Glyburide*.
- **B. Metformin Hydrochloride:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Metformin Hydrochloride*.

ASSAY

Change to read:

- **Glyburide**

Solution A:

■ **Buffer:** ■ 1S (*USP39*)

28.8 g/L of monobasic ammonium phosphate

Mobile phase: Acetonitrile and *Solution A*

■ **Buffer:** ■ 1S (*USP39*)

(40:60). Adjust with 1 N sodium hydroxide to a pH of 5.3.

Diluent: Acetonitrile and water (50:50)

Standard stock solution: 0.25 mg/mL of USP Glyburide RS prepared as follows.

■ Transfer a weighed amount of USP Glyburide RS to a suitable volumetric flask. ■ 1S (USP39)

Dissolve first in the acetonitrile, using 50% of the final volume, and then dilute with water to volume.

Standard solution: 0.025 mg/mL of USP Glyburide RS in *Diluent*, from the *Standard stock solution*

System suitability solution 1: Prepare a solution containing 0.025 mg/mL of USP Glyburide Related Compound A RS in *Diluent*. Transfer 50 µL of this solution to a 50-mL volumetric flask, and dilute with *Standard solution* to volume.

System suitability solution 2: 5.0 mg/mL of USP Metformin Hydrochloride RS in *System suitability solution 1*

Sample solution: Dissolve NLT 5 Tablets in *Diluent* by stirring with a magnetic stirring bar for at least 1 h. Dilute to obtain a solution containing 0.025 mg/mL of glyburide, based on the label claim. Centrifuge a portion of this solution at 3000 rpm for 10 min and use the clear supernatant. [Note—Retain a portion of this solution for the *Assay for Metformin Hydrochloride*.]

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 100 µL

■ **Run time:** 1.25 times the retention time of glyburide ■ 1S (USP39)

System suitability

Sample: *System suitability solution 2*

[Note—The relative retention time for glyburide related compound A is about 0.30 with respect to glyburide.]

Suitability requirements

Capacity factor, k': NLT 7 for the peak due to glyburide

Column efficiency: NLT 3000 theoretical plates for the peak due to glyburide

■ 1S (USP39)

Relative standard deviation: NMT 1.5% for the glyburide peak; NMT 10% for the glyburide related compound A peak

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms for about 1.25 times the retention time of the glyburide peak.

■ 1S (USP39)

Calculate the percentage of the labeled amount of glyburide (C₂₃H₂₈ClN₃O₅S) in the portion of

Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response

■ of glyburide ■_{1S} (USP39)
from the *Sample solution*

r_S = peak response

■ of glyburide ■_{1S} (USP39)
from the *Standard solution*

C_S = concentration of USP Glyburide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of glyburide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of glyburide

Change to read:

● **Metformin Hydrochloride**

Solution A:

■ **Buffer:** ■_{1S} (USP39)

Transfer 1.0 g each of sodium heptanesulfonate and sodium chloride to a 2000-mL volumetric flask. Add 1800 mL of water, and adjust with 0.06 M phosphoric acid to a pH of 3.85. Dilute with water to volume.

Mobile phase: Acetonitrile and *Solution A*

■ *Buffer* ■_{1S} (USP39)

(10:90)

■ [

Note—To improve the separation, the composition of acetonitrile and *Buffer* may be changed to 5:95, if necessary.] ■_{1S} (USP39)

Diluent: Acetonitrile and water (1:40)

Standard solution: 0.25 mg/mL of USP Metformin Hydrochloride RS in *Diluent*. [Note—Sonicate to achieve complete dissolution, if necessary.]

System suitability stock solution: 25 µg/mL each of USP Metformin Related Compound B RS and USP Metformin Related Compound C RS in *Diluent*

System suitability solution: Transfer 0.5 mL of the *System suitability stock solution* to a 50-mL volumetric flask, and dilute with *Standard solution* to volume.

Sample solution: Dilute with water a portion of the retained *Sample solution* from the *Assay for Glyburide* to obtain 0.25 mg/mL of metformin hydrochloride based on the label claim.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 218 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 5 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for metformin related compound B, metformin, and

metformin related compound C are about 0.86, 1.0, and 2.1–2.3, respectively. Metformin related compound C can have a variable retention time.

- The composition of acetonitrile and *Buffer* in *Mobile phase* may be changed to 5:95, if it elutes at a relative retention time of less than 2.1. ■1S (USP39)

]

Suitability requirements

Resolution: NLT 1.5 between metformin related compound B and metformin

Tailing factor: 0.8–2.0 for the metformin peak

Relative standard deviation: NMT 1.5% for the metformin peak; NMT 10% each for the metformin related compound B and metformin related compound C peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of

- metformin hydrochloride ■1S (USP39)

($C_4H_{11}N_5 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response

- of metformin ■1S (USP39)
- from the *Sample solution*

r_S = peak response

- of metformin ■1S (USP39)
- from the *Standard solution*

C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metformin hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of metformin hydrochloride

PERFORMANCE TESTS

Change to read:

● Dissolution (711)

Glyburide

Medium: 0.05 M boric acid and 0.05 M potassium chloride solution. Prepare by dissolving 3.09 g of boric acid and 3.73 g of potassium chloride in 250 mL of water, adjust with 1 N sodium hydroxide to a pH of 9.5, and dilute with water to 1 L; 500 mL.

Apparatus 2: 75 rpm

Time: 30 min

Standard solution: ~~Transfer 10 mg of USP Glyburide RS to a 100 mL volumetric flask. Dissolve in 20 mL of acetonitrile, and dilute with *Medium* to volume.~~

- 0.1 mg/mL of USP Glyburide RS prepared as follows. Transfer a weighed amount of USP Glyburide RS to a suitable volumetric flask, dissolve first in acetonitrile, using 20% of the final volume, then dilute with *Medium* to volume. ■1S (USP39)

Dilute further with *Medium* to obtain a solution having a glyburide concentration, in mg/mL, of ($L/500$), where L is the label claim of glyburide in mg/Tablet.

Sample solution: Sample per *Dissolution* 〈 711 〉. Pass a portion of the solution under test through a polypropylene filter of 0.45- μ m pore size or a glass fiber filter of 1- μ m pore size. Dilute with *Medium*, if necessary.

■ ~~1S (USP39)~~

Solution A:

■ **Buffer:** ~~1S (USP39)~~

~~28.7 mg/mL~~

■ ~~28.8 g/L 1S (USP39)~~

~~of monobasic ammonium phosphate in water~~

Mobile phase: ~~Solution A and acetonitrile (1:1).~~

■ ~~Acetonitrile and Buffer (1:1). 1S (USP39)~~

Adjust with 1 N sodium hydroxide to a pH of 5.3.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Column temperature: 30^o

Flow rate: 1.5 mL/min

Injection volume: 200 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: ~~NLT 5000~~

■ ~~1S (USP39)~~

Tailing factor: 0.8–2.0

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of

■ ~~glyburide 1S (USP39)~~

(C₂₃H₂₈ClN₃O₅S) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

~~r_U = peak response from the *Sample solution*~~

~~r_S = peak response from the *Standard solution*~~

~~C_S = concentration of USP Glyburide RS in the *Standard solution* (mg/mL)~~

~~C_S = nominal concentration of glyburide in the *Sample solution* (mg/mL)~~

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of glyburide from the *Sample solution* r_S = peak response of glyburide from the *Standard solution* C_S = concentration of USP Glyburide RS in the *Standard solution* (mg/mL) L

= label claim of glyburide (mg/Tablet)

 V = volume of *Medium*, 500 mL

■ 1S (USP39)

Tolerances: NLT 85% (Q) of the labeled amount of glyburide is dissolved.**Metformin hydrochloride****Medium:** 0.05 M phosphate buffer, pH 6.8. Prepare by dissolving 6.8 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 0.2 N sodium hydroxide to a pH of 6.8 ± 0.1 ; 1000 mL.**Apparatus 2:** 50 rpm**Time:** 30 min**Standard solution:** Dissolve a quantity of USP Metformin Hydrochloride RS in *Medium*, ~~Dilute~~ further, if necessary, with *Medium*

■ 1S (USP39)

to obtain a solution having a metformin hydrochloride concentration, in mg/mL, of $(L/1000)$, where L is the label claim of metformin hydrochloride in mg/Tablet.■ Dilute further, if necessary, with *Medium*. ■ 1S (USP39)**Sample solution:** Sample per *Dissolution* (711). Pass a portion of the solution under test through a polypropylene filter of 0.45- μ m pore size or a glass fiber filter of 1- μ m pore size. Dilute with *Medium*, if necessary,■ to a concentration similar to that of the *Standard solution*. ■ 1S (USP39)**Instrumental conditions**(See *Spectrophotometry and Light-Scattering* (851).)**Mode:** UV-Vis**Analytical wavelength:** 232 nm**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of

■ metformin hydrochloride ■ 1S (USP39)

(C₄H₁₁N₅·HCl) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metformin hydrochloride in the *Sample solution* (mg/mL)

■

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A_U

= absorbance of the *Sample solution*

A_S

= absorbance of the *Standard solution*

C_S

= concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)

L

= label claim (mg/Tablet)

V

= volume of *Medium*, 1000 mL

D

= dilution factor for the *Sample solution*

■ 1S (USP39)

Tolerances: NLT 85% (Q) of the labeled amount of metformin hydrochloride is dissolved.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements for *Weight Variation* for metformin hydrochloride and for *Content Uniformity* for glyburide

IMPURITIES

Change to read:

- **Glyburide**

Solution A₇

■ **Buffer**, ■ 1S (USP39)

Mobile phase, Diluent, Sample solution, Chromatographic system, and System suitability:

Proceed as directed in the *Assay for Glyburide*.

Standard solution: Dilute 1.0 mL of the *Standard solution* from the *Assay for Glyburide* with *Diluent* to 100 mL.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each glyburide impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100 \quad \text{1S (USP39)}$$

r_U = peak response of each glyburide impurity from the *Sample solution*

r_S = peak response of glyburide from the *Standard solution*

C_S = concentration of USP Glyburide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of glyburide in the *Sample solution* (mg/mL)

F = relative response factor, 0.8

1.2 1S (USP39)

for glyburide related compound A, 1.0 for all other impurities

Acceptance criteria

[Note—Disregard any peak less than 0.05%, and disregard any peak observed in the blank.]

Glyburide related compound A: NMT 1.0%

Any other individual impurities: NMT 0.2%

Total impurities: NMT 0.50%, excluding glyburide related compound A

Change to read:

• Metformin Hydrochloride

Solution A₇

1 Buffer, 1S (USP39)

Mobile phase, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay for *Metformin Hydrochloride*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each metformin impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each metformin impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria

[Note—Disregard any peak less than 0.05%, and disregard any peak observed in the blank.]

Individual metformin impurities: NMT 0.1%

Total impurities: NMT 0.5 %

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

Change to read:

- **USP Reference Standards** { 11 }

USP Glyburide RS

USP Glyburide Related Compound A RS

4-[2-(5-Chloro-2-methoxybenzamido)ethyl]benzenesulfonamide.

■

$C_{16}H_{17}ClN_2O_4S$ 368.84 ■ 1S (USP39)

USP Metformin Hydrochloride RS

USP Metformin Related Compound B RS

1-Methylbiguanide hydrochloride.

$C_3H_9N_5 \cdot HCl$ 151.60

USP Metformin Related Compound C RS

Dimethylmelamine, or *N,N*-dimethyl- [1,3,5]triazine-2,4,6-triamine.

$C_5H_{10}N_6$ 154.17

BRIEFING

Hydralazine Hydrochloride, *USP 38* page 3766. As a part of USP monograph modernization efforts, the following changes are proposed:

1. In the test for *Organic Impurities*, the preparation of the *Standard stock solution* and the *Standard solution* are added.
2. In the test for *Organic Impurities*, four specified impurities, corresponding acceptance criteria, and calculations are included to be consistent with the FDA-approved specifications.
3. An individual unspecified impurity specification in accordance with ICH guidelines and the corresponding calculation are included in the test for *Organic Impurities*.
4. Four new Reference Standards are added to the *USP Reference Standards* section.
5. The system suitability requirement for the *Relative standard deviation* in the test for *Organic Impurities* is added.
6. The reagent phthalazine in the *System suitability solution* in the *Assay* is replaced with USP Phthalazine RS.
7. The test for *Water-Insoluble Substances* is deleted as the corresponding dosage form monograph for Injection adequately tests for the presence of particulate matter.

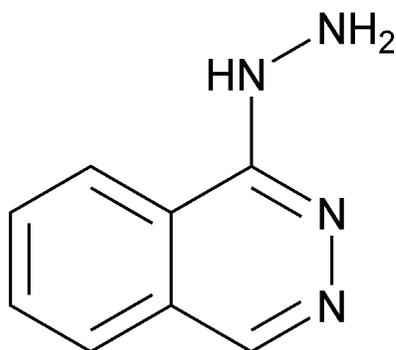
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: S. Ramakrishna.)

Correspondence Number—C140008

Comment deadline: May 31, 2015

Hydralazine Hydrochloride



• HCl

$C_8H_8N_4 \cdot HCl$ 196.64

Phthalazine, 1-hydrazino-, monohydrochloride;
1-Hydrazinophthalazine monohydrochloride [304-20-1].

DEFINITION

Hydralazine Hydrochloride contains NLT 98.0% and NMT 102.0% of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈 197M 〉
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General, Chloride** 〈 191 〉
Sample solution: 0.25 mg/mL of Hydralazine Hydrochloride in water
Acceptance criteria: Meets the requirements

ASSAY

Change to read:

- **Procedure**

Mobile phase: Dissolve 1.44 g of sodium dodecyl sulfate and 0.75 g of tetrabutylammonium bromide in 770 mL of water, and add 230 mL of acetonitrile. Adjust with 0.1 N sulfuric acid to a pH of 3.0.

System suitability stock solution: 0.25 mg/mL of USP Hydralazine Hydrochloride RS and 0.05 mg/mL of phthalazine

■ USP Phthalazine RS ■_{1S} (USP39)

in 0.1 N acetic acid

System suitability solution: 25 µg/mL of USP Hydralazine Hydrochloride RS and 5 µg/mL of phthalazine

■ USP Phthalazine RS ■_{1S} (USP39)

in 0.1 N acetic acid from *System suitability stock solution*

Standard stock solution: 0.4 mg/mL of USP Hydralazine Hydrochloride RS in 0.1 N acetic acid

Standard solution: 40 µg/mL of USP Hydralazine Hydrochloride RS in 0.1 N acetic acid from *Standard stock solution*

Sample stock solution: 0.4 mg/mL of Hydralazine Hydrochloride in 0.1 N acetic acid

Sample solution: 40 µg/mL of Hydralazine Hydrochloride in 0.1 N acetic acid prepared as follows. Transfer a suitable amount of *Sample stock solution* to a suitable volumetric flask. Dilute with 0.1 N acetic acid to volume, and filter, discarding the first 10 mL of the filtrate.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.0-mm × 25-cm; 10-µm packing L10

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for phthalazine and hydralazine hydrochloride are 0.65 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between the phthalazine and hydralazine peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%

■ 0.73%, *Standard solution* ■ 1S (USP39)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$) in the portion of Hydralazine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydralazine from the *Sample solution*

r_S = peak response of hydralazine from the *Standard solution*

C_S = concentration of USP Hydralazine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = concentration of Hydralazine Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Delete the following:

-

• **Heavy Metals, Method II** 〈 231 〉

: NMT 20 ppm • (Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

Mobile phase and **System suitability solution:** Proceed as directed in the *Assay*.

■ **Standard stock solution:** 0.1 mg/mL each of USP Hydralazine Hydrochloride RS, USP Phthalazine RS, USP 1-Phthalazinone RS, and USP 1-Chlorophthalazine RS, and 0.2 mg/mL of USP 2-Formyl Benzoic Acid RS in 0.1 N acetic acid. Initially add 0.1 N acetic acid to fill about 60% of the total volume, sonicate to dissolve, cool to room temperature, and dilute with 0.1 N acetic acid to final volume.

Standard solution: 0.001 mg/mL of USP Hydralazine Hydrochloride RS, USP Phthalazine RS, USP 1-Phthalazinone RS, and USP 1-Chlorophthalazine RS, and 0.002 mg/mL of USP 2-Formyl Benzoic Acid RS in 0.1 N acetic acid from *Standard stock solution* ■_{1S} (USP39)

Sample solution: 0.5 mg/mL of Hydralazine Hydrochloride in 0.1 N acetic acid prepared as follows. Transfer a suitable amount of Hydralazine Hydrochloride to a suitable volumetric flask. Add 0.1 N acetic acid to fill about 60% of the total volume, and sonicate to dissolve. Cool, and dilute with 0.1 N acetic acid to volume.

Chromatographic system: Proceed as directed in the *Assay*, except use an *Injection volume* of 20 µL.

System suitability

Samples: *System suitability solution*

■ and *Standard solution* ■_{1S} (USP39)

~~[Note—The relative retention times for phthalazine and hydralazine hydrochloride are 0.65 and 1.0, respectively.]~~

■ ■_{1S} (USP39)

Suitability requirements

Resolution: NLT 4.0 between the phthalazine and hydralazine peaks,

■ *System suitability solution*

Relative standard deviation: NMT 5.0% for each peak, *Standard solution* ■_{1S} (USP39)

Analysis

Samples:

■ *Standard solution* and ■_{1S} (USP39)

Sample solution

Calculate the percentage of each peak, other than the solvent peak and the hydralazine peak, in the portion of Hydralazine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each peak

r_T = sum of the responses of all the peaks, excluding that of the solvent peak

■ Calculate the percentage of each specified impurity in the portion of Hydralazine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of each specified impurity from the *Sample solution*

r_S = peak response of the corresponding specified impurity from the *Standard solution* C_S = concentration of the corresponding specified impurity in the *Standard solution* (mg/mL) C_U = concentration of Hydralazine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Hydralazine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of any unspecified impurity from the *Sample solution* r_S = peak response of hydralazine from the *Standard solution* C_S = concentration of USP Hydralazine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = concentration of Hydralazine Hydrochloride in the *Sample solution* (mg/mL)

■ 1S (USP39)

Acceptance criteria:**Total impurities:** NMT 1.0%

■ See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
1-Phthalazinone	0.55	0.15
2-Formyl benzoic acid	0.60	0.15
Phthalazine	0.75	0.15
1-Chlorophthalazine	0.83	0.15
Hydralazine	1.00	—
Any unspecified impurity	—	0.10
Total impurities ^a	—	1.0
a Total impurities include specified and unspecified impurities.		

■ 1S (USP39)

- **Limit of Hydrazine**

Buffer: Dissolve 5.82 g of dibasic sodium phosphate and 3.81 g of monobasic potassium phosphate in 1 L of water, and adjust with either 1 N sodium hydroxide or 1 N phosphoric acid to a pH of 7.0 ± 0.1 .

Mobile phase: Dissolve 300 mg of edetate disodium in 300 mL of water in a 1-L volumetric flask. Dilute with acetonitrile to volume.

Benzaldehyde solution: Transfer 1.0 mL of benzaldehyde to a 100-mL volumetric flask,

and dilute with a mixture of methanol and water (9:1) to volume.

Acetonitrile solution: Transfer 300 mL of water to a 1000-mL volumetric flask, and dilute with acetonitrile to volume.

Standard stock solution: 0.65 mg/mL of hydrazine dihydrochloride in water

Standard solution 1: 0.325 µg/mL of hydrazine dihydrochloride in water from *Standard stock solution*

Standard solution 2: Transfer 1.0 mL of *Standard solution 1* to a 10-mL flask. Add 4.0 mL of *Benzaldehyde solution*, and shake by mechanical means for 20 min.

Standard solution 3: Transfer 2.0 mL of *Standard solution 2* to a 5-mL volumetric flask, and dilute with *Acetonitrile solution* to volume.

Extraction column: Use a freshly conditioned solid phase extraction column containing benzenesulfonic acid strong cation-exchange packing with a sorbent-mass to column volume ratio of 500 mg per 3 mL, or equivalent. The column is conditioned as follows. Wash the column with two 2.0-mL portions of hexanes, and dry under vacuum for 2 min. Wash the column with two 2.0-mL portions of methanol, two 2.0-mL portions of water, and two 2.0-mL portions of *Buffer*. At no time after the hexanes wash should the column be allowed to dry out.

Sample solution: Transfer about 20 mg of Hydralazine Hydrochloride to a 10-mL reaction vessel, and dissolve in 1.0 mL of water. Add 4.0 mL of *Benzaldehyde solution*, and shake by mechanical means for 20 min. Pipet 2.0 mL of this solution into the *Extraction column* and elute into a 5-mL volumetric flask. Wash the *Extraction column* with two 1.5-mL portions of *Acetonitrile solution*, collecting the washings with the eluate, and dilute with *Acetonitrile solution* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 310 nm

Column: 4.0-mm × 25-cm; 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for hydralazine and hydrazine are about 1.0 and 1.5, respectively.]

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of hydrazine in the portion of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$) taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of hydrazine from the *Sample solution*

r_S = peak response of hydrazine from the *Standard solution*

C_S = concentration of hydrazine dihydrochloride in the *Standard solution* (µg/mL)

C_U = concentration of Hydralazine Hydrochloride in the *Sample solution* (µg/mL)

$M_{i\bar{r}}$ molecular weight of hydrazine, 32.05

$M_{i\bar{z}}$ molecular weight of hydrazine dihydrochloride, 104.97

Acceptance criteria: NMT 0.001%

SPECIFIC TESTS

- **pH** 〈 791 〉

Sample solution: 20 mg/mL of Hydralazine Hydrochloride in water

Acceptance criteria: 3.5–4.2

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 110° for 15 h.

Acceptance criteria: NMT 0.5%

Delete the following:

- **Water-Insoluble Substances**

Sample: 2.0 g

Analysis: Transfer the *Sample* to a 250 mL conical flask. Add 100 mL of water, and shake by mechanical means for 30 min. Filter the solution through a tared sintered glass crucible, and wash into the crucible any undissolved residue remaining in the flask. Wash the residue with three 10 mL portions of water, dry at 105° for 3 h, cool, and weigh.

Acceptance criteria: Weight of the residue does not exceed 10 mg (0.5%). ■1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.

Change to read:

- **USP Reference Standards** 〈 11 〉

- USP 1-Chlorophthalazine RS

$C_8H_5N_2Cl$ 164.59

- USP 2-Formyl Benzoic Acid RS

$C_8H_6O_3$ 150.13 ■1S (USP39)

- USP Hydralazine Hydrochloride RS

- USP Phthalazine RS

$C_8H_6N_2$ 130.15

- USP 1-Phthalazinone RS

$C_8H_6N_2O$ 146.15

- 1S (USP39)

BRIEFING

Hydralazine Hydrochloride Tablets, USP 38 page 3769. As part of USP monograph modernization efforts, it is proposed to include a validated HPLC-based *Organic Impurities* test procedure. The proposed test procedure is validated using the Nucleosil CN brand of

L10 column in which hydralazine elutes at about 7 min. A Reference Standard for USP Phthalazine RS is in development and hence the reagent phthalazine in the *System suitability solution* in the *Assay* is replaced with USP Phthalazine RS. The *USP Reference Standards* section is updated to include the Reference Standard for phthalazine.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: S. Ramakrishna.)

Correspondence Number—C125038

Comment deadline: May 31, 2015

Hydralazine Hydrochloride Tablets

DEFINITION

Hydralazine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$).

IDENTIFICATION

• **A. Infrared Absorption** (197K)

Sample: Transfer an amount equivalent to about 100 mg of hydralazine hydrochloride from finely powdered Tablets to a glass-stoppered flask. Add 40 mL of 1 N hydrochloric acid, shake by mechanical means for 5 min, and filter. Discard the first few mL of the filtrate. Place 20 mL of the filtrate in a separator, wash with 10 mL of methylene chloride, and discard the methylene chloride washing. Mix the aqueous solution in the separator with 2 mL of 14-mg/mL sodium nitrite solution, add 10 mL of methylene chloride, shake by mechanical means for 5 min, and allow the layers to separate. Pass the methylene chloride layer through a filter of anhydrous sodium sulfate previously washed with methylene chloride and collect the solution in a 50-mL beaker. Evaporate to dryness with the aid of gentle heat and a stream of dry nitrogen.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **Procedure**

Mobile phase: Dissolve 1.44 g of sodium dodecyl sulfate and 0.75 g of tetrabutylammonium bromide in 770 mL of water and add 230 mL of acetonitrile. Adjust with 0.1 N sulfuric acid to a pH of 3.0.

■ **Diluent:** 0.1 N acetic acid ■ 1S (USP39)

~~**System suitability stock solution:** 0.25 mg/mL of USP Hydralazine Hydrochloride RS and 0.05 mg/mL of phthalazine in 0.1 N acetic acid~~

■ 1S (USP39)

System suitability solution: 25 µg/mL of USP Hydralazine Hydrochloride RS and 5 µg/mL of

~~phthalazine in 0.1 N acetic acid from *System suitability stock solution*~~

■ USP Phthalazine RS in *Diluent*. ■ 1S (USP39)

~~**Standard stock solution:** 0.4 mg/mL of USP Hydralazine Hydrochloride RS in 0.1 N acetic acid~~
 ■ 1S (USP39)

~~**Standard solution:** 40 µg/mL of USP Hydralazine Hydrochloride RS in 0.1 N acetic acid from *Standard stock solution*~~

■ *Diluent*. ■ 1S (USP39)

~~**Sample stock solution:** Nominally 0.4 mg/mL of hydralazine hydrochloride from NLT 20 finely powdered Tablets in 0.1 N acetic acid~~

■ *Diluent*. ■ 1S (USP39)

Centrifuge the solution

■ and use the clear supernatant. ■ 1S (USP39)

~~**Sample solution:** Nominally 40 µg/mL of hydralazine hydrochloride in 0.1 N acetic acid from the clear liquid of~~

■ *Diluent*. ■ 1S (USP39)

from *Sample stock solution*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.0-mm × 25-cm; 10-µm packing L10

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for phthalazine and hydralazine are about 0.65 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between the phthalazine and hydralazine peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydralazine hydrochloride (C₈H₈N₄·HCl) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydralazine from the *Sample solution*

r_S = peak response of hydralazine from the *Standard solution*

C_S = concentration of USP Hydralazine Hydrochloride RS in the *Standard solution* (µg/mL)

$C_{\bar{f}}$ nominal concentration of hydralazine hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution 〈 711 〉

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: A known concentration of USP Hydralazine Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter and dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 260 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of hydralazine hydrochloride ($\text{C}_8\text{H}_8\text{N}_4\cdot\text{HCl}$) dissolved.

Tolerances: NLT 75% (Q) of the labeled amount of hydralazine hydrochloride ($\text{C}_8\text{H}_8\text{N}_4\cdot\text{HCl}$) is dissolved.

• Uniformity of Dosage Units 〈 905 〉: Meet the requirements

IMPURITIES

Add the following:

• Organic Impurities

Diluent and System suitability solution: Proceed as directed in the *Assay*.

Mobile phase: Dissolve 1.44 g of sodium dodecyl sulfate and 0.75 g of tetrabutylammonium bromide in 770 mL of water, adjust with 0.1 N sulfuric acid to a pH of 3.0, and add 230 mL of acetonitrile.

Standard stock solution: 0.1 mg/mL of USP Hydralazine Hydrochloride RS in *Diluent*, prepared as follows. To a suitable amount of USP Hydralazine Hydrochloride RS, add 60% of the total volume of *Diluent*, sonicate to dissolve, cool to room temperature, and then dilute with *Diluent* to volume.

Standard solution: 0.001 mg/mL of USP Hydralazine Hydrochloride RS in *Diluent* from *Standard stock solution*

Sample solution: Nominally 0.5 mg/mL of hydralazine hydrochloride from NLT 20 powdered Tablets in *Diluent*, prepared as follows. To a suitable amount of powdered Tablets, add 60% of the total volume of *Diluent* and sonicate for 15 min with occasional swirling. Cool to room temperature, dilute with *Diluent* to volume, and pass through a suitable filter of 0.45- μm pore size.

Chromatographic system: Proceed as directed in the *Assay* except for the following.

Injection volume: 20 μL

Run time: NLT 5.8 times the retention time of hydralazine

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between the phthalazine and hydralazine peaks, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any unspecified degradation product from the *Sample solution*

r_S peak response of hydralazine from the *Standard solution*

C_S concentration of USP Hydralazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of hydralazine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
1-Phthalazinone ^a	0.55	—
2-Formylbenzoic acid ^a	0.60	—
Phthalazine ^a	0.75	—
1-Chlorophthalazine ^a	0.83	—
Hydralazine	1.00	—
Any unspecified degradation product	—	0.20
Total impurities ^b	—	1.5
^a Process related impurity.		

^b Total impurities include process related impurities and degradation products.

■1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

- **USP Reference Standards** { 11 }

USP Hydralazine Hydrochloride RS

■ USP Phthalazine RS

C₈H₆N₂ 130.15 ■1S (USP39)

BRIEFING

Hydroxyzine Hydrochloride Oral Solution, *USP 38* page 3814. As part of the USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Add a stability-indicating liquid chromatographic procedure as a test for *Organic Impurities* and replace the existing *Assay* with a similar procedure. These procedures were validated using the Acquity UPLC HSS C18 SB brand of L1 column. The typical retention time for hydroxyzine is about 15.6 min.
2. Replace *Identification test A*, which is based on TLC, with a test based on the retention time agreement using the proposed *Assay*.
3. Add *Identification test B* based on the UV spectral agreement for the main peak from the proposed *Assay*.
4. Include a storage requirement in the *Packaging and Storage* section based on the information for an approved drug product.
5. Add USP 4-Chlorobenzophenone RS and USP Hydroxyzine Related Compound A RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R.-H. Yeh, D. Min.)

Correspondence Number—C117375

Comment deadline: May 31, 2015

Hydroxyzine Hydrochloride Oral Solution**DEFINITION**

Hydroxyzine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$).

IDENTIFICATION**Change to read:**• **A.**

~~**Standard solution:** 0.35 mg/mL of USP Hydroxyzine Hydrochloride RS in methanol~~

~~**Sample solution:** Nominally 0.4 mg/mL of hydroxyzine hydrochloride from Oral Solution in methanol~~

~~**Chromatographic system**~~

~~(See *Chromatography* (621); *Thin-Layer Chromatography*.)~~

~~**Mode:** TLC~~

~~**Adsorbent:** Thin-layer chromatographic plate, coated with a 0.25-mm layer of chromatographic silica gel, and dried in air for 30 min, followed by drying under vacuum at 140° for 30 min~~

~~**Application volume:** 100 µL~~

~~**Developing solvent system:** Toluene, alcohol, and ammonium hydroxide (150:95:1)~~

~~**Spray reagent:** Potassium iodoplatinate TS~~

~~**Analysis**~~

~~**Samples:** Standard solution and Sample solution~~

~~Apply the solutions on a chromatographic plate, and allow the spots to dry. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots by lightly spraying with *Spray reagent*.~~

~~**Acceptance criteria:** The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.~~

- The retention time of the major peak of the *Sample solution* corresponds to that of the hydroxyzine peak of the *Standard solution*, as obtained in the Assay. ■ 1S (USP39)

Add the following:

- ● **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ■ 1S (USP39)

ASSAY

Change to read:

● **Procedure**

~~**Solution A:** 6.8 g/L of monobasic potassium phosphate in water~~

~~**Mobile phase:** Methanol and *Solution A* (50:50). Pass through a polytetrafluoroethylene membrane filter of NMT 5- μ m pore size.~~

~~**Standard solution:** 100 μ g/mL of USP Hydroxyzine Hydrochloride RS in water~~

~~**Sample solution:** Nominally 100 μ g/mL of hydroxyzine hydrochloride from Oral Solution in water prepared as follows. Transfer a suitable volume of Oral Solution to an appropriate volumetric flask. Dilute with water to volume, and pass a portion through a polytetrafluoroethylene membrane filter of NMT 5- μ m pore size.~~

~~**Chromatographic system**~~

~~(See *Chromatography* (621), *System Suitability*.)~~

~~**Mode:** LC~~

~~**Detector:** UV 232 nm~~

~~**Column:** 4.6 mm \times 25 cm; packing L9~~

~~**Flow rate:** 2.0 mL/min~~

~~**Injection volume:** 20 μ L~~

~~**System suitability**~~

~~**Sample:** *Standard solution*~~

~~**Suitability requirements**~~

~~**Relative standard deviation:** NMT 2.5%~~

- **Solution A:** Trifluoroacetic acid and water (0.1: 99.9)

Solution B: Acetonitrile and trifluoroacetic acid (99.5: 0.5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
12	60	40
16	60	40
21	20	80
25	20	80
26	90	10
30	90	10

Diluent: Acetonitrile and water (30:70)

Standard solution: 0.05 mg/mL of USP Hydroxyzine Hydrochloride RS in *Diluent*

Sample stock solution: Nominally 0.5 mg/mL of hydroxyzine hydrochloride from Oral Solution in *Diluent* prepared as follows. Transfer a portion of Oral Solution, equivalent to 25 mg of hydroxyzine hydrochloride, to a 50-mL volumetric flask. Dissolve and dilute with *Diluent* to volume.

Sample solution: Nominally 0.05 mg/mL of hydroxyzine hydrochloride from *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 230 nm. For *Identification test B*, use a diode array detector in the range of 200–400 nm.

Column: 2.1-mm × 15-cm; 1.8-μm packing L1

Column temperature: 30°

Flow rate: 0.3 mL/min

Injection volume: 2 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of hydroxyzine from the *Sample solution*

r_S

= peak response of hydroxyzine from the *Standard solution*

C_S

= concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of hydroxyzine hydrochloride in the *Sample solution* (mg/mL)

■ 1S (USP39)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Uniformity of Dosage Units** 〈 905 〉
For single-unit containers
Acceptance criteria: Meets the requirements
- **Deliverable Volume** 〈 698 〉
For multiple-unit containers
Acceptance criteria: Meets the requirements

IMPURITIES

Add the following:

■ • Organic Impurities

Solution A, Solution B, Mobile phase, and Diluent: Proceed as directed in the *Assay*.

Standard stock solution: 25.0 µg/mL each of USP Hydroxyzine Hydrochloride RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS in *Diluent*

Standard solution: 1.0 µg/mL each of USP Hydroxyzine Hydrochloride RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS from *Standard stock solution* in *Diluent*

Sample solution: Nominally 500 µg/mL of hydroxyzine hydrochloride from Oral Solution in *Diluent* prepared as follows. Transfer a portion of Oral Solution, equivalent to 25 mg of hydroxyzine hydrochloride, to a 50-mL volumetric flask, dissolve, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detectors

Hydroxyzine related compound A and hydroxyzine hydrochloride: UV 230 nm

4-Chlorobenzophenone: UV 254 nm

Column: 2.1-mm × 15-cm; 1.8-µm packing L1

Column temperature: 30°

Flow rate: 0.3 mL/min

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between hydroxyzine related compound A and hydroxyzine
Relative standard deviation: NMT 3.0% for hydroxyzine related compound A, hydroxyzine, and 4-chlorobenzophenone

Analysis

Samples: *Standard solution and Sample solution*

For impurities detected at UV 230 nm

Calculate the percentage of any individual unspecified degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any individual unspecified degradation product from the *Sample solution*

r_S peak response of hydroxyzine from the *Standard solution*

C_S concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U nominal concentration of hydroxyzine hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

For 4-chlorobenzophenone detected at UV 254 nm

Calculate the percentage of 4-chlorobenzophenone in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of 4-chlorobenzophenone from the *Sample solution*

r_S peak response of 4-chlorobenzophenone from the *Standard solution*

C_S concentration of USP 4-Chlorobenzophenone RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U nominal concentration of hydroxyzine hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See Table 2. Disregard any peak below 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Hydroxyzine related compound A ^a	0.94	—
Hydroxyzine	1.0	—
4-Chlorobenzophenone	1.4	0.2
Any individual unspecified degradation product	—	0.3
Total degradation products	—	0.5

^a This is a process impurity that is controlled in the drug substance. It is not to be reported or included in the total degradation products.

■ 1S (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

● **Packaging and Storage:** Preserve in tight, light-resistant containers.

■ Store at controlled room temperature. ■ 1S (USP39)

Change to read:

- **USP Reference Standards** { 11 }

- USP 4-Chlorobenzophenone RS
4-Chlorobenzophenone.
 $C_{13}H_9ClO$ 216.66 ■ 1S (USP39)

USP Hydroxyzine Hydrochloride RS

- USP Hydroxyzine Related Compound A RS
1-[(4-Chlorophenyl)phenylmethyl]piperazine.
 $C_{17}H_{19}ClN_2$ 286.80 ■ 1S (USP39)

BRIEFING

Imipramine Hydrochloride Tablets, USP 38 page 3844. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Replace the spectrophotometric *Assay* procedure with a stability-indicating liquid chromatographic procedure using the Acquity UPLC BEH C8 brand of L7 column. The retention time of imipramine in the analysis is about 10.5 min.
2. Add *Identification* test *B* using a retention time agreement based on the proposed method for the *Assay*.
3. Add a specific equation in the test for *Dissolution* to provide additional information.
4. Add a liquid chromatographic procedure as the test for *Organic Impurities* that uses the same chromatographic parameters as the proposed *Assay*.
5. Add a storage requirement in the *Packaging and Storage* section that is consistent with FDA-approved drug products.
6. Add USP Desipramine Hydrochloride RS, USP Depramine RS, and USP Iminodibenzyl RS to the *Reference Standards* section to support the proposed procedures in the *Assay* and the test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: D.A. Porter.)

Correspondence Number—C120149

Comment deadline: May 31, 2015

Imipramine Hydrochloride Tablets**DEFINITION**

Imipramine Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$).

IDENTIFICATION**Change to read:**

•

■ **A.** ■ 1S (USP39)

Infrared Absorption 〈 197K〉

Sample: Powder a suitable number of Tablets, equivalent to 100 mg of imipramine hydrochloride, and macerate the powder with 10 mL of chloroform. Filter the chloroform extract through paper into a wide-mouth test tube, and evaporate the filtrate to 3 mL. Carefully add ether until the liquid becomes turbid, heat on a steam bath to produce a clear solution, cool, and allow to stand. The precipitate that is formed may be recrystallized from acetone. Filter the crystalline precipitate, wash with ether, and dry under vacuum at 105° for 30 min. Use the precipitate.

Acceptance criteria: Meet the requirements

Add the following:

- • **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

ASSAY

Change to read:

• **Procedure**

Diluent A: 0.5 N hydrochloric acid

Diluent B: hydrochloric acid in water (1 in 25)

Standard solution: 25 µg/mL USP Imipramine Hydrochloride RS in *Diluent A*

Sample stock solution: Nominally 0.5 mg/mL of imipramine hydrochloride from Tablets prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 100 mg of imipramine hydrochloride, to a 200-mL volumetric flask; add 100 mL of *Diluent B*, and shake vigorously by mechanical means for 1 h. Dilute with *Diluent B* to volume, pass through a suitable and filter, and discard the first 20 mL of the filtrate.

Sample solution: Pipet 5 mL of the *Sample stock solution* into a separator, add 10 mL of 1 N sodium hydroxide, and extract with four 20-mL portions of ether, shaking each portion for 2 min and collecting the extracts in a second separator. Extract the combined ether extracts with four 20-mL portions of *Diluent A*, and combine the extracts in a 250-mL beaker. Aerate this solution with nitrogen to remove residual ether, then transfer to a 100-mL volumetric flask, and rinse the beaker with *Diluent A*, collecting the rinsings in the flask. Add *Diluent A* to volume.

Instrumental conditions-

(See *Spectrophotometry and Light Scattering* 〈 851〉.)

Mode: UV-Vis

Analytical wavelength: 250 nm

Cell: 1 cm

Analysis-

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

~~A_U~~ absorbance of the *Sample solution*

~~A_S~~ absorbance of the *Standard solution*

~~C_S~~ concentration of USP Imipramine Hydrochloride RS in the *Standard solution* (mg/mL)

~~C_U~~ nominal concentration of imipramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: ~~93.0%–107.0%~~

■ **Solution A:** 0.02 M ammonium bicarbonate in water. Adjust with 28%–30% ammonia solution to a pH of 8.0.

Solution B: Acetonitrile and methanol (70:30)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
1.0	90	10
5.0	60	40
7.0	60	40
10.0	48	52
13.0	48	52
14.0	20	80
16.0	20	80
16.1	90	10
18.0	90	10

Diluent: Acetonitrile and water (40:60)

Standard solution: 0.25 mg/mL of USP Imipramine Hydrochloride RS in *Diluent*

Sample stock solution: Nominally 1.0 mg/mL of imipramine hydrochloride from Tablets prepared as follows. Powder NLT 10 Tablets and transfer a sufficient portion of powder to a volumetric flask. Add 75% of the final flask volume of *Diluent*. Sonicate as needed to aid in dissolution. Allow to cool to room temperature, then dilute with *Diluent* to volume. Centrifuge and use the supernatant. [Note—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]

Sample solution: Nominally 0.25 mg/mL of imipramine from *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 2.1-mm × 10-cm, 1.7-μm packing L7

Column temperature: 35°

Flow rate: 0.4 mL/min

Injection volume: 2 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Imipramine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of imipramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0% \blacksquare 1S (USP39)

PERFORMANCE TESTS

Change to read:

• **Dissolution** 〈 711 〉

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Mode: UV-Vis

Analytical wavelength: maximum absorbance at about 250 nm

\blacksquare UV 250 nm \blacksquare 1S (USP39)

Standard solution: USP Imipramine Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* to a suitable concentration.

Analysis

Samples: *Standard solution* and *Sample solution*

~~Determine the amounts of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) dissolved.~~

\blacksquare Calculate the percentage of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$)

dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U

= absorbance of the *Sample solution*

A_S

= absorbance of the *Standard solution*

C_S

= concentration of USP Imipramine Hydrochloride RS in the *Standard solution* (mg/mL)

D

= dilution factor for the *Sample solution*, if needed

V

= volume of *Medium*, 900 mL

L

= label claim (mg/Tablet)

■ 1S (USP39)

Tolerances: NLT 75% (Q) of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) is dissolved.

Change to read:

● **Uniformity of Dosage Units** 〈 905 〉

Procedure for content uniformity

Diluent: Hydrochloric acid and water (1:100)

Standard solution: 0.025 mg/mL of USP Imipramine Hydrochloride RS in *Diluent*

Sample stock solution: Transfer 1 finely powdered Tablet to a 100-mL volumetric flask with the aid of 70 mL of *Diluent* and shake by mechanical means for 30 min. Dilute with *Diluent* to volume, and filter, if necessary, discarding the first 20 mL of the filtrate.

Sample solution: Nominally 0.025 mg/mL of imipramine hydrochloride from *Sample stock solution* in *Diluent*

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* 〈 851 〉.)

Mode: UV-Vis

Analytical wavelength: maximum absorbance at about 250 nm

■ UV 250 nm ■ 1S (USP39)

Cell: 1 cm

Blank: *Diluent*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U absorbance of the *Sample solution*

A_S absorbance of the *Standard solution*

C_S concentration of USP Imipramine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of imipramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

Add the following:

Organic Impurities

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.002 mg/mL each of USP Imipramine Hydrochloride RS, USP Depramine RS, USP Desipramine Hydrochloride RS, and USP Iminodibenzyl RS in *Diluent*. Sonicate if necessary to aid dissolution.

Sample solution: Nominally 1.0 mg/mL of imipramine hydrochloride from Tablets prepared as follows. Powder NLT 10 Tablets and transfer a portion to a volumetric flask. Add about 75% of the final flask volume of *Diluent*. Sonicate as necessary to aid dissolution. Allow to cool to room temperature, then dilute with *Diluent* to volume. Centrifuge and use the supernatant. [Note—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between the depramine and desipramine peaks

Relative standard deviation: NMT 2.5% each for depramine, desipramine, imipramine, and iminodibenzyl

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each identified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_S peak response of each corresponding Reference Standard from the *Standard solution*

C_S concentration of each corresponding Reference Standard in the *Standard solution* (mg/mL)

C_U nominal concentration of imipramine hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each unspecified degradation product from the *Sample solution*

r_S peak response of imipramine from the *Standard solution*

C_S concentration of USP Imipramine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of imipramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desipramine	0.82	0.10
Depramine	0.84	0.10
Imipramine	1.0	—
Iminodibenzyl	1.18	0.10
Any individual unspecified degradation product	—	0.20
Total degradation products	—	1.5

■1S (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers.
- Store at controlled room temperature. ■1S (USP39)

Change to read:

● USP Reference Standards < 11 >

■ USP Depramine RS

3-(5*H*-Dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine hydrochloride.

C₁₉H₂₂N₂·HCl 314.85

USP Desipramine Hydrochloride RS

10,11-Dihydro-5*H*-[3-(methylamino)propyl]-5*H*-dibenz[*b,f*]azepine monohydrochloride.

C₁₈H₂₂N₂·HCl 302.84

USP Iminodibenzyl RS

10,11-Dihydro-5*H*-dibenzo[*b,f*]azepine.

C₁₄H₁₃N 195.28 ■1S (USP39)

USP Imipramine Hydrochloride RS

BRIEFING

Indapamide, *USP 38* page 3849. As part of the USP monograph modernization effort, the following revisions are proposed:

1. Revise the acceptance criterion in the *Definition* from NLT 98.0% and NMT 101.0% to NLT 98.0% and NMT 102.0%, which is typical for chromatographic procedures.
2. Replace the UV-based *Identification* test *B* with a retention time agreement for indapamide based on the *Assay*.
3. Replace the existing HPLC *Assay* procedure, which depends on an internal standard, with a stability-indicating HPLC procedure with conditions similar to the proposed *Organic Impurities* test. The proposed liquid chromatographic procedure is based on an analysis performed with the YMC-Pack Pro C8 brand of L7 column. The typical retention time for indapamide is about 22.8 min.
4. Replace the current TLC-based *Organic Impurities* procedure with a validated liquid chromatography based test procedure.
5. Add two new Reference Standards and an existing Reference Standard to the *USP Reference Standards* section: USP Indapamide Related Compound A RS, USP Indapamide Related Compound B RS, and USP Indapamide Related Compound C RS, in support of the newly proposed *Organic Impurities* procedure.

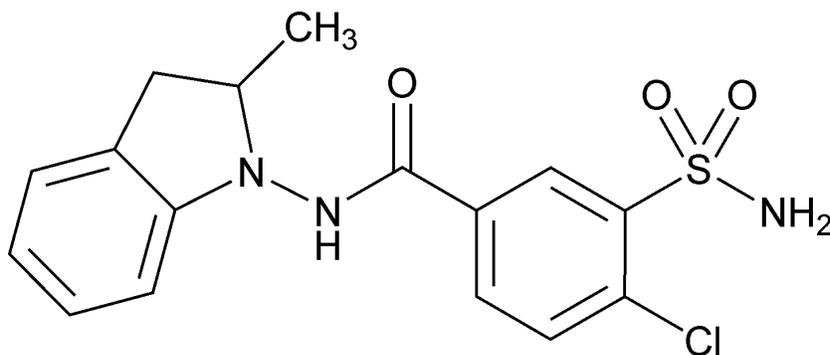
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: C. Anthony.)

Correspondence Number—C138904

Comment deadline: May 31, 2015

Indapamide



$C_{16}H_{16}ClN_3O_3S$ 365.83

Benzamide, 3-(aminosulfonyl)-4-chloro-*N*-(2,3-dihydro-2-methyl-1*H*-indol-1-yl)-; 4-Chloro-*N*-(2-methyl-1-indoliny)-3-sulfamoylbenzamide [26807-65-8].

DEFINITION

Change to read:

Indapamide contains NLT 98.0% and NMT ~~101.0%~~

■ 102.0% ■ 1S (*USP39*)

of indapamide ($C_{16}H_{16}ClN_3O_3S$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** ~~(197K)~~

Delete the following:

- **B. Ultraviolet Absorption** ~~(197U)~~

Solution: 5 µg/mL in methanol

Acceptance criteria: Meets the requirements. ■ 1S (USP39)

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

ASSAY**Change to read:**

- **Procedure**

Mobile phase: ~~Acetonitrile, methanol, water, and glacial acetic acid (175:175:650:1)~~

Internal standard solution: ~~5.0 mg/mL of *p*-chloroacetanilide in methanol~~

Standard solution: ~~1.0 mg/mL of USP Indapamide RS; and 0.25 mg/mL of internal standard from *Internal standard solution* in *Mobile phase* prepared as follows. Dissolve an appropriately weighed quantity of USP Indapamide RS in an appropriate volume of *Internal standard solution* in a suitable flask and dilute to volume with *Mobile phase*.~~

Sample solution: ~~1 mg/mL of Indapamide; and 0.25 mg/mL of internal standard from *Internal standard solution* in *Mobile phase* prepared as follows. Dissolve an appropriately weighed quantity of Indapamide in 5% of a suitable flask volume of *Internal standard solution* and dilute to volume with *Mobile phase*.~~

Chromatographic system

~~(See *Chromatography* (621), *System Suitability*.)~~

Mode: ~~LC~~

Detector: ~~UV 254 nm~~

Column: ~~4 mm × 30 cm; packing L1~~

Flow rate: ~~2 mL/min~~

Injection volume: ~~5 µL~~

System suitability

Sample: ~~*Standard solution*~~

~~[Note—The relative retention times for *p*-chloroacetanilide and indapamide are about 0.65 and 1.0, respectively.]~~

Suitability requirements

Resolution: ~~NLT 2.0 between any peak of interest and any adjacent peak~~

Tailing factor: ~~NMT 2.0 for the analyte peak~~

Relative standard deviation: ~~NMT 2.0%~~

Analysis

Samples: ~~*Standard solution* and *Sample solution*~~

Calculate the percentage of indapamide ($C_{16}H_{16}ClN_3O_3S$) in the portion of Indapamide

taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U peak area ratio of indapamide to the internal standard from the *Sample solution*

R_S peak area ratio of indapamide to the internal standard from the *Standard solution*

C_S concentration of USP Indapamide RS in the *Standard solution* (mg/mL)

C_U concentration of Indapamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.0% on the dried basis

■ **Solution A:** 10 mM ammonium acetate solution, adjusted with glacial acetic acid to a pH of 5.0

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
3.0	90	10
27	50	50
33	50	50
35	90	10
40	90	10

Diluent: Methanol and water (70:30)

Standard solution: 0.1 mg/mL of USP Indapamide RS in *Diluent*

Sample solution: 0.1 mg/mL of Indapamide in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of indapamide (C₁₆H₁₆ClN₃O₃S) in the portion of Indapamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

 r_S

= peak response from the *Standard solution*

 C_S

= concentration of USP Indapamide RS in the *Standard solution* (mg/mL)

 C_U

= concentration of Indapamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ■_{1S} (USP39)

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Delete the following:

■ • **Chromatographic Purity**

~~{Note—Minimize exposure to light while weighing the samples and spotting on the thin-layer chromatographic plate. Use low-actinic glassware or wrap the glassware with aluminum foil and protect all the chromatographic solutions from light. Place the chromatographic tanks in a dark room or cover them with aluminum foil during the development. The paperlined chamber should be saturated with solvent vapor for 1 h before development of the plates. }~~

~~**Standard solution A:** 0.30 mg/mL of USP Indapamide RS in methanol~~

~~**Standard solution B:** 0.15 mg/mL of USP Indapamide RS in methanol from *Standard solution A*~~

~~**Standard solution C:** 0.075 mg/mL of USP Indapamide RS in methanol from *Standard solution B*~~

~~**Sample solution:** 30 mg/mL of Indapamide in methanol~~

~~**Chromatographic system**~~

~~{See *Chromatography* 〈 621 〉, *Thin-Layer Chromatography*.}~~

~~**Mode:** TLC~~

~~**Adsorbent:** 0.25-mm layer of chromatographic silica-gel mixture~~

~~**Application volume:** 10 µL~~

~~**Developing solvent system:** Toluene, ethyl acetate, and glacial acetic acid (70:30:1)~~

~~**Analysis:** Apply separately the *Sample solution* and *Standard solution A*, *Standard solution B*, and *Standard solution C* to a suitable thin-layer chromatographic plate. Position the plate in a chromatographic chamber, and develop the chromatograms in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry under a current of air. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots observed in the chromatogram of the *Sample solution*~~

with those of the principal spots in the chromatograms of *Standard solution A*, *Standard solution B*, and *Standard solution C*.

Acceptance criteria: No secondary spot from the chromatograms of the *Sample solution* is larger or more intense than the principal spot of *Standard solution B* (0.5%), and the sum of the intensities of the secondary spots from the *Sample solution* corresponds to NMT 2.0%. ■ 1S (USP39)

Add the following:

■ ● Organic Impurities

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.001 mg/mL each of USP Indapamide RS, USP Indapamide Related Compound A RS, USP Indapamide Related Compound B RS, and USP Indapamide Related Compound C RS in *Diluent*

Sample solution: 1.0 mg/mL of Indapamide in *Diluent*

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between indapamide related compound C and indapamide; NLT 2.0 between indapamide related compound B and indapamide related compound A

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of indapamide related compound A, indapamide related compound B, and indapamide related compound C in the portion of Indapamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of indapamide related compound A, indapamide related compound B, or indapamide related compound C from the *Sample solution*

r_S peak response of the corresponding related compound from the *Standard solution*

C_S concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

C_U concentration of Indapamide in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Indapamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any unspecified impurity from the *Sample solution*

r_S peak response of indapamide from the *Standard solution*

C_S concentration of USP Indapamide RS in the *Standard solution* (mg/mL)

C_U concentration of Indapamide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Indapamide related compound C	0.87	0.5
Indapamide	1.0	—
Indapamide related compound B	1.1	0.5
Indapamide related compound A	1.2	0.5
Individual unspecified impurity	—	0.10
Total impurities	—	2.0

■ 1S (USP39)

SPECIFIC TESTS

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 3.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Indapamide RS

- USP Indapamide Related Compound A RS

4-Chloro-*N*-(2-methyl-1*H*-indol-1-yl)-3-sulfamoylbenzamide.

C₁₆H₁₄ClN₃O₃S 363.82

USP Indapamide Related Compound B RS

2-Methyl-1-nitrosoindoline.

C₉H₁₀N₂O 162.19

USP Indapamide Related Compound C RS

2-Methylindoline.

C₉H₁₁N 133.19 ■ 1S (USP39)

BRIEFING

Indapamide Tablets, USP 38 page 3850. As part of the USP monograph modernization effort, the following revisions are proposed:

1. Replace the existing *Assay* procedure with an HPLC procedure that is consistent with the proposed *Organic Impurities* method. The proposed *Assay* is based on an analysis performed with the YMC-Pack Pro C8 brand of L7 column. The typical retention time for indapamide is about 22.8 min.
2. Add a validated HPLC procedure for *Organic Impurities* similar to the proposed *Assay* method.
3. Add two new Reference Standards and an existing Reference Standard, introduced by the proposed HPLC procedure, to the *USP Reference Standards* section: USP

Indapamide Related Compound A RS, USP Indapamide Related Compound B RS, and USP Indapamide Related Compound C RS.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM2: C. Anthony.)

Correspondence Number—C115769

Comment deadline: May 31, 2015

Indapamide Tablets

DEFINITION

Indapamide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of indapamide ($C_{16}H_{16}ClN_3O_3S$).

IDENTIFICATION

• A. Infrared Absorption (197K)

Sample: Crush a quantity of Tablets, equivalent to 15 mg of indapamide, remove and discard any coating material, and finely powder the remaining Tablet cores. Agitate the powdered Tablets with two 30-mL portions of 0.2 N sodium hydroxide in a centrifuge tube for 10 min. Centrifuge each mixture, and combine the supernatants in a 250-mL separator. Acidify the liquid with 12 mL of dilute hydrochloric acid (1 in 10). Extract the acidic solution with two 4.0-mL portions of ether, filter the extracts through anhydrous sodium sulfate contained in a filter paper, and evaporate the ether, with the aid of a current of dry air, on a water bath. Dry the crystals at 105° for 1 h.

Acceptance criteria: The crystals so obtained meet the requirements.

- B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• Procedure

Solution A: 1.54 mg/mL of sodium 1-octanesulfonate in water

Mobile phase: Acetonitrile, *Solution A*, and glacial acetic acid (30:70:1)

Internal standard solution: 0.25 mg/mL of 2'-chloroacetophenone in acetonitrile

Standard stock solution: 0.1 mg/mL of USP Indapamide RS in acetonitrile

Standard solution: 0.01 mg/mL of USP Indapamide RS from *Standard stock solution*, *Internal standard solution*, and a mixture of water and acetonitrile (50:10)

Sample stock solution: Equivalent to 0.05 mg/mL of indapamide from Tablets prepared as follows. Transfer NLT 20 finely powdered Tablets, equivalent to 2.5 mg to a 50-mL volumetric flask. Add 25 mL of acetonitrile and sonicate for 20 min. Cool, dilute with acetonitrile to volume, and mix. Transfer this solution to a 50-mL centrifuge tube, and centrifuge at 2000 rpm for about 10 min.

Sample solution: Equivalent to 0.01 mg/mL of indapamide from *Sample stock solution*, *Internal standard solution*, and mixture of water and acetonitrile (70:4)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 4.5 mm × 10 cm; 3-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for indapamide and the internal standard are about 1.0 and 1.18, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the analyte peak and the internal standard peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of $C_{16}H_{16}ClN_3O_3S$ in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U peak response ratio of indapamide to the internal standard from the *Sample solution*

R_S peak response ratio of indapamide to the internal standard from the *Standard solution*

C_S concentration of USP Indapamide RS in the *Standard solution* (mg/mL)

C_U nominal concentration of indapamide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

■ **Solution A:** 10 mM ammonium acetate solution. Adjust with glacial acetic acid to a pH of 5.0.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
3.0	90	10
27	50	50
33	50	50
35	90	10
40	90	10

Diluent: Methanol and water (70:30)

Standard solution: 0.1 mg/mL of USP Indapamide RS in *Diluent*

Sample stock solution: Nominally 0.5 mg/mL of indapamide in *Diluent* prepared as follows. Transfer 25 mg of indapamide, from NLT 20 finely powdered Tablets, to a 50-

mL volumetric flask. Dissolve in 35 mL of *Diluent* by sonicating for 15 min with intermittent shaking. Dilute with *Diluent* to volume. Centrifuge the solution for 10 min.

Sample solution: Nominally 0.1 mg/mL of indapamide in *Diluent* prepared as follows.

Pipette 5.0 mL of the clear supernatant into a 25-mL volumetric flask and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of indapamide (C₁₆H₁₆ClN₃O₃S) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Indapamide RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of indapamide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% ■ 1S (USP39)

PERFORMANCE TESTS

● Dissolution 〈 711 〉

Medium: 0.05 M pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Solution A: 1.54 mg/mL of sodium 1-octanesulfonate in water

Mobile phase: Acetonitrile, *Solution A*, and glacial acetic acid (30:70:1)

Standard solution: USP Indapamide RS in methanol, and dilute, if necessary, with a mixture of *Medium* and methanol (99:1) to obtain a solution with a known concentration equivalent to the solution under test

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of indapamide (C₁₆H₁₆ClN₃O₃S) dissolved.

Tolerances: NLT 75% (Q) of the labeled amount of indapamide (C₁₆H₁₆ClN₃O₃S) is dissolved.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

Add the following:

■ • Organic Impurities

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 2 µg/mL each of USP Indapamide RS, USP Indapamide Related Compound A RS, USP Indapamide Related Compound B RS, and USP Indapamide Related Compound C RS in *Diluent*

Sample solution: Nominally 1.0 mg/mL of indapamide in *Diluent* prepared as follows. Transfer an amount equivalent to 20 mg of indapamide, from NLT 20 finely powdered Tablets, to a 20-mL volumetric flask. Dissolve in 15 mL of *Diluent* by sonicating for 15 min with intermittent shaking. Dilute with *Diluent* to volume. Centrifuge the solution for 10 min and use the supernatant.

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between indapamide related compound C and indapamide; NLT 2.0 between indapamide related compound B and indapamide related compound A

Relative standard deviation: NMT 3.0% for any peak

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of indapamide related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of indapamide related compound A from the *Sample solution*

r_S peak response of indapamide related compound A from the *Standard solution*

C_S concentration of USP Indapamide Related Compound A RS in the *Standard solution* (mg/mL)

C_U nominal concentration of indapamide in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any individual unspecified impurity from the *Sample solution*

r_S peak response of indapamide from the *Standard solution*

C_S concentration of USP Indapamide RS in the *Standard solution* (mg/mL)

C_U nominal concentration of indapamide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Indapamide related compound C	0.89	— ^a
Indapamide	1.0	—
Indapamide related compound B	1.11	— ^a
Indapamide related compound A	1.15	0.2
Any individual unspecified impurity	—	0.1
Total impurities	—	1.0

^a Indapamide related compound B and indapamide related compound C are process impurities that are both controlled in the drug substance. They are not reported in the drug product and should not be included in the total impurities. Indapamide related compound A is both a degradant and a process impurity.

■ 1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** { 11 }

USP Indapamide RS

■ USP Indapamide Related Compound A RS

4-Chloro-*N*-(2-methyl-1*H*-indol-1-yl)-3-sulfamoylbenzamide.

$C_{16}H_{14}ClN_3O_3S$ 363.82

USP Indapamide Related Compound B RS

2-Methyl-1-nitrosoindoline.

$C_9H_{10}N_2O$ 162.19

USP Indapamide Related Compound C RS

2-Methylindoline.

$C_9H_{11}N$ 133.19

■ 1S (USP39)

BRIEFING

Isoleucine, USP 38 page 3951. As part of the USP modernization effort, it is proposed to replace the TLC *Related Compounds* test with an HPLC *Related Compounds* test to better characterize the organic impurities. The liquid chromatographic procedure in the test for *Related Compounds* is based on analyses performed with the Phenomenex Luna C18 brand of L1 column. Typical retention times for isoleucine and leucine are 4.0 and 4.4 min, respectively.

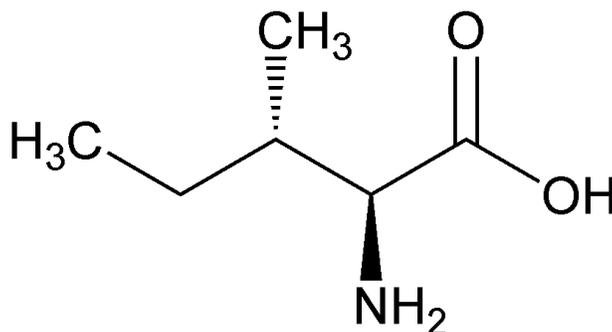
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(DS: H. Dinh.)

Correspondence Number—C135172

Comment deadline: May 31, 2015

Isoleucine



$C_6H_{13}NO_2$ 131.17

l-Isoleucine [73-32-5].

DEFINITION

Isoleucine contains NLT 98.5% and NMT 101.5% of *l*-isoleucine ($C_6H_{13}NO_2$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** { 197K }

ASSAY● **Procedure****Sample:** 130 mg of Isoleucine**Blank:** Mix 3 mL of formic acid and 50 mL of glacial acetic acid.**Titrimetric system**(See *Titrimetry* 〈 541 〉.)**Mode:** Direct titration**Titrant:** 0.1 N perchloric acid VS**Endpoint detection:** Potentiometric**Analysis:** Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid.Titrate with the *Titrant*. Perform the blank determination.Calculate the percentage of isoleucine ($C_6H_{13}NO_2$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

 V_S *Titrant* volume consumed by the *Sample* (mL) V_B *Titrant* volume consumed by the *Blank* (mL) N = actual normality of the *Titrant* (mEq/mL) F = equivalency factor, 131.2 mg/mEq W = *Sample* weight (mg)**Acceptance criteria:** 98.5%–101.5% on the dried basis**IMPURITIES**● **Residue on Ignition** 〈 281 〉: NMT 0.3%● **Chloride and Sulfate, Chloride** 〈 221 〉**Standard solution:** 0.50 mL of 0.020 N hydrochloric acid**Sample:** 0.73 g of Isoleucine**Acceptance criteria:** NMT 0.05%● **Chloride and Sulfate, Sulfate** 〈 221 〉**Standard solution:** 0.10 mL of 0.020 N sulfuric acid**Sample:** 0.33 g of Isoleucine**Acceptance criteria:** NMT 0.03%● **Iron** 〈 241 〉: NMT 30 ppm**Delete the following:**

●

● **Heavy Metals, Method I** 〈 231 〉

: NMT 15 ppm (Official 1-Dec-2015)

Change to read:● **Related Compounds**~~**System suitability solution:** 0.4 mg/mL each of USP I Isoleucine RS and USP I Valine RS in 0.1 N hydrochloric acid~~~~**Standard solution:** 0.05 mg/mL of USP I Isoleucine RS in 0.1 N hydrochloric acid. [Note — This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]~~

~~**Sample solution:** 10 mg/mL of Isoleucine in 0.1 N hydrochloric acid~~

~~**Chromatographic system**~~

~~(See *Chromatography* 〈 621 〉, *Thin-Layer Chromatography*.)~~

~~**Mode:** TLC~~

~~**Adsorbent:** 0.25-mm layer of chromatographic silica-gel mixture~~

~~**Application volume:** 5 μ L~~

~~**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (3:1:1)~~

~~**Spray reagent:** 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)~~

~~**System suitability**~~

~~**Suitability requirements:** The chromatogram of the *System suitability solution* exhibits two clearly separated spots.~~

~~**Analysis**~~

~~**Samples:** *System suitability solution*, *Standard solution*, and *Sample solution*~~

~~After air-drying the plate, spray with *Spray reagent*, and heat between 100^o and 105^o for 15 min. Examine the plate under white light.~~

~~**Acceptance criteria:** Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.~~

~~**Individual impurities:** NMT 0.5%~~

~~**Total impurities:** NMT 2.0%~~

■ **Buffer solution:** 0.2 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.8.

Mobile phase: Acetonitrile and *Buffer solution* (2:98)

System suitability solution: 0.25 mg/mL each of USP I-Leucine RS and USP I-Isoleucine RS in *Mobile phase*

Standard solution: 0.025 mg/mL of USP I-Leucine RS in *Mobile phase*

Sample solution: 5.0 mg/mL of Isoleucine in *Mobile phase*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 15-cm; 3- μ m packing L1

Column temperature: 40^o

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for isoleucine and leucine are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between leucine and isoleucine

Relative standard deviation: NMT 2.0% for each of leucine and isoleucine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of leucine in the portion of Isoleucine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of leucine from the *Sample solution*

r_S

= peak response of leucine from the *Standard solution*

C_S

= concentration of USP I-Leucine RS in the *Standard solution* (mg/mL)

C_U

= concentration of Isoleucine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Isoleucine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U

= peak response of any unspecified impurity from the *Sample solution*

r_T

= sum of the peak responses for all the peaks from the *Sample solution*

Acceptance criteria

Leucine: NMT 0.5%

Any unspecified impurity: NMT 0.2%

Total unspecified impurities: NMT 1.0%

■ 1S (USP39)

SPECIFIC TESTS

- **Optical Rotation, Specific Rotation** 〈 781S 〉
Sample solution: 40 mg/mL in 6 N hydrochloric acid
Acceptance criteria: +38.9° to +41.8°
- **pH** 〈 791 〉
Sample solution: 10 mg/mL in water
Acceptance criteria: 5.5–7.0
- **Loss on Drying** 〈 731 〉
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** < 11 >

USP L-Isoleucine RS

USP L-Valine RS

■ USP L-Leucine RS

■ 1S (USP39)

BRIEFING

Leucine, USP 38 page 4049. As part of the USP modernization effort, it is proposed to replace the TLC *Related Compounds* test with an HPLC *Related Compounds* test to better characterize the organic impurities. The liquid chromatographic procedure in the test for *Related Compounds* is based on analyses performed with the Phenomenex Luna C18 brand of L1 column. Typical retention times for isoleucine and leucine are 4.0 and 4.4 min, respectively.

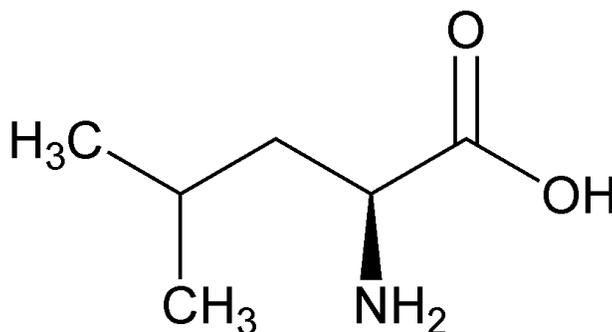
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(DS: H. Dinh.)

Correspondence Number—C135153

Comment deadline: May 31, 2015

Leucine



C₆H₁₃NO₂ 131.17

L-Leucine [61-90-5].

DEFINITION

Leucine contains NLT 98.5% and NMT 101.5% of L-leucine (C₆H₁₃NO₂), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** < 197K >

ASSAY

- **Procedure**

Sample: 130 mg of Leucine

Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* 〈 541 〉.)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid.

Titrate with the *Titrant*. Perform the *Blank* determination.

Calculate the percentage of leucine ($C_6H_{13}NO_2$) in the portion of the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S *Titrant* volume consumed by the *Sample* (mL)

V_B *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 131.2 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.4%

- **Chloride and Sulfate, Chloride** 〈 221 〉

Standard solution: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.73 g of Leucine

Acceptance criteria: NMT 0.05%

- **Chloride and Sulfate, Sulfate** 〈 221 〉

Standard solution: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.33 g of Leucine

Acceptance criteria: NMT 0.03%

- **Iron** 〈 241 〉: NMT 30 ppm

Delete the following:

-

- **Heavy Metals, Method II** 〈 231 〉

: NMT 15 ppm (Official 1-Dec-2015)

Change to read:

- **Related Compounds**

~~**System suitability solution:** 0.4 mg/mL each of USP I Leucine RS and USP I Valine RS in 0.1 N hydrochloric acid~~

~~**Standard solution:** 0.05 mg/mL of USP I Leucine RS in 0.1 N hydrochloric acid. [Note —This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]~~

~~**Sample solution:** 10 mg/mL of Leucine in 0.1 N hydrochloric acid~~

Chromatographic system

(See *Chromatography* \langle 621 \rangle , *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)

Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2-N acetic acid (95:5)

System suitability

Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

Analysis

Samples: *System suitability solution*, *Standard solution*, and *Sample solution*

After air-drying the plate, spray with *Spray reagent*, and heat between 100 $^{\circ}$ and 105 $^{\circ}$ for 15 min. Examine the plate under white light.

Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

■ **Buffer solution:** 0.2 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.8.

Mobile phase: Acetonitrile and *Buffer solution* (2:98)

System suitability solution: 0.25 mg/mL each of USP I-Leucine RS and USP I-Isoleucine RS in *Mobile phase*

Standard solution: 0.025 mg/mL of USP I-Isoleucine RS in *Mobile phase*

Sample solution: 5.0 mg/mL of Leucine in *Mobile phase*

Chromatographic system

(See *Chromatography* \langle 621 \rangle , *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 15-cm; 3- μ m packing L1

Column temperature: 40 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for isoleucine and leucine are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between leucine and isoleucine

Relative standard deviation: NMT 2.0% each for leucine and isoleucine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of isoleucine in the portion of Leucine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of isoleucine from the *Sample solution*

r_S

= peak response of isoleucine from the *Standard solution*

C_S

= concentration of USP I-Isoleucine RS in the *Standard solution* (mg/mL)

C_U

= concentration of Leucine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Leucine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U

= peak response of any unspecified impurity from the *Sample solution*

r_T

= sum of the peak responses of all the peaks from the *Sample solution*

Acceptance criteria

Isoleucine: NMT 0.8%

Any unspecified impurity: NMT 0.2%

Total unspecified impurities: NMT 1.0%

■ 1S (USP39)

SPECIFIC TESTS

- **Optical Rotation, Specific Rotation** 〈 781S 〉
Sample solution: 40 mg/mL in 6 N hydrochloric acid
Acceptance criteria: +14.9° to +17.3°
- **pH** 〈 791 〉
Sample solution: 10 mg/mL in water
Acceptance criteria: 5.5–7.0
- **Loss on Drying** 〈 731 〉
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP I-Valine RS

■ USP I-Isoleucine RS

- 1S (USP39)

USP I-Leucine RS

BRIEFING

Leucovorin Calcium Compounded Oral Suspension. Because there is currently no existing *USP* monograph for this dosage form, a new compounded preparation monograph is proposed based on a validated stability-indicating method used to assess stability. The liquid chromatographic procedure in the *Assay* is based on analyses validated using the Poroshell 120 SB-C8 brand of L7 column. The typical retention time for leucovorin calcium is about 20.3 min.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C154508

Comment deadline: May 31, 2015

Add the following:

■ **Leucovorin Calcium Compounded Oral Suspension**

DEFINITION

Leucovorin Calcium Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$).

Prepare Leucovorin Calcium Compounded Oral Suspension containing 5 mg/mL of leucovorin as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈 795 〉).

Leucovorin Calcium tablets ^a equivalent to	500 mg of leucovorin
Sodium Hydroxide (1 N)	To adjust pH to 7.1–7.6
Syrup, a sufficient quantity to make	100 mL
^a Leucovorin calcium 25-mg tablets, Teva Pharmaceuticals, Sellersville, PA.	

Place the *Leucovorin Calcium* tablets into a suitable container. Wet the tablets with a small amount of *Syrup* and triturate to make a smooth paste. Add the *Syrup* to make the contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the *Syrup*. Adjust with *Sodium Hydroxide (1 N)* to a pH of 7.1–7.6. Add sufficient *Syrup* to bring to final volume. Shake to mix well. [Note—pH may decrease to 6.1 after bringing to final volume with *Syrup* without affecting the stability of the preparation.]

ASSAY

- **Procedure**

Solution A: Methanol and 5 mM tetrabutylammonium phosphate (20:80). Adjust with tetrabutylammonium hydroxide to a pH of 6.6.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Methanol (%)	Solution A (%)
0	0	100
20	10	90
20.1	0	100
30	0	100

Diluent: Methanol and water (20:80)

Standard solution: 0.05 mg/mL of leucovorin prepared from USP Leucovorin Calcium RS and *Diluent*. Vortex and sonicate until dissolved.

Sample solution: Transfer 1.0 mL of Oral Suspension to a 100-mL volumetric flask, and rinse the pipette with about 2 mL of *Diluent*. Dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV-Vis 290 nm

Column: 4.6-mm × 15-cm; 2.7-μm packing L7

Column temperature: 55°

Flow rate: 0.75 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

[Note—The retention time for leucovorin is about 20.3 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of leucovorin (C₂₀H₂₃N₇O₇) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of leucovorin from USP Leucovorin Calcium RS in the *Standard solution* (mg/mL)

C_U nominal concentration of leucovorin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** 〈 791 〉: 6.1–7.1

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant plastic containers. Store in a refrigerator (2°–8°) or at controlled room temperature.
- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded when stored in a refrigerator (2°–8°); NMT 30 days after the date on which it was compounded when stored at controlled room temperature
- **Labeling:** Label it to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP Reference Standards** 〈 11 〉

USP Leucovorin Calcium RS

■ 1S (USP39)

BRIEFING

Levothyroxine Sodium, *USP 38* page 4089. In preparation for the omission of the flame tests from general chapter *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to replace the use of the flame test in *Identification* test *B* with the pyroantimonate precipitation test currently described in 〈 191 〉. This test is also consistent with test (a) in 2.3.1. *Identification reactions of ions and functional groups* in the *European Pharmacopoeia*. The preparation of the *Sample solution* is adopted from the monograph for *Levothyroxine Sodium* in the *European Pharmacopoeia*.

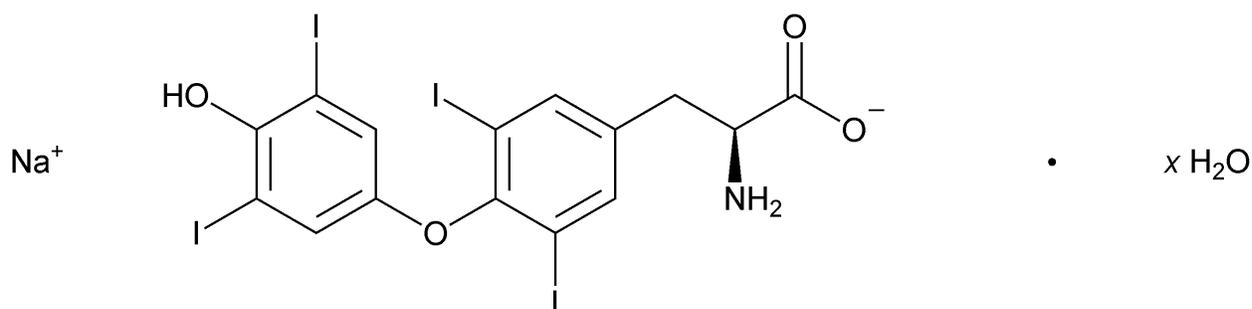
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: R.S. Prasad.)

Correspondence Number—C154668

Comment deadline: May 31, 2015

Levothyroxine Sodium



$C_{15}H_{10}I_4NNaO_4 \cdot xH_2O$ (anhydrous) 798.85

l-Tyrosine, *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-, monosodium salt, hydrate;

Monosodium *l*-thyroxine hydrate [25416-65-3].

Anhydrous [55-03-8].

DEFINITION

Levothyroxine Sodium is the sodium salt of l-3,3',5,5'-tetraiodothyronine. It contains NLT 97.0% and NMT 103.0% of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈 197 〉: [Note—Methods described in *Infrared Absorption* 〈 197K 〉 or 〈 197A 〉 may be used.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **C. Identification Tests—General, Sodium** 〈 191 〉

Sample solution: ~~Ignite 50 mg in a platinum dish over a flame and cool the residue. Add a 1 N potassium hydroxide solution dropwise until the residue is dissolved.~~

- To 200 mg add 2 mL of 1 N sulfuric acid. Heat on a water bath and then carefully heat over an open flame, increasing the temperature gradually up to about 600°. Continue the ignition until most of the particles have disappeared. Dissolve the residue in 2 mL of water.

■ 1S (USP39)

Acceptance criteria: The *Sample solution* meets the requirements of the flame

■ pyroantimonate precipitation ■ 1S (USP39)

test.

ASSAY

- **Procedure**

Mobile phase: Acetonitrile and water (4:6) that contains 0.5 mL of phosphoric acid in each 1000 mL

Solution A: 400 mg of sodium hydroxide in 500 mL of water. Cool and add 500 mL of methanol.

Levothyroxine stock solution: 0.4 mg/mL of USP Levothyroxine RS in *Solution A*

Liothyronine stock solution: 0.4 mg/mL of liothyronine from USP Liothyronine RS in *Solution A*. Make a 1:100 dilution of this solution using *Mobile phase*.

Standard solution: 10 µg/mL of levothyroxine from *Levothyroxine stock solution* and 0.2 µg/mL of liothyronine from *Liothyronine stock solution* in *Mobile phase*

Sample solution: 10 µg/mL of Levothyroxine Sodium in *Mobile phase*. [Note—A small amount of 0.01 M methanolic sodium hydroxide can be used to facilitate the dissolution of the sample.]

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; packing L10

Flow rate: 1.5 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between liothyronine and levothyroxine

Relative standard deviation: NMT 2.0% for levothyroxine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of levothyroxine from the *Sample solution*

r_S = peak response of levothyroxine from the *Standard solution*

C_S = concentration of USP Levothyroxine RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of Levothyroxine Sodium in the *Sample solution* ($\mu\text{g/mL}$)

M_{r1} = molecular weight of levothyroxine sodium, 798.85

M_{r2} = molecular weight of levothyroxine, 776.87

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

[Note—On the basis of the synthetic route, perform either *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2*. *Procedure 2* is recommended when related compounds listed in *Table 3* may be present.]

• **Organic Impurities, Procedure 1**

Diluent: Acetonitrile and water (1:1)

Solution A: Dilute 5 mL of phosphoric acid with *Diluent* to 100.0 mL.

Mobile phase: Dissolve 1.0 g of sodium 1-heptanesulfonate in 200 mL of water. Add 200 mL of acetonitrile, 400 mL of methanol, and 1.0 mL of phosphoric acid. Dilute with water to 1 L.

Standard stock solution 1: Transfer 25 mg of USP Levothyroxine RS to a 100-mL volumetric flask. Add 50 mL of *Diluent* and 1 drop of 10 N sodium hydroxide, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Standard stock solution 2: Transfer 25 mg of USP Liothyronine RS to a 100-mL volumetric flask. Add 50 mL of *Diluent* and 1 drop of 10 N sodium hydroxide, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

System suitability solution: Transfer 5.0 mL of *Standard stock solution 1* and 5.0 mL of *Standard stock solution 2* to a 100-mL volumetric flask. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Standard solution: Pipet 4.0 mL of the *System suitability solution* into a 100-mL volumetric flask. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Sample solution: Transfer 25 mg of Levothyroxine Sodium to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Blank solution: Transfer 7 mL of *Solution A* to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 15 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between levothyroxine and liothyronine, *System suitability solution*

Relative standard deviation: NMT 2.0% for the levothyroxine peak, *Standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank solution*

[Note—Record the chromatograms for at least six times the retention time of the levothyroxine peak. Verify that no peaks elute in the *Blank solution* at the expected retention times for levothyroxine and related compounds.]

Calculate the area percentage of each related compound in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of levothyroxine from the *Standard solution*

C_S = concentration of levothyroxine in the *Standard solution* (mg/mL)

C_U = concentration of Levothyroxine Sodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of levothyroxine sodium, 798.85

M_{r2} = molecular weight of levothyroxine, 776.87

[Note—The relative response factor for the impurities listed in *Table 1* is 1.00. Any unspecified impurity peaks should be assigned a relative response factor of 1.00.]

Disregard peaks corresponding to those of the *Blank solution*, and disregard peaks corresponding to less than 0.03%.

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Liothyronine	0.65–0.70	1.0
β-Hydroxy-T4 ^a	0.71–0.76	0.15
Levothyroxine	1.0	—
T4-Hydroxyacetic acid ^b	1.13–1.28	0.15
N-Formyl-T4 ^c and T4-acetamide ^d	1.47–1.53	0.15

<i>N</i> -Acetyl-T4 ^e	1.50–1.86	0.20
T4-Acetic acid ^f	2.42–2.51	0.30
T4-Aldehyde ^g	3.17–3.45	0.15
T4-Benzoic acid ^h	3.46–3.70	0.15
Individual unspecified impurity	—	0.10
Total impurities	—	2.0

a *O*-(4-Hydroxy-3,5-diiodophenyl)-3,5-diiodo-β-hydroxy-L-tyrosine.
b 2-Hydroxy-2-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetic acid.
c *N*-Formyl-*O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.
d 2-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetamide.
e *N*-Acetyl-*O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.
f 2-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetic acid.
g 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzaldehyde.
h 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzoic acid.

● **Organic Impurities, Procedure 2**

Solution A: Dissolve 9.7 g of sulfamic acid in 2000 mL of water. Add 1.5 g of sodium hydroxide, mix to dissolve, and adjust with 2 N sodium hydroxide to a pH of 2.0.

Solution B: Acetonitrile

Diluent 1: Methanol and *Solution A* (90:10)

Diluent 2: Acetonitrile and *Solution A* (30:70); mix with *Diluent 1* (1:1).

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	70	30
10	70	30
40	20	80
50	20	80
53	70	30
75	70	30

Identification solution: Dissolve 5.0 mg of USP Levothyroxine for Peak Identification RS in 4.5 mL of methanol. Add 0.5 mL of *Solution A*. Further dilute a portion of this solution with *Diluent 2* to obtain a solution containing about 0.2 mg/mL.

Standard stock solution: 0.1 mg/mL each of USP Levothyroxine RS and USP Liothyronine RS in *Diluent 1*

Standard solution: 0.002 mg/mL each of USP Levothyroxine RS and USP Liothyronine RS, prepared using the *Standard stock solution* in *Diluent 2*

Sensitivity solution: 0.0002 mg/mL each of USP Levothyroxine RS and USP Liothyronine RS, prepared using the *Standard solution* in *Diluent 2*

Sample solution: Dissolve an amount of Levothyroxine Sodium in *Diluent 1* to obtain a solution with a known concentration of about 1.0 mg/mL. Further dilute a portion of this solution with *Diluent 2* to obtain a solution with a known concentration of about 0.2 mg/mL.

Blank solution: Use *Diluent 2*.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.0-mm × 15-cm; 3-μm packing L1

Flow rate: 1.0 mL/min

Injection volume: 25 μL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 5 between levothyroxine and liothyronine, *Standard solution*

Signal-to-noise ratio: NLT 5 for each peak from the *Sensitivity solution*, calculated as follows:

$$\text{Result} = (2H)/h$$

H = measured height of the peak

h = amplitude of the average measured baseline noise

Analysis

Samples: *Blank solution*, *Standard solution*, *Identification solution*, and *Sample solution*

[Note—Identify the components on the basis of their relative retention times as listed in *Table 3*.]

Calculate the percentage of liothyronine sodium in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of liothyronine from the *Sample solution*

r_S = peak response of liothyronine from the *Standard solution*

C_S = concentration of liothyronine in the *Standard solution* (mg/mL)

C_U = concentration of Levothyroxine Sodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of liothyronine sodium, 672.96

M_{r2} = molecular weight of liothyronine, 650.98

Calculate the percentage of any other impurity in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of any impurity from the *Sample solution*

r_S = peak response of levothyroxine from the *Standard solution*

C_S = concentration of levothyroxine in the *Standard solution* (mg/mL)

C_U = concentration of Levothyroxine Sodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of levothyroxine sodium, 798.85

M_{r2} = molecular weight of levothyroxine, 776.87

[Note—The relative response factor for the impurities listed in *Table 3* is 1.00. Any

unspecified impurity peaks should be assigned a relative response factor of 1.00.]

Disregard peaks corresponding to those of the *Blank solution*, and disregard peaks corresponding to less than 0.03%.

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Liothyronine	0.65	1.0
Monochlorotriiodothyronine ^a	0.94	0.15
Levothyroxine <i>N</i> -methylamide ^b	0.97	0.15
Levothyroxine	1.0	—
Triiodothyroacetic acid, or T3-acetic acid ^c	1.57	0.15
<i>O</i> -(4-Hydroxy-3,5-diiodophenyl)thyroxine, or T6 ^d	1.61	0.50
<i>O</i> -Methyl-tetraiodothyroethylamine, or T4-amine <i>O</i> -methyl ^e	1.76	0.30
T4-Acetic acid ^f	1.79	0.30
Individual unspecified impurity	—	0.10
Total impurities	—	2.0

a (S)-2-Amino-3-[3-chloro-4-(4-hydroxy-3,5-diiodophenoxy)-5-iodophenyl]propanoic acid.
b (S)-2-Amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]-*N*-methylpropanamide.
c [4-(4-Hydroxy-3-iodophenoxy)-3,5-diiodophenyl]acetic acid.
d (S)-2-Amino-3-[4-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenoxy]-3,5-diiodophenyl]propanoic acid.
e 2-[4-(3,5-Diiodo-4-methoxyphenoxy)-3,5-diiodophenyl]ethanamine.
f 2-(4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetic acid.

SPECIFIC TESTS

- **Optical Rotation**, *Specific Rotation* 〈 781S 〉
Sample solution: Equivalent to 30 mg/mL of anhydrous Levothyroxine Sodium in alcohol and 1 N sodium hydroxide (2:1)
Acceptance criteria: -5° to -6°
- **Water Determination**, *Method I* 〈 921 〉: NMT 11.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, protected from light. Store as stated in the labeling instructions.
- **Labeling:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.
- **USP Reference Standards** 〈 11 〉
USP Levothyroxine RS

O-(4-Hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.

$C_{15}H_{11}I_4NO_4$ 776.87

USP Levothyroxine for Peak Identification RS

Levothyroxine sodium spiked with liothyronine, triiodothyroacetic acid, and tetraiodothyroacetic acid.

USP Levothyroxine Sodium RS

USP Liothyronine RS

O-(4-Hydroxy-3-iodophenyl)-3,5-diiodo-L-tyrosine.

$C_{15}H_{12}I_3NO_4$ 650.98

BRIEFING

Losartan Potassium Tablets, *USP 38* page 4152. Based on the comments received, it is proposed to include a chromatographic procedure for *Dissolution, Test 1*. The liquid chromatographic procedure for analysis is based on a 5- μ m, Inertsil ODS-3 brand of L1 column. The typical retention time for losartan is about 6 min under the specified conditions.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: S. Ramakrishna.)

Correspondence Number—C154346

Comment deadline: May 31, 2015

Losartan Potassium Tablets

DEFINITION

Losartan Potassium Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: 1.25 mg/mL of monobasic potassium phosphate and 1.5 mg/mL of dibasic sodium phosphate in water. The resulting pH is approximately 7.0. Pass the solution through a PTFE or equivalent filter of 0.45- μ m pore size, and degas before use.

Solution A: Acetonitrile and *Buffer* (15:85)

Solution B: Use acetonitrile.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
10	40	60
11	80	20

Time (min)	Solution A (%)	Solution B (%)
15	80	20

System suitability stock solution: Dissolve 12 mg of USP Losartan Potassium RS in a 50-mL volumetric flask, first using 5 mL of water, followed by 5 mL of 0.1 N hydrochloric acid. Place the flask in a 105° oven for 1–2 h, and allow to cool to room temperature. Pipet 5 mL of 0.1 N sodium hydroxide into the flask, and dilute with water to volume. Adjust with either 0.1 N hydrochloric acid or 0.1 N sodium hydroxide to a pH of 6.0. [Note—The resulting solution contains the 1*H*-dimer and 2*H*-dimer, and the resulting solution may be cloudy.]

System suitability solution: Add 3 mL of acetonitrile to 7 mL of *System suitability stock solution* to clear the cloudy solution, and mix well.

Standard solution: 0.25 mg/mL of USP Losartan Potassium RS in *Solution A*. Pass through a PTFE or equivalent filter of 0.45- μ m pore size.

Sample stock solution: Transfer 10 Tablets to a 500-mL volumetric flask, add *Solution A* to fill the flask to about 50% of the final volume, and sonicate with intermittent shaking for 15 min. Sonicate for an additional 10 min. Dilute with *Solution A* to volume, and mix well.

Sample solution: 0.25 mg/mL of losartan potassium in *Solution A* from the *Sample stock solution*. Mix well. Pass an aliquot of the solution through a PTFE filter of 0.45- μ m pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 3.9-mm \times 15-cm; 5- μ m packing L7

Flow rate: 1.0 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for the losartan, 1*H*-dimer, and 2*H*-dimer peaks; *System suitability solution*

Resolution: NLT 2.0 between the 1*H*-dimer and 2*H*-dimer, *System suitability solution*

Column efficiency: NLT 3000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of losartan potassium (C₂₂H₂₂ClKN₆O) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of losartan from the *Sample solution*

r_S = peak response of losartan from the *Standard solution*

C_S concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)

C_T nominal concentration of losartan potassium in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

Change to read:

• Dissolution 〈 711 〉

Test 1

Medium: Water; 900 mL, deaerated

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: $(L/1000)$ mg/mL of USP Losartan Potassium RS in *Medium*, where L is the Tablet label claim, in mg

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Analysis: Determine the amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved by using ~~UV absorption at the wavelength of maximum absorbance at about 256 nm on portions of the *Sample solution* in comparison with the *Standard solution*, using *Medium* as blank. Use the appropriate cell size as listed in *Table 2*, or make the appropriate dilution of the solutions with *Medium* to be within the linearity range of the spectrophotometer.~~

■ one of the following procedures:

Spectrophotometric procedure

Analytical wavelength: Maximum absorbance at about 256 nm

Blank: *Medium*

Cell path length: See *Table 2* or make the appropriate dilution of the solutions with *Medium* to be within the linearity range of the spectrophotometer. ■ 1S (USP39)

Table 2

Tablet Strength (mg/Tablet)	Cell Size (cm)
25	1.0
50	0.5
100	0.2

Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

■ Chromatographic procedure

Solution A: 0.1% v/v phosphoric acid in water

Mobile phase: Acetonitrile and *Solution A* (40:60)

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.0-mm × 25-cm; 5-μm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: NLT 1.5 times the retention time of losartan

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)

L

= label claim (mg/Tablet)

V

= volume of *Medium*, 900 mL

■ 1S (USP39)

Tolerances: NLT 75% (Q) of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets *USP Dissolution Test 2*.

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 30 min

Buffer: 1.4 g/L of anhydrous monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.3 ± 0.1 .

Mobile phase: Methanol, acetonitrile, and *Buffer* (20:20:60)

Standard solution: 0.028 mg/mL of USP Losartan Potassium RS in *Medium*

Sample solution

For Tablets labeled to contain 25 mg: Pass a portion of the solution under test through a

suitable filter of 0.45- μm pore size.

For Tablets labeled to contain 50 and 100 mg: Pass a portion of the solution under test through a suitable filter of 0.45- μm pore size. Further dilute the filtrate with *Medium* to prepare a 0.028-mg/mL solution.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm \times 15-cm; 5- μm packing L10

Column temperature: 45 $^{\circ}$

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of losartan potassium ($\text{C}_{22}\text{H}_{22}\text{ClKN}_6\text{O}$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (Q) of the labeled amount of losartan potassium ($\text{C}_{22}\text{H}_{22}\text{ClKN}_6\text{O}$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

Medium: Water; 900 mL, deaerated

Apparatus 2: 50 rpm

Time: 30 min for 25-mg and 50-mg Tablet strengths, and 45 min for 100-mg Tablet strength

Buffer: 0.025 M phosphoric acid. Adjust with 1 N sodium hydroxide to a pH of 2.15.

Mobile phase: Acetonitrile and *Buffer* (400:600)

Standard stock solution: 0.27 mg/mL of USP Losartan Potassium RS prepared as follows. Add methanol to USP Losartan Potassium RS to fill about 10% of the volume of the flask, and add *Medium* to fill about 50% of the volume of the flask. Sonicate for NLT 15 min. Cool to room temperature, and dilute with *Medium* to volume.

Standard solution: Prepare as directed in *Table 3* from the *Standard stock solution*.

Table 3

Tablet Strength (mg/Tablet)	Concentration (mg/mL)
25	0.027
50	0.054
100	0.108

Sample solution: Pass a portion of the solution under test through a suitable polyethylene filter of 10- μ m pore size.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 10-cm; 3.5- μ m packing L7

Column temperature: 40 $^{\circ}$

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of losartan from the *Sample solution*

r_S = peak response of losartan from the *Standard solution*

C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) is dissolved for 25-mg and 50-mg Tablet strengths. NLT 80% (Q) of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) is dissolved for 100-mg Tablet strength.

• Uniformity of Dosage Units 〈 905 〉

Procedure for content uniformity

Buffer: Dissolve 1.36 mg/mL of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

Diluent: Dissolve 17.42 g of dibasic potassium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 8.0. Dilute with water to a volume of 1000 mL, and mix well. Further dilute with water (1 in 10), and mix well.

Mobile phase: Acetonitrile and *Buffer* (60:40)

Standard solution: 0.05 mg/mL of USP Losartan Potassium RS in *Diluent*

Sample stock solution: Transfer 1 Tablet to a 100-mL volumetric flask, add about 65 mL

of *Diluent*, and shake mechanically for 30 min. Dilute with *Diluent* to volume, and mix well.

Sample solution: 0.05 mg/mL of losartan potassium in *Diluent* from the *Sample stock solution*. Filter an aliquot of the solution, and use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 10-μm packing L7

Flow rate: 1.4 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) in the portion of the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of losartan from the *Sample solution*

r_S peak response of losartan from the *Standard solution*

C_S concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)

C_U nominal concentration of losartan potassium in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• Organic Impurities

Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Prepare as directed in the *Assay*.

Standard stock solution: Use the *Standard solution*, prepared as directed in the *Assay*.

Standard solution: 2.5 μg/mL of USP Losartan Potassium RS in *Solution A* from the *Standard stock solution*

Sensitivity solution: Dilute 1 mL of the *Standard solution* to 10 mL in *Solution A*.

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Tailing factor: NMT 2.0 for the losartan, 1*H*-dimer, and 2*H*-dimer peaks; *System suitability solution*

Resolution: NLT 2.0 between the 1*H*-dimer and 2*H*-dimer, *System suitability solution*

Column efficiency: NLT 3000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10 for the losartan peak from the first injection. If this is not met, then the *Signal-to-noise ratio* must be greater than 3 with a relative standard deviation of area counts less than 25% for three replicate injections, *Sensitivity solution*.

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—Identify the peaks using the relative retention times provided in *Table 4*.]

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of losartan from the *Standard solution*

C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of losartan potassium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Losartan	1.0	—
1 <i>H</i> -Dimer ^a	2.4	0.5
2 <i>H</i> -Dimer ^b	2.9	0.5
Total impurities ^c	—	1.0

^a 5-[4'-({2-Butyl-5-[(5-{4'-[(2-butyl-4-chloro-5-hydroxymethyl-1*H*-imidazol-1-yl)methyl]biphenyl-2-yl})-1*H*-tetrazol-1-yl)methyl]-4-chloro-1*H*-imidazol-1-yl)methyl]biphenyl-2-yl]tetrazol, potassium salt.

^b 5-[4'-({2-Butyl-5-[(5-{4'-[(2-butyl-4-chloro-5-hydroxymethyl-1*H*-imidazol-1-yl)methyl]biphenyl-2-yl})-2*H*-tetrazol-2-yl)methyl]-4-chloro-1*H*-imidazol-1-yl)methyl]biphenyl-2-yl]tetrazol, potassium salt.

^c The total impurities include the sum of all the specified impurities and the sum of all the unspecified impurities. Disregard peaks less than 0.1%.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store in tightly closed containers, protected from light, at controlled room temperature.
- **Labeling:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP Reference Standards** { 11 }
USP Losartan Potassium RS

BRIEFING

Methylprednisolone, *USP 38* page 4351. As part of the USP monograph modernization effort, the following revisions are proposed:

1. Add an *Identification* test based on HPLC with the retention time match for

methylprednisolone.

2. Delete the *Ultraviolet Absorption* test for *Identification* because it is nonspecific and the monograph now has two orthogonal identification tests.
3. Delete the wet chemical test as it is nonspecific and adds no value to ensuring the quality of the drug substance.
4. Replace the existing *Assay* with a validated stability-indicating HPLC procedure which also is used in the newly introduced test for *Organic Impurities*. The proposed liquid chromatographic procedure is based on analyses performed with the Acquity UPLC BEH C18 brand of L1 column.
5. Add a test for *Organic Impurities* based on the HPLC procedure and performed with the Acquity UPLC BEH C18 brand of L1 column. The typical retention time for methylprednisolone is about 11.1 min.
6. Add the following three new Reference Standards, introduced by the proposed HPLC procedure, to the *USP Reference Standards* section: USP Methylprednisolone Related Compound A RS, USP Methylprednisolone Related Compound C RS, and USP Methylprednisolone Related Compound D RS.

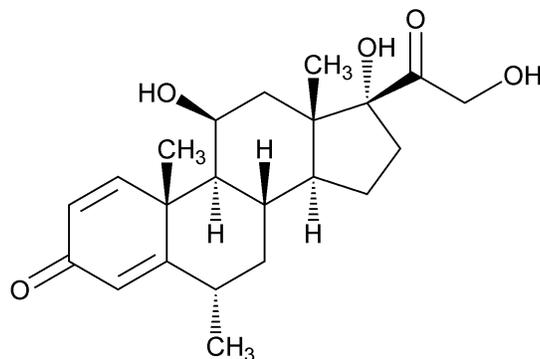
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: C. Anthony.)

Correspondence Number—C139785

Comment deadline: May 31, 2015

Methylprednisolone



$C_{22}H_{30}O_5$ 374.47

Pregna-1,4-diene-3,20-dione, 11,17,21-trihydroxy-6-methyl-, (6 α ,11 β)-;
11 β ,17,21-Trihydroxy-6 α -methylpregna-1,4-diene-3,20-dione [83-43-2].

DEFINITION

Methylprednisolone contains NLT 97.0% and NMT 103.0% of methylprednisolone ($C_{22}H_{30}O_5$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)

~~Delete the following:~~

~~Delete the following:~~

- ~~B. Ultraviolet Absorption~~ ~~(197U)~~

~~Analytical wavelength:~~ 243 nm

~~Sample solution:~~ 10 µg/mL in alcohol

~~Acceptance criteria:~~ Absorptivities, calculated on the dried basis, do not differ by more than 3.0%. ■ 1S (USP39)

~~Delete the following:~~

- ~~C.~~

~~Sample:~~ 5 mg

~~Analysis:~~ Dissolve the *Sample* in 2 mL of sulfuric acid.

~~Acceptance criteria:~~ A red color is produced. ■ 1S (USP39)

~~Add the following:~~

- ~~B.~~ The retention time of the major peak for methylprednisolone of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

ASSAY~~Change to read:~~

- ~~Procedure~~

~~Mobile phase:~~ Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)

~~Solution A:~~ Chloroform and glacial acetic acid (97:3)

~~Internal standard solution:~~ 0.2 mg/mL of prednisone in *Solution A*

~~Standard solution:~~ 0.2 mg/mL of USP Methylprednisolone RS in the *Internal standard solution*

~~Sample solution:~~ 0.2 mg/mL of Methylprednisolone in the *Internal standard solution*

~~Chromatographic system~~

~~(See *Chromatography* (621), *System Suitability*.)~~

~~Mode:~~ LC

~~Detector:~~ UV 254 nm

~~Column:~~ 4.0 mm × 25.0 cm; packing L3

~~Flow rate:~~ 1 mL/min

~~Injection volume:~~ 10 µL

~~System suitability~~

~~Sample:~~ *Standard solution*

~~[Note—The relative retention times for prednisone and methylprednisolone are about 0.7 and 1.0, respectively.]~~

~~Suitability requirements~~

~~Resolution:~~ NLT 4.0 between methylprednisolone and prednisone

~~Relative standard deviation:~~ NMT 2.0%

~~Analysis~~

~~Samples:~~ *Standard solution* and *Sample solution*

Calculate the percentage of methylprednisolone ($C_{22}H_{30}O_5$) in the portion of Methylprednisolone taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U peak response ratio of methylprednisolone to prednisone from the *Sample solution*

R_S peak response ratio of methylprednisolone to prednisone from the *Standard solution*

C_S concentration of USP Methylprednisolone RS in the *Standard solution* (mg/mL)

C_U concentration of Methylprednisolone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

■ **Solution A:** 0.1% Formic acid

Solution B: Methanol

Diluent: *Solution B* and water (1:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	50	50
3.0	50	50
17.0	30	70
22.0	30	70
22.1	50	50
25.0	50	50

System suitability solution: 5 µg/mL each of USP Methylprednisolone RS and USP Methylprednisolone Related Compound C RS in *Diluent*, prepared as follows. Dissolve suitable quantities of USP Methylprednisolone RS and USP Methylprednisolone Related Compound C RS in methanol in a volumetric flask, sonicate, and dilute with *Diluent* to volume.

Standard solution: 0.1 mg/mL of USP Methylprednisolone RS in *Diluent*, prepared as follows. Transfer an appropriate quantity of USP Methylprednisolone RS to a suitable volumetric flask, dissolve in 2.5% of the flask volume of methanol, sonicate, and dilute with *Diluent* to volume.

Sample solution: 0.1 mg/mL of Methylprednisolone in *Diluent* prepared as follows. Transfer an appropriate quantity of Methylprednisolone to a suitable volumetric flask and dissolve in 25% of the flask volume of methanol, sonicate and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: 254 nm

Column: 2.1-mm × 15-cm; 1.7-µm packing L1

Temperatures

Column: 40°

Sample: 4°

Flow rate: 0.2 mL/min

Injection volume: 2 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for methylprednisolone and methylprednisolone related compound C are 1.0 and 1.07, respectively.]

Suitability requirements

Resolution: NLT 2.0 between methylprednisolone and methylprednisolone related compound C, *System suitability solution*

Tailing factor: NMT 2.0 for methylprednisolone, *Standard solution*

Relative standard deviation: NMT 0.73% for methylprednisolone, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylprednisolone ($C_{22}H_{30}O_5$) in the portion of Methylprednisolone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Methylprednisolone RS in the *Standard solution* (mg/mL)

C_U

= concentration of Methylprednisolone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis \blacksquare_{1S} (USP39)

IMPURITIES

- **Residue on Ignition** $\langle 281 \rangle$: NMT 0.2%

Change to read:

- **Organic Impurities**

Mobile phase: ~~Tetrahydrofuran, dimethyl sulfoxide, butanol, and water (40:10:1:149)~~

Diluent: ~~Tetrahydrofuran, glacial acetic acid, and water (25:3:72)~~

Standard solution: ~~0.01 mg/mL of USP Methylprednisolone RS in Diluent~~

Sample solution: ~~1.0 mg/mL of Methylprednisolone in Diluent~~

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: ~~LC~~

~~**Detector:** UV 254 nm~~

~~**Column:** 4.6 mm × 20 cm; packing L1~~

~~**Flow rate:** 1 mL/min~~

~~**Injection volume:** 10 µL~~

~~**System suitability**~~

~~**Sample:** *Standard solution*~~

~~**Suitability requirements**~~

~~**Column efficiency:** NLT 800 theoretical plates~~

~~**Relative standard deviation:** NMT 5.0%~~

~~**Analysis**~~

~~**Samples:** *Standard solution* and *Sample solution*~~

Calculate the percentage of each impurity in the portion of Methylprednisolone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

~~r_U~~ peak response for each impurity from the *Sample solution*

~~r_S~~ peak response for methylprednisolone from the *Standard solution*

~~C_S~~ concentration of USP Methylprednisolone RS in the *Standard solution* (mg/mL)

~~C_U~~ concentration of Methylprednisolone in the *Sample solution* (mg/mL)

~~**Acceptance criteria**~~

~~**Any individual impurity:** NMT 1.0%~~

~~**Total impurities:** NMT 2.0%~~

■ Solution A, Solution B, Diluent, Mobile phase, and Chromatographic system:

Proceed as directed in the Assay.

Standard solution: 5 µg/mL each of USP Methylprednisolone RS, USP

Methylprednisolone Related Compound A RS, USP Methylprednisolone Related Compound C RS, and USP Methylprednisolone Related Compound D RS in *Diluent*, prepared as follows. Dissolve suitable quantities of USP Methylprednisolone RS, USP

Methylprednisolone Related Compound A RS, USP Methylprednisolone Related Compound C RS, and USP Methylprednisolone Related Compound D RS in methanol in a volumetric flask, sonicate, and dilute with *Diluent* to volume.

Sample solution: 0.5 mg/mL of Methylprednisolone in *Diluent* prepared as follows.

Transfer an appropriate amount of Methylprednisolone to a suitable volumetric flask and dissolve in 25% of the flask volume of methanol, sonicate, and dilute with *Diluent* to volume.

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between methylprednisolone and methylprednisolone related compound C

Relative standard deviation: NMT 2.0% for methylprednisolone, methylprednisolone related compound A, methylprednisolone related compound C, and methylprednisolone related compound D

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylprednisolone related compound A, methylprednisolone

related compound C, or methylprednisolone related compound D in the portion of Methylprednisolone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of methylprednisolone related compound A, methylprednisolone related compound C, or methylprednisolone related compound D from the *Sample solution*

r_S

= peak response of the corresponding related compound from the *Standard solution*

C_S

= concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

C_U

= concentration of Methylprednisolone in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Methylprednisolone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of any unspecified impurity from the *Sample solution*

r_S

= peak response of methylprednisolone from the *Standard solution*

C_S

= concentration of USP Methylprednisolone RS in the *Standard solution* (mg/mL)

C_U

= concentration of Methylprednisolone in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methylprednisolone related compound A	0.81	1.0
Methylprednisolone	1.0	—
Methylprednisolone related compound C	1.07	1.0

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methylprednisolone related compound D	1.49	1.0
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

■ 1S (USP39)

SPECIFIC TESTS

- **Optical Rotation, Specific Rotation** 〈 781S 〉

Sample solution: 5 mg/mL in dioxane

Acceptance criteria: +79° to +86°

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Methylprednisolone RS

- USP Methylprednisolone Related Compound A RS

17,21-Dihydroxy-6 α -methylpregna-1,4-diene-3,11,20-trione.

C₂₂H₂₈O₅ 372.45

USP Methylprednisolone Related Compound C RS

11 β -Hydroxy-6 α -methylandrosta-1,4-diene-3,17-dione.

C₂₀H₂₆O₃ 314.42

USP Methylprednisolone Related Compound D RS

11 β ,20-Dihydroxy-6 α -methylpregna-1,4,17(20)-triene-3,21-dione.

C₂₂H₂₈O₄ 356.46

■ 1S (USP39)

BRIEFING

Methylprednisolone Tablets, USP 38 page 4352. As part of the USP monograph modernization effort, the following revisions are proposed:

1. Add an *Identification* test based on HPLC with the retention time match for methylprednisolone.
2. Delete the wet chemical test as it is nonspecific and adds no value to ensuring the quality of the drug substance.
3. Replace the existing *Assay* with a validated stability-indicating UHPLC procedure which also is used in the newly introduced test for *Organic Impurities*. The proposed liquid chromatographic procedure is based on analyses performed with the Acquity

UPLC BEH C18 brand of L1 column.

4. Add a test for *Organic Impurities* based on the UHPLC procedure and performed with the Acquity UPLC BEH C18 brand of L1 column. The typical retention time for methylprednisolone is about 10.7 min.
5. Prednisone is replaced with USP Prednisone RS in the test for *Uniformity of Dosage Units*, and USP Prednisone RS is added to the *USP Reference Standards* section.
6. Add the following three new Reference Standards, introduced by the proposed HPLC procedure, to the *USP Reference Standards* section: USP Methylprednisolone Related Compound A RS, USP Methylprednisolone Related Compound C RS, and USP Methylprednisolone Related Compound D RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: C. Anthony.)

Correspondence Number—C120149

Comment deadline: May 31, 2015

Methylprednisolone Tablets

DEFINITION

Methylprednisolone Tablets contain NLT 92.5% and NMT 107.5% of the labeled amount of methylprednisolone ($C_{22}H_{30}O_5$).

IDENTIFICATION

Change to read:

- **A.**

■ **Infrared Absorption** ~~(197K)~~ ■_{1S} (USP39)

~~**Sample:** Digest 40 mg of methylprednisolone from powdered Tablets with 25 mL of solvent hexane for 15 min. Filter, and discard the filtrate. Digest the residue with 25 mL of chloroform for 15 min. Filter, evaporate the filtrate to dryness, and dry at 105° for 2 h. Use the residue in Analysis 1 and Analysis 2~~

~~**Analysis 1:** Infrared Absorption ~~(197K)~~~~

~~**Acceptance criteria 1:** Meets the requirements~~

~~**Analysis 2:** Dissolve 5 mg of *Sample* in 2 mL of sulfuric acid~~

~~**Acceptance criteria 2:** a red color is produced~~

■ **Sample:** Digest 40 mg of methylprednisolone from powdered Tablets with 25 mL of solvent hexane for 15 min. Filter, and discard the filtrate. Digest the residue with 25 mL of chloroform for 15 min. Filter, evaporate the filtrate to dryness, and dry at 105° for 2 h.

Acceptance criteria: Meets the requirements ■_{1S} (USP39)

Add the following:

- **B.** The retention time of the major peak for methylprednisolone of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■_{1S} (USP39)

ASSAY**Change to read:**● **Procedure**

Mobile phase: ~~Butyl chloride, water saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)~~

Solution A: ~~chloroform and glacial acetic acid (97:3)~~

Internal standard solution: ~~0.2 mg/mL of prednisone in *Solution A*~~

Standard solution: ~~0.2 mg/mL of USP Methylprednisolone RS in the *Internal standard solution*~~

Sample solution: ~~Transfer the equivalent to 10 mg of methylprednisolone from powdered Tablets (NLT 20 Tablets) to a suitable container. Add 2.5 mL of water and swirl to form a fine slurry. Add 50.0 mL of *Internal standard solution*, and shake for 15 min. Filter or centrifuge a portion of the liquid so obtained, if necessary, and analyze the clear solution.~~

Chromatographic system

~~(See *Chromatography* (621), *System Suitability*.)~~

Mode: ~~LC~~

Detector: ~~UV 254 nm~~

Column: ~~4.0 mm × 25.0 cm; packing L3~~

Flow rate: ~~1 mL/min~~

Injection volume: ~~10 µL~~

System suitability

Sample: ~~*Standard solution*~~

~~[Note—The relative retention times for prednisone and methylprednisolone are about 0.7 and 1.0, respectively.]~~

Suitability requirements

Resolution: ~~NLT 4.0 between methylprednisolone and prednisone~~

Relative standard deviation: ~~NMT 2.0%~~

Analysis

Samples: ~~*Standard solution* and *Sample solution*~~

~~Calculate the percentage of the labeled amount of methylprednisolone ($C_{22}H_{30}O_5$) in the portion of Tablets taken:~~

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

~~R_U peak response ratio of methylprednisolone to prednisone from the *Sample solution*~~

~~R_S peak response ratio of methylprednisolone to prednisone from the *Standard solution*~~

~~C_S concentration of USP Methylprednisolone RS in the *Standard solution* (mg/mL)~~

~~C_U nominal concentration of methylprednisolone in the *Sample solution* (mg/mL)~~

Acceptance criteria: ~~92.5%–107.5%~~

■ **Solution A:** 0.1% Formic acid

Solution B: Methanol

Diluent: *Solution B* and water (1:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	50	50
3.0	50	50
17.0	30	70
22.0	30	70
22.1	50	50
25.0	50	50

System suitability solution: 2.5 µg/mL each of USP Methylprednisolone RS and USP Methylprednisolone Related Compound C RS in *Diluent*, prepared as follows. Dissolve suitable quantities of USP Methylprednisolone RS and USP Methylprednisolone Related Compound C RS in methanol in a volumetric flask and dilute with *Diluent* to volume.

Standard solution: 0.1 mg/mL of USP Methylprednisolone RS in *Diluent*, prepared as follows. Dissolve a suitable quantity of USP Methylprednisolone RS in methanol in a volumetric flask and dilute with *Diluent* to volume.

Sample solution: Nominally 0.1 mg/mL of methylprednisolone in *Diluent* prepared as follows. Transfer an appropriate amount of methylprednisolone from NLT 20 finely powdered Tablets to a suitable volumetric flask and add 10% of the flask volume of water. Allow the powder to stand for 2 min and then swirl the flask to disperse the powder. Add 50% of the flask volume of methanol and shake for 15 min. Dilute with *Diluent* to the mark, shake for 5 min, and filter.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: 254 nm

Column: 2.1-mm × 15-cm; 1.7-µm packing L1

Temperatures

Column: 40°

Autosampler: 4°

Flow rate: 0.2 mL/min

Injection volume: 2 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for methylprednisolone and methylprednisolone related compound C are 1.0 and 1.08, respectively.]

Suitability requirements

Resolution: NLT 2.0 between methylprednisolone and methylprednisolone related compound C, *System suitability solution*

Tailing factor: NMT 2.0 for methylprednisolone, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylprednisolone (C₂₂H₃₀O₅) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Methylprednisolone RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of methylprednisolone in the *Sample solution* (mg/mL)

Acceptance criteria: 92.5%–107.5% $\pm 1S$ (USP39)

PERFORMANCE TESTS

• Dissolution 〈 711 〉

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solutions: 20 µg/mL of USP Methylprednisolone RS in alcohol and water prepared as follows. Dissolve a suitable quantity of USP Methylprednisolone RS in 0.1% the flask volume of methanol and dilute with water to volume. Prepare quantitative dilutions of this solution for the development of a standard curve.

Sample solution: Filtered aliquots removed from the *Medium* and suitably diluted, if necessary.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 246 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Standard solutions* and *Sample solution*

Using a standard curve, representing the absorbance versus concentration of the *Standard solutions*, determine the amount of methylprednisolone dissolved in the *Sample solution*.

Acceptance criteria: NLT 70% (Q) of the labeled amount of methylprednisolone ($C_{22}H_{30}O_5$) is dissolved.

• Uniformity of Dosage Units 〈 905 〉

Procedure for content uniformity

Mobile phase: Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol,

and glacial acetic acid (95:95:14:7:6)

Solution A: Chloroform and glacial acetic acid (97:3)

Internal standard solution: 0.2 mg/mL of USP Prednisone RS in *Solution A*

Standard solution: 0.2 mg/mL of USP Methylprednisolone RS in the *Internal standard solution*

Sample solution: Place 1 Tablet in a suitable container. For tablet-labeled strengths of 10 mg or less, add 0.5 mL of water. For tablet-labeled strengths greater than 10 mg, add 1.0 mL of water. Allow the Tablet to stand for 2 min, then swirl the container to disperse the Tablet. Add 5.0 mL of *Internal standard solution* for each mg of labeled Tablet strength, shake for 15 min, and filter or centrifuge a portion of the resulting solution to obtain a clear solution. Use the clear solution.

Chromatographic system

(See *Chromatography* 〈621〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.0-mm × 25.0-cm; packing L3

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for prednisone and methylprednisolone are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between methylprednisolone and prednisone

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylprednisolone (C₂₂H₃₀O₅) in the Tablet taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U peak response ratio of methylprednisolone to prednisone from the *Sample solution*

R_S peak response ratio of methylprednisolone to prednisone from the *Standard solution*

C_S concentration of USP Methylprednisolone RS in the *Standard solution* (mg/mL)

C_U nominal concentration of methylprednisolone in the *Sample solution* (mg/mL)

Acceptance criteria: Meets the requirements

IMPURITIES

Add the following:

Organic Impurities

Solution A, Solution B, Diluent, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 2.5 µg/mL each of USP Methylprednisolone RS, USP Methylprednisolone Related Compound A RS, USP Methylprednisolone Related Compound C RS, and USP Methylprednisolone Related Compound D RS in *Diluent*, prepared as follows. Dissolve

suitable quantities of USP Methylprednisolone RS, USP Methylprednisolone Related Compound A RS, USP Methylprednisolone Related Compound C RS, and USP Methylprednisolone Related Compound D RS in methanol in a volumetric flask and dilute with *Diluent* to volume.

Sample solution: Nominally 0.5 mg/ mL of methylprednisolone in *Diluent* prepared as follows. Transfer an appropriate amount of methylprednisolone from NLT 20 finely powdered Tablets to a suitable volumetric flask, and add 10% of the flask volume of water. Allow the powder to stand for 2 min and then swirl the flask to disperse the powder. Add 50% of the flask volume of methanol and shake for 15 min. Dilute with *Diluent* to the mark, shake for 5 min, and filter.

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between methylprednisolone and methylprednisolone related compound C

Relative standard deviation: NMT 2.0% for methylprednisolone, methylprednisolone related compound A, methylprednisolone related compound C, and methylprednisolone related compound D

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of methylprednisolone related compound A, methylprednisolone related compound C, or methylprednisolone related compound D in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of methylprednisolone related compound A, methylprednisolone related compound C, or methylprednisolone related compound D from the *Sample solution*

r_S peak response of the corresponding related compound from the *Standard solution*

C_S concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

C_U nominal concentration of methylprednisolone in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any unspecified impurity from the *Sample solution*

r_S peak response of methylprednisolone from the *Standard solution*

C_S concentration of USP Methylprednisolone RS in the *Standard solution* (mg/mL)

C_U nominal concentration of methylprednisolone in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methylprednisolone related compound A ^a	0.80	0.20
Methylprednisolone	1.0	—
Methylprednisolone related compound C ^b	1.08	—
Methylprednisolone related compound D ^a	1.51	0.20
Any individual unspecified impurity	—	0.1
Total impurities	—	2.0
^a Process impurities as well as possible degradants.		

^b Process impurity, included for identification only.

■ 1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

Change to read:

- **USP Reference Standards** { 11 }

USP Methylprednisolone RS

- USP Methylprednisolone Related Compound A RS

17,21-Dihydroxy-6 α -methylpregna-1,4-diene-3,11,20-trione.

C₂₂H₂₈O₅ 372.46

USP Methylprednisolone Related Compound C RS

11 β -Hydroxy-6 α -methylandrosta-1,4-diene-3,17-dione.

C₂₀H₂₆O₃ 314.43

USP Methylprednisolone Related Compound D RS

11 β ,20-Dihydroxy-6 α -methylpregna-1,4,17(20)-triene-3,21-dione.

C₂₂H₂₈O₄ 356.46

USP Prednisone RS

■ 1S (USP39)

BRIEFING

Miconazole Compounded Ophthalmic Solution. Because there is no existing *USP* monograph for this dosage form, a new compounded preparation monograph is proposed based on a validated stability-indicating method used to assess stability. The liquid chromatographic procedure in the *Assay* is based on analyses validated using the Luna C18(2) brand of L1 column. The typical retention time for miconazole is about 18.0 min.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C154508

Comment deadline: May 31, 2015

Add the following:**■ Miconazole Compounded Ophthalmic Solution****DEFINITION**

Miconazole Compounded Ophthalmic Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of miconazole ($C_{18}H_{14}Cl_4N_2O$).

Prepare Miconazole Compounded Ophthalmic Solution 1% (10 mg/mL) as follows (see *Pharmaceutical Compounding—Sterile Preparations* 〈 797 〉).

Miconazole	1 g
Polyoxyl 40 Hydrogenated Castor Oil	11.5 mL
Lactic Acid Solution (88%)	0.4 mL
Sterile Water for Injection, a sufficient amount to make	100 mL

Add the *Miconazole* to a sterile container and gradually add the *Polyoxyl 40 Hydrogenated Castor Oil*. Mix into a smooth viscous mixture. Add the *Lactic Acid Solution* (88%) and mix thoroughly. Add 80 mL of the *Sterile Water for Injection* and stir vigorously until the *Miconazole* is completely dissolved. Transfer the contents stepwise and quantitatively to a sterile calibrated container and bring to final volume with *Sterile Water for Injection*. Pass through a sterile filter of 0.22- μ m pore size into an empty sterile dropper bottle. [Note—Room temperature *Sterile Water for Injection* should be used to assist in solubilization.]

ASSAY**• Procedure**

Mobile phase: Dissolve 5.7 g of ammonium acetate in 380 mL of water, and add 320 mL of methanol and 300 mL of acetonitrile. Mix well.

Standard solution: 0.05 mg/mL of miconazole prepared from USP Miconazole RS in methanol

Sample solution: Transfer 0.5 mL of Ophthalmic Solution to a 100-mL volumetric flask, dilute with methanol to volume, and vortex to mix.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV-Vis 230 nm

Column: 2.0-mm \times 10-cm; 2.5- μ m packing L1

Column temperature: 55 $^{\circ}$

Flow rate: 0.35 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

[Note—The retention time for miconazole is about 18.0 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of miconazole ($C_{18}H_{14}Cl_4N_2O$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of miconazole from the *Sample solution*

r_S = peak response of miconazole from the *Standard solution*

C_S = concentration of USP Miconazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of miconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** $\langle 791 \rangle$: 2.9–3.9
- **Sterility Tests** $\langle 71 \rangle$: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **Particulate Matter in Ophthalmic Solutions** $\langle 789 \rangle$: It meets the requirements.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in sterile plastic ophthalmic dropper bottles. Store in a refrigerator (2° – 8°) or at controlled room temperature.
- **Beyond-Use Date:** In the absence of performing and completing a sterility test, the storage conditions for *High-Risk Level CSPs in Pharmaceutical Compounding—Sterile Preparations* $\langle 797 \rangle$ apply. After successful completion of sterility testing, NMT 30 days after the date on which it was compounded when stored in a refrigerator (2° – 8°); NMT 21 days after the date on which it was compounded when stored at controlled room temperature.
- **Labeling:** Label it to indicate that it is for ophthalmic use only and to state the *Beyond-Use Date*.
- **USP Reference Standards** $\langle 11 \rangle$
 USP Miconazole RS
 ■ 1S (USP39)

BRIEFING

Miconazole Nitrate Topical Powder, *USP 38* page 4395. As part of the USP monograph modernization effort, it is proposed to make the following changes:

1. Add a stability-indicating HPLC test for *Organic Impurities*. The HPLC procedure in the test for *Organic Impurities* is based on analyses performed with the Kinetex Phenyl-Hexyl brand of L11 column manufactured by Phenomenex. The typical retention time for miconazole is 25 min.
2. Replace the GC procedure that requires the use of an internal standard for the *Assay* with an advanced HPLC method. The method for the proposed *Assay* uses the same HPLC parameters as those used for the proposed test for *Organic Impurities*.
3. Add *Identification* test *B* based on retention time agreement in the proposed *Assay*.

4. Add an additional storage requirement to the *Packaging and Storage* section.
5. Add USP Miconazole Related Compound F RS to the *USP Reference Standards* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM1: D. Min.)

Correspondence Number—C132337

Comment deadline: May 31, 2015

Miconazole Nitrate Topical Powder

DEFINITION

Miconazole Nitrate Topical Powder contains NLT 90.0% and NMT 110.0% of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$).

IDENTIFICATION

• **A.**

Sample: Transfer nominally 100 mg of miconazole nitrate from Topical Powder to a 50-mL beaker, disperse in 40 mL of methanol, and mix for a minimum of 5 min. Allow to settle for 5–10 min and filter into a 100-mL beaker. Evaporate on a steam bath to dryness. Dry the residue at 105° for 10 min.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue obtained from the *Sample* exhibits maxima only at the same wavelengths as those of a similar preparation of USP Miconazole Nitrate RS.

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

ASSAY

Change to read:

• **Procedure**

~~**Internal standard solution:** 0.5 mg/mL of cholestane in chloroform~~

~~**Standard solution:** 2 mg/mL of USP Miconazole Nitrate RS, prepared as follows. Transfer 5.0 mL of 0.8 mg/mL of USP Miconazole Nitrate RS in a mixture of chloroform and methanol (1:1) to a test tube, and add 2.0 mL of *Internal standard solution*. Evaporate to dryness at a temperature not higher than 40° with the aid of a current of nitrogen. Dissolve the residue in 2.0 mL of a mixture of chloroform and methanol (1:1).~~

~~**Sample solution:** Nominally 2 mg/mL of miconazole nitrate, prepared as follows. Transfer an equivalent to 20 mg of miconazole nitrate from Topical Powder to a 50-mL centrifuge tube. Add 25.0 mL of methanol, and shake by mechanical means for 30 min to dissolve the miconazole nitrate. Centrifuge to obtain a clear supernatant. Transfer 5.0 mL of this solution to a test tube, add 2.0 mL of *Internal standard solution*, and evaporate to dryness at a temperature not higher than 40° with the aid of a current of nitrogen.~~

Dissolve the residue in 2.0 mL of a mixture of chloroform and methanol (1:1).

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.2-m glass; packed with 3% phase G32 on support S1A

Temperatures

Column: 250 °

Injection port: 250 °

Detector: 300 °

Carrier gas: Helium

Flow rate: 50 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for cholestane and miconazole nitrate are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the cholestane and miconazole nitrate peaks

Relative standard deviation: NMT 3.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of miconazole nitrate

(C₁₈H₁₄Cl₄N₂O·HNO₃) in the portion of Topical Powder taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U peak response ratio of miconazole nitrate to cholestane from the *Sample solution*

R_S peak response ratio of miconazole nitrate to cholestane from the *Standard solution*

C_S concentration of USP Miconazole Nitrate RS in the *Standard solution* (mg/mL)

C_U nominal concentration of miconazole nitrate in the *Sample solution* (mg/mL)

■ Solution A:

Methanol, water, and 1 M triethylammonium acetate (300:700:10)

Solution B: Acetonitrile, methanol, and 1 M triethylammonium acetate (250:750:2)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
5	70	30
10	46	54
27	46	54
30	25	75

Time (min)	Solution A (%)	Solution B (%)
35	25	75
36	70	30
40	70	30

Diluent: Methanol and water (70:30)

System suitability solution: 0.1 mg/mL of USP Miconazole Nitrate RS and 6 µg/mL of USP Miconazole Related Compound F RS in *Diluent*. Sonication may be needed to aid dissolution.

Standard solution: 0.1 mg/mL of USP Miconazole Nitrate RS in *Diluent*. Sonication may be needed to aid dissolution.

Sample solution: Nominally 0.1 mg/mL of miconazole nitrate in *Diluent* prepared as follows. Transfer an appropriate amount of miconazole nitrate from a portion of the Topical Powder to a suitable volumetric flask. Add *Diluent* equivalent to 80% of the flask volume and sonicate for 30 min. Dilute with *Diluent* to volume. Centrifuge a portion of the solution for 10 min. Pass the supernatant through a suitable filter of 0.2-µm pore size. Use the filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 10-cm; 2.6-µm packing L11

Column temperature: 40°

Flow rate: 0.8 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

System suitability requirements

Resolution: NLT 2.0 between miconazole related compound F and miconazole, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$) in the portion of Topical Powder taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of miconazole from the *Sample solution*

r_S

= peak response of miconazole from the *Standard solution*

C_S = concentration of USP Miconazole Nitrate RS in the *Standard solution* (mg/mL) C_U = nominal concentration of miconazole nitrate in the *Sample solution* (mg/mL)

■ 1S (USP39)

Acceptance criteria: 90.0%–110.0%**IMPURITIES****Add the following:**■ ● **Organic Impurities****Solution A, Solution B, Mobile phase, Diluent, System suitability solution,** and**Chromatographic system:** Proceed as directed in the *Assay*.**Standard solution:** 1.2 µg/mL of USP Miconazole Nitrate RS in *Diluent***Sample solution:** Nominally 600 µg/mL of miconazole nitrate in *Diluent* prepared as follows.Transfer a portion of Topical Powder to a suitable volumetric flask. Add *Diluent* equivalent to 80% of the flask volume and sonicate for 30 min. Dilute with *Diluent* to volume.

Centrifuge a portion of the solution for 10 min. Pass the supernatant through a suitable filter of 0.2-µm pore size. Use the filtrate.

System suitability**Samples:** *System suitability solution* and *Standard solution*[Note—See *Table 2* for relative retention times.]**System suitability requirements****Resolution:** NLT 2.0 between miconazole related compound F and miconazole, *System suitability solution***Relative standard deviation:** NMT 2.5%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified impurity in the portion of Topical Powder taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak response of any individual unspecified impurity from the *Sample solution* r_S peak response of miconazole from the *Standard solution* C_S concentration of USP Miconazole Nitrate RS in the *Standard solution* (µg/mL) C_U nominal concentration of miconazole nitrate in the *Sample solution* (µg/mL)**Acceptance criteria:** See *Table 2*. Disregard limit: 0.03%.**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deschlorobenzyl econazole ^{a,j}	0.22	—
Miconazole quaternary salt ^{b,j}	0.57	—
Miconazole benzyl analog ^{c,j}	0.65	—
Miconazole related compound C ^{d,j}	0.74	—
Miconazole related compound I ^{e,j}	0.76	—
Econazole nitrate ^{f,j}	0.78	—
Miconazole 2,6-isomer ^{g,j}	0.87	—
Miconazole 2,5-isomer ^{h,j}	0.94	—
Miconazole related compound F ^{i,j}	0.96	—
Miconazole	1.0	—
Any individual unspecified impurity	—	0.25
Total impurities	—	0.5

^a 1-(2,4-Dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol.

^b 2-(3-{2-[(4-Chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazol-3-ium-1-yl)-2-methylpropanoate.

^c 1-[2-(Benzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.

^d 2-[(2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethan-1-amine.

^e 1-{2-[(2-Chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.

^f 1-{2-[(4-Chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.

^g 1-{2-[(2,6-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.

^h 1-{2-[(2,5-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.

ⁱ 1-{2-[(3,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.

^j These are process impurities which are included in the table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and should not be included in the total impurities.

■ 1S (USP39)

PERFORMANCE TESTS

- **Minimum Fill** < 755 >: Meets the requirements

SPECIFIC TESTS

- **Microbial Enumeration Tests** 〈 61 〉 and **Tests for Specified Microorganisms** 〈 62 〉: The total count does not exceed 10^2 cfu/g. It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in well-closed containers.
- Store at controlled room temperature. ■ 1S (USP39)

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Miconazole Nitrate RS

- USP Miconazole Related Compound F RS

1-{2-[(3,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1H-imidazole.
 $C_{18}H_{14}Cl_4N_2O$ 416.13

- 1S (USP39)

BRIEFING

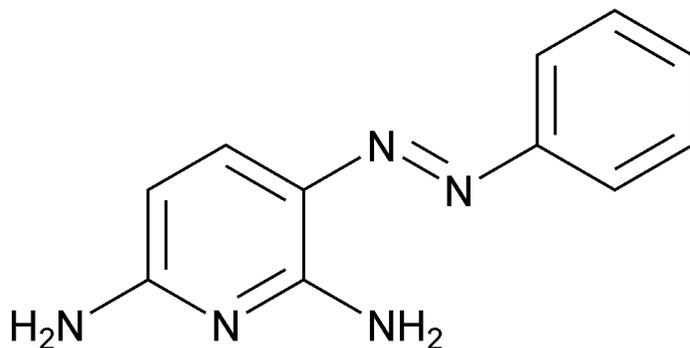
Phenazopyridine Hydrochloride, USP 38 page 4828. As part of USP monograph modernization efforts, the following revisions are proposed:

1. Replace the TLC procedure using hazardous solvent (chloroform) in *Identification* test C with the retention time agreement proposed in the HPLC Assay procedure.
2. Add the *Identification* test D for the counter ion.
3. Replace the UV procedure in the *Assay* with a stability-indicating HPLC procedure. The liquid chromatographic procedure is based on analyses performed with the Luna C18 (2) brand of L1 column. The typical retention time for phenazopyridine is about 21 min.
4. Revise the acceptance criteria in the *Definition* and *Assay* from "NLT 99.0% and NMT 101.0%" to "NLT 98.0% and NMT 102.0%", which are typical for chromatographic procedures.
5. Replace the *Organic Impurities* test by TLC with a validated HPLC procedure. The liquid chromatographic procedure is similar to the *Assay* procedure.
6. The acceptance criteria for 2,6-diaminopyridine and the individual unspecified impurities are proposed at 0.2% and 0.1%, respectively, based on sponsor's submission. Stakeholders with different impurities and/or limits than those in this revision are strongly encouraged to submit their FDA approved acceptance criteria, along with the list of specified degradation products, for the Expert Committee's consideration.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM2: S. Ramachandran.)

Correspondence Number—C132945; C136493

Comment deadline: May 31, 2015**Phenazopyridine Hydrochloride**

• HCl

C₁₁H₁₁N₅·HCl 249.70

2,6-Pyridinediamine, 3-(phenylazo)-, monohydrochloride;
 2,6-Diamino-3-(phenylazo)pyridine monohydrochloride [136-40-3].

DEFINITION**Change to read:**Phenazopyridine Hydrochloride contains ~~NLT 99.0% and NMT 101.0%~~

■ NLT 98.0% and NMT 102.0% ■_{1S} (USP39)

of phenazopyridine hydrochloride (C₁₁H₁₁N₅·HCl), calculated on the dried basis.**IDENTIFICATION**

- **A. Infrared Absorption** (197K)
- **B. Ultraviolet Absorption** (197U)
 - Medium:** Sulfuric acid in alcohol (1 in 360)
 - Sample solution:** 5 µg/mL of Phenazopyridine Hydrochloride in *Medium*
 - Acceptance criteria:** Meets the requirements

Delete the following:

■ • ~~C.~~

~~**Standard solution:** 0.02 mg/mL of USP Phenazopyridine Hydrochloride RS in the same medium as that in the *Sample solution*~~

~~**Sample stock solution:** 0.2 mg/mL of Phenazopyridine Hydrochloride in alcohol~~

~~**Sample solution:** Dilute the *Sample stock solution* with chloroform to obtain a solution with known concentration at 0.02 mg/mL.~~

Chromatographic system

~~(See *Chromatography* (621), *Thin-Layer Chromatography*.)~~

~~**Adsorbent:** 0.25-mm layer of chromatographic silica gel~~

~~**Application volume:** 10 µL~~

~~**Developing solvent system:** Chloroform, ethyl acetate, and methanol (85:10:5)~~

~~**Spray reagent:** 2-N hydrochloric acid~~

Analysis**Samples:** ~~Standard solution and Sample solution~~~~Develop in *Developing solvent system*. Locate the spots by spraying the plate lightly with *Spray reagent*.~~**Acceptance criteria:** ~~The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. ■ 1S (USP39)~~**Add the following:**

- • **C.** The retention time of the phenazopyridine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

Add the following:

- • **D. Identification Tests—General, Chloride (191):** Meets the requirements ■ 1S (USP39)

ASSAY**Change to read:**● **Procedure****Standard solution:** ~~5 µg/mL of USP Phenazopyridine Hydrochloride RS in the same medium as that in the *Sample solution*~~**Sample solution:** ~~Transfer about 100 mg of Phenazopyridine Hydrochloride to a 200-mL volumetric flask. Add about 100 mL of a mixture of sulfuric acid and alcohol (1 in 360); heat gently on a steam bath for 10 min, shake by mechanical means to dissolve, cool to room temperature, and dilute with alcoholic sulfuric acid to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with alcoholic sulfuric acid to volume, and mix. Transfer 5.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with alcoholic sulfuric acid to volume, and mix.~~**Instrumental conditions****Mode:** ~~UV~~**Analytical wavelength:** ~~390 nm (maximum absorbance)~~**Cell:** ~~1 cm~~**Blank:** ~~Dilute alcoholic sulfuric acid (1 in 360)~~**Analysis****Samples:** ~~Standard solution and Sample solution~~Calculate the percentage of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$) in the portion of Phenazopyridine Hydrochloride taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

 ~~A_S absorbance of the *Sample solution*~~ ~~A_U absorbance of the *Standard solution*~~ ~~C_S concentration of USP Phenazopyridine Hydrochloride RS in the *Standard solution* (µg/mL)~~ ~~C_U concentration of the *Sample solution* (µg/mL)~~

Acceptance criteria: ~~99.0%–101.0% on the dried basis~~

■ **Solution A:** 20 mM ammonium acetate in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2	95	5
15	50	50
20	50	50
28	30	70
33	30	70
35	95	5
40	95	5

Diluent: Acetonitrile and water (10:90)

Standard solution: 0.03 mg/mL of USP Phenazopyridine Hydrochloride RS in *Diluent*

Sample solution: 0.03 mg/mL of Phenazopyridine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$) in the portion of Phenazopyridine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Phenazopyridine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= concentration of Phenazopyridine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ■_{1S} (USP39)

IMPURITIES

- **Residue on Ignition** (281): NMT 0.2%
- **Water-Insoluble Substances**

Sample: 2 g of Phenazopyridine Hydrochloride

Analysis: Dissolve the *Sample* in 200 mL of water, heat to boiling, and then heat in a covered container on a steam bath for 1 h. Filter through a tared, fine-porosity, sintered-glass crucible, wash thoroughly with water, and dry at 105° to constant weight.

Acceptance criteria: The weight of the residue does not exceed 0.1% of the weight of Phenazopyridine Hydrochloride taken.

Delete the following:

●

● **Heavy Metals, Method II** (231)

: NMT 20 ppm ● (Official 1-Dec-2015)

Delete the following:

● **Ordinary Impurities** (466)

Standard solutions: 0.04, 0.02, and 0.01 mg/mL of USP Phenazopyridine Hydrochloride RS in alcohol

Sample solution: 2.0 mg/mL in alcohol

Eluant: Chloroform, ethyl acetate, and methanol (85:10:5)

Visualization: Spray the plate with 5 N hydrochloric acid.

Acceptance criteria: Meets the requirements ■_{1S} (USP39)

Add the following:

● **Organic Impurities**

Solution A, Solution B, Mobile phase, and Diluent: Proceed as directed in the *Assay*.

Sensitivity solution: 0.25 µg/mL each of USP Phenazopyridine Hydrochloride RS and 2,6-diaminopyridine in *Diluent*

Standard solution: 0.5 µg/mL of USP Phenazopyridine Hydrochloride RS and 1.0 µg/mL of 2,6-diaminopyridine in *Diluent*

Sample solution: 0.5 mg/mL of Phenazopyridine Hydrochloride in *Diluent*

Chromatographic system: Proceed as directed in the *Assay* except for the *Detector*.

Detector: UV 240 nm

System suitability

Samples: *Sensitivity solution* and *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for the phenazopyridine peak, *Standard solution*

Relative standard deviation: NMT 3.0% for the phenazopyridine peak, *Standard solution*

Signal-to-noise ratio: NLT 30 for the phenazopyridine peak, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 2,6-diaminopyridine in the portion of Phenazopyridine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 2,6-diaminopyridine from the *Sample solution*

r_S = peak response of 2,6-diaminopyridine from the *Standard solution*

C_S = concentration of 2,6-diaminopyridine in the *Standard solution* (mg/mL)

C_U = concentration of Phenazopyridine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Phenazopyridine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual unspecified impurity from the *Sample solution*

r_S = peak response of phenazopyridine from the *Standard solution*

C_S = concentration of USP Phenazopyridine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Phenazopyridine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
2,6-Diaminopyridine	0.37	0.2
Phenazopyridine	1.00	—
Individual unspecified impurity	—	0.10
Total impurities	—	2.0

■ 1S (USP39)

SPECIFIC TESTS

- **Loss on Drying** (731)

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

- **USP Reference Standards** 〈 11 〉

USP Phenazopyridine Hydrochloride RS

BRIEFING

Phenazopyridine Hydrochloride Tablets, *USP 38* page 4829. As part of USP monograph modernization efforts, the following revisions are proposed:

1. Replace *Identification* test *A* using hazardous solvent (chloroform) with a UV procedure.
2. Add an orthogonal *Identification* test *B* based on the retention time agreement proposed in the *HPLC Assay* procedure.
3. Add a validated stability-indicating HPLC procedure for the *Organic Impurities* test. The liquid chromatographic procedure is based on analyses performed using the Luna C18 (2) brand of L1 column. The typical retention time for phenazopyridine is about 21 min.
4. Replace the *Assay* by HPLC with an HPLC procedure that uses the same parameters as the proposed test for *Organic Impurities*.
5. The limit for unspecified impurities is based on ICH guidelines. Stakeholders are encouraged to submit their FDA-approved acceptance criteria, along with the list of specified degradation products, for the Expert Committee's consideration.
6. Add the storage temperature to the *Packaging and Storage* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: S. Ramachandran.)

Correspondence Number—C139182

Comment deadline: May 31, 2015

Phenazopyridine Hydrochloride Tablets

DEFINITION

Phenazopyridine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$).

IDENTIFICATION

Delete the following:

● ~~A.~~

~~**Analysis:** Transfer a quantity of finely ground Tablets, nominally equivalent to 50 mg of phenazopyridine hydrochloride, to a 125 mL separator, add 50 mL of water, 1 mL of 1 N hydrochloric acid, and 5 mL of a saturated sodium chloride solution, and shake to dissolve. Extract with two 25 mL portions of chloroform, and discard the chloroform. Add 5 mL of 1 N sodium hydroxide to the aqueous solution, and extract with one 50 mL portion of chloroform. Transfer the chloroform layer to a second 125 mL separator, and wash with one 50 mL portion of 0.1 N sodium hydroxide. Filter the chloroform layer through a pledget of cotton previously washed with chloroform. Add 5 drops of hydrochloric acid to the filtrate, and evaporate on a steam bath under a current of air to dryness. Add 5 mL of~~

alcohol, and evaporate. Dry the residue at 105° for 4 h.

Acceptance criteria: The IR-absorption spectrum of a potassium bromide dispersion of the dried residue exhibits maxima only at the same wavelengths as that of a similar preparation of USP Phenazopyridine Hydrochloride RS. ■1S (USP39)

Add the following:

- **A. Infrared Absorption (197K):** The UV spectrum of the phenazopyridine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ■1S (USP39)

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ■1S (USP39)

ASSAY

Change to read:

● **Procedure**

Buffer: 2.64 g of dibasic ammonium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 3.0, dilute with water to 1000 mL, and mix.

Mobile phase: Methanol and *Buffer* (500:500)

Standard solution: 0.5 mg/mL of USP Phenazopyridine Hydrochloride RS. Initially add 50% final volume of methanol, and swirl to dissolve. Dilute with *Buffer* to volume, and pass through a filter of 0.5 µm or finer pore size.

Sample solution: Transfer an amount nominally equivalent to 100 mg of phenazopyridine hydrochloride from finely powdered Tablets (NLT 20) to a 200-mL volumetric flask. Add 100 mL of methanol, and sonicate for 10 min. Add 75 mL of *Buffer*, and sonicate for an additional 10 min with occasional mixing. Dilute with *Buffer* to volume, and mix. Pass this solution through a filter of 0.5 µm or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1400 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of phenazopyridine from the *Sample solution*

r_S peak response of phenazopyridine from the *Standard solution*

C_S concentration of USP Phenazopyridine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of phenazopyridine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

■ **Solution A:** 20 mM ammonium acetate in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2	95	5
15	50	50
20	50	50
28	30	70
33	30	70
35	95	5
40	95	5

Diluent: Acetonitrile and water (10:90)

Standard solution: 0.03 mg/mL of USP Phenazopyridine Hydrochloride RS in *Diluent*

Sample stock solution: Nominally 0.3 mg/mL of phenazopyridine hydrochloride from NLT 20 finely powdered Tablets in *Diluent*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add *Diluent* equivalent to 75% of the flask volume and sonicate for 15 min. Allow the solution to cool to room temperature, dilute with *Diluent* to volume, and centrifuge.

Sample solution: Nominally equivalent to 0.03 mg/mL of phenazopyridine hydrochloride in *Diluent* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm. For *Identification test A*, use a diode array detector in the range of 200–600 nm.

Column: 4.6-mm × 25-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements**Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 1.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Phenazopyridine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = nominal concentration of phenazopyridine hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0% $\pm 1S$ (USP39)**PERFORMANCE TESTS**● **Dissolution** 〈 711 〉**Medium:** Water; 900 mL**Apparatus 2:** 50 rpm**Time:** 45 min**Standard solution:** USP Phenazopyridine Hydrochloride RS in *Medium***Sample solution:** Filter portions of the solution under test and suitably dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.**Instrumental conditions****Mode:** UV**Analytical wavelength:** 422 nm**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the quantity of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$) dissolved by using UV absorption from the *Sample solution* in comparison with the *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$) is dissolved.● **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

IMPURITIES**Add the following:****■ • Organic Impurities**

Solution A, Solution B, Mobile phase, and Diluent: Proceed as directed in the *Assay*.

Sensitivity solution: 0.25 µg/mL of USP Phenazopyridine Hydrochloride RS in *Diluent*

Standard solution: 0.001 mg/mL of USP Phenazopyridine Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.5 mg/mL of phenazopyridine hydrochloride from NLT 20 finely powdered Tablets in *Diluent*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add *Diluent* equivalent to 60% of the flask volume and sonicate for 15 min. Allow the solution to cool to room temperature and dilute with *Diluent* to volume. Centrifuge the solution and dilute the supernatant with *Diluent* to obtain 0.5 mg/mL of phenazopyridine hydrochloride.

Chromatographic system: Proceed as directed in the *Assay* except for the *Detector*.

Detector: UV 240 nm

System suitability

Samples: *Sensitivity solution* and *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for the phenazopyridine peak, *Standard solution*

Relative standard deviation: NMT 3.0% for the phenazopyridine peak, *Standard solution*

Signal-to-noise ratio: NLT 30 for the phenazopyridine peak, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified impurity in the portion of the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each individual unspecified impurity from the *Sample solution*

r_S peak response of phenazopyridine from the *Standard solution*

C_S concentration of USP Phenazopyridine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of phenazopyridine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
2,6-Diaminopyridine ^a	0.37	—
Phenazopyridine	1.00	—
Individual unspecified impurity	—	0.2
Total impurities	—	2.0

^a For identification only. These are process impurities monitored in the drug substance and are not included in the total impurities.

■ 1S (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers.
- Store at controlled room temperature. ■ 1S (USP39)

- **USP Reference Standards** { 11 }

USP Phenazopyridine Hydrochloride RS

BRIEFING

Rabeprazole Sodium, PF 38(2) [Mar.–Apr. 2012]. On the basis of comments received, the earlier proposal presented in PF 38(2) is cancelled and replaced with a new proposal based on the monographs for *Rabeprazole Sodium* and *Rabeprazole Sodium Hydrate* proposed in *Pharmeuropa 26.3*.

1. In the *Definition* and *Assay* sections, the *Acceptance criteria* include both the anhydrous and hydrated forms.
2. A procedure for polymorphic equalization is added to *Identification* test A.
3. This proposal includes a new HPLC procedure for the *Assay* and *Organic Impurities* test which was shown to separate impurities listed in the earlier PF proposal and also listed in the draft Pending monograph posted for public comments on the USP website. The HPLC procedure in the *Assay* and the test for *Organic Impurities* is based on analyses performed with the Hypersil BDS C18 brand of L1 column. The typical retention time for rabeprazole is about 19 min.
4. The tests for *Loss on Drying* and *Water Determination* are included to accommodate specifications suitable for anhydrous and hydrated forms. The labeling statement is added to distinguish these two forms.

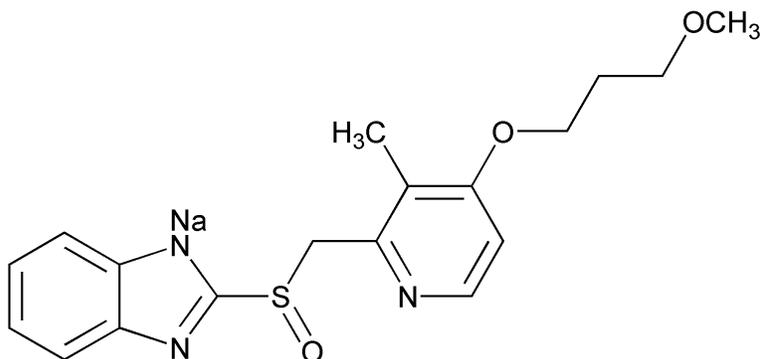
(SM3: E. Gonikberg, R.S. Prasad.)

Correspondence Number—C152244; C90062; C150117; C134108; C119887; C118815

Comment deadline: May 31, 2015

Add the following:

■ Rabeprazole Sodium



C₁₈H₂₀N₃NaO₃S

381.42

1*H*-Benzimidazole, 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]methyl]sulfinyl]-, sodium salt;
 2-[[[4-(3-Methoxypropoxy)-3-methyl-2-pyridyl]methyl]sulfinyl]benzimidazole sodium salt [117976-90-6].

DEFINITION

Rabeprazole Sodium contains NLT 98.0% and NMT 102.0% of rabeprazole sodium ($C_{18}H_{20}N_3NaO_3S$), calculated on the dried basis. The hydrated form contains NLT 98.0% and NMT 102.0% of rabeprazole sodium ($C_{18}H_{20}N_3NaO_3S$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈 197 〉

[Note—Methods described in *Infrared Absorption* 〈 197K 〉, 〈 197M 〉, or 〈 197A 〉 may be used. If the spectra obtained in the solid state shows differences, dissolve the substance to be examined and the reference substance separately in methanol, evaporate to dryness, and record new spectra using the residues.]

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- **C. Identification Tests—General, Sodium** 〈 191 〉

ASSAY

- **Procedure**

[Note—Protect solutions containing rabeprazole from light.]

Buffer 1: 4.35 g/L of dibasic potassium phosphate. Adjust with phosphoric acid to a pH of 7.0.

Solution A: Acetonitrile and *Buffer 1* (5:95)

Solution B: Methanol

Solution C: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	100	0	0
2	100	0	0
7	85	0	15
27	30	40	30
32	15	55	30

Return to original conditions and re-equilibrate the system for 10 min.

Buffer 2: Dissolve 17.4 g of dibasic potassium phosphate in 950 mL of water, adjust using a 2 N solution of potassium hydroxide to a pH of 11.3, and dilute with water to 1000 mL.

Pass through a suitable filter of 0.45- μ m pore size.

Diluent: Methanol and *Buffer 2* (20:80)

Standard solution: 0.1 mg/mL of USP Rabeprazole Sodium RS in *Diluent*

Sample solution: 0.1 mg/mL of Rabeprazole Sodium in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Temperatures

Autosampler: 6°

Column: 45°

Flow rate: 1 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of rabeprazole sodium (C₁₈H₂₀N₃NaO₃S) in the portion of Rabeprazole Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Rabeprazole Sodium RS in the *Standard solution* (mg/mL)

C_U concentration of Rabeprazole Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis for the anhydrous form or on the anhydrous basis for the hydrated form

IMPURITIES

• **Organic Impurities**

[Note—Protect solutions containing rabeprazole from light.]

Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the Assay.

System suitability solution: 1.0 mg/mL of USP Rabeprazole Sodium RS and 0.002 mg/mL each of USP Rabeprazole Related Compound A RS, USP Rabeprazole Related Compound B RS, USP Rabeprazole Related Compound C RS, USP Rabeprazole Related Compound D RS, USP Rabeprazole Related Compound E RS, and USP Rabeprazole Related Compound F RS in *Diluent*

Sensitivity solution: 0.5 μg/mL of USP Rabeprazole Sodium RS in *Diluent*

Standard solution: 0.001 mg/mL of USP Rabeprazole Sodium RS in *Diluent*

Sample solution: 1.0 mg/mL of Rabeprazole Sodium in *Diluent*

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between rabeprazole related compound D and rabeprazole related compound F, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Peak-to-valley ratio: NLT 1.5 for rabeprazole related compound F and rabeprazole, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Rabeprazole Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_S peak response of rabeprazole from the *Standard solution*

C_S concentration of USP Rabeprazole Sodium RS in the *Standard solution* (mg/mL)

C_U concentration of Rabeprazole Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pyridinone analog (rabeprazole related compound A)	0.32	0.15
Benzimidazolol ^a	0.47	0.10
Mercaptobenzimidazole (rabeprazole related compound C)	0.50	0.10
Rabeprazole sulfone <i>N</i> -oxide ^b	0.74	0.10
Rabeprazole <i>N</i> -oxide (rabeprazole related compound B)	0.76	0.15
Methoxy analog ^c	0.82	0.15
Rabeprazole sulfone (rabeprazole related compound D)	0.90	0.8
Chloro analog (rabeprazole related compound F)	0.98	0.10
Rabeprazole	1.0	—
Methoxy sulfide analog ^d	1.04	0.10
Rabeprazole sulfide (rabeprazole related compound E)	1.24	0.15
Any other individual impurity	—	0.10
Total impurities	—	1.0

^a 1*H*-Benzimidazol-2-ol.

^b 2-[[[(1*H*-Benzimidazol-2-yl)sulfonyl]methyl]-4-(3-methoxypropoxy)-3-methylpyridine 1-oxide.

^c 2-[[[(4-Methoxy-3-methylpyridin-2-yl)methyl]sulfinyl]benzimidazole.

d 2-{{[(4-Methoxy-3-methylpyridin-2-yl)methyl]thio}benzimidazole.

SPECIFIC TESTS

- **Loss on Drying** 〈 731 〉

[Note—Perform this test for the anhydrous form.]

Analysis: Dry under vacuum over phosphorus pentoxide for 24 h.

Acceptance criteria: NMT 1.0%

- **Water Determination, Method 1** 〈 921 〉

[Note—Perform this test where it is labeled as a hydrated form.]

[Note—Hydranal composite 5 is a suitable titrant.]

Sample: 0.2 g

Acceptance criteria: NMT 7.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at room temperature.

- **Labeling:** Where it is the hydrated form, the label so indicates.

- **USP Reference Standards** 〈 11 〉

USP Rabeprazole Sodium RS

USP Rabeprazole Related Compound A RS

Sodium 1-(1*H*-benzimidazol-2-yl)-3-methyl-4-oxo-1,4-dihydropyridine-2-carboxylate.

$C_{14}H_{10}N_3NaO_3$ 291.24

USP Rabeprazole Related Compound B RS

2-{{[(1*H*-Benzimidazol-2-yl)sulfinyl]methyl}-4-(3-methoxypropoxy)-3-methylpyridine 1-oxide.

$C_{18}H_{21}N_3O_4S$ 375.44

USP Rabeprazole Related Compound C RS

1*H*-Benzimidazole-2-thiol.

$C_7H_6N_2S$ 150.20

USP Rabeprazole Related Compound D RS

2-{{[4-(3-Methoxypropoxy)-3-methyl-2-pyridyl]methyl}sulfonyl}benzimidazole.

$C_{18}H_{21}N_3O_4S$ 375.44

USP Rabeprazole Related Compound E RS

2-{{[4-(3-Methoxypropoxy)-3-methyl-2-pyridyl]methylthio}benzimidazole.

$C_{18}H_{21}N_3O_2S$ 343.44

USP Rabeprazole Related Compound F RS

2-{{[(4-Chloro-3-methyl-2-pyridyl)methyl]sulfinyl}benzimidazole.

$C_{14}H_{12}ClN_3OS$ 305.78

■ 1S (USP39)

BRIEFING

Rimexolone Ophthalmic Suspension, USP 38 page 5181. On the basis of comments received, it is proposed to make the following changes:

1. Clarify the preparation of *Standard solution* and add the column particle size in the

Assay based on the validated method.

2. Delete the reference to *Viscosity—Capillary Viscometer Methods* 〈 911 〉 as an option for measuring viscosity and clarify the procedure and acceptance criteria to be consistent with the validated method and FDA-approved product specifications.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: H. Cai.)

Correspondence Number—C142146

Comment deadline: May 31, 2015

Rimexolone Ophthalmic Suspension

DEFINITION

Rimexolone Ophthalmic Suspension is a sterile suspension of Rimexolone in a suitable aqueous medium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of rimexolone ($C_{24}H_{34}O_3$). It may contain suitable stabilizers, buffers, and antimicrobial agents.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

Mobile phase: Acetonitrile and water (60:40)

■ **Standard stock solution:** 1 mg/mL of USP Rimexolone RS in methanol ■ 1S (USP39)

Standard solution: 0.2 mg/mL of USP Rimexolone RS, prepared by dissolving a suitable quantity in methanol and diluting with *Mobile phase* to volume

■ in *Mobile phase* from *Standard stock solution* ■ 1S (USP39)

Sample stock solution: Nominally 1 mg/mL of rimexolone, prepared as follows. Transfer an amount nominally equivalent to 25 mg of rimexolone from a portion of Ophthalmic Suspension to a 25-mL volumetric flask. Dilute with methanol to volume and sonicate for 2 min.

Sample solution: Nominally 0.2 mg/mL of rimexolone from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 4.6-mm × 25-cm;

■ 5- μ m ■ 1S (USP39)

packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor, k' : NLT 1.5

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of rimexolone ($C_{24}H_{34}O_3$) in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Rimexolone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of rimexolone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **Sterility Tests** $\langle 71 \rangle$: Meets the requirements
- **pH** $\langle 791 \rangle$: 6.0–8.0

Delete the following:

- ~~**Viscosity—Capillary Methods** $\langle 911 \rangle$ or **Viscosity—Rotational Methods** $\langle 912 \rangle$: 50–350 centipoises ■ 1S (USP39)~~

Add the following:

- **Viscosity—Rotational Methods** $\langle 912 \rangle$

Analysis: Equip a cone-and-plate rheometer¹ following *Method III*. The shear rate under the test condition is 11.5 s^{-1} and temperature is 25° .

Acceptance criteria: 15–200 mPa·s ■ 1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** $\langle 11 \rangle$
USP Rimexolone RS

Brookfield cone-and-plate rheometer, with spindle CP-42 (#42), is operated at 3 rpm. Any other equivalent rheometer is suitable as well. ■_{1S} (USP39)

BRIEFING

Risedronate Sodium, USP 38 page 5188. In preparation for the omission of the flame tests from *Identification Tests—General* 〈 191 〉, proposed in PF 41(2) [Mar.–Apr. 2015], it is proposed to replace the use of the flame test in *Identification* test B with the pyroantimonate precipitation test currently described in 〈 191 〉. This test is also consistent with test (a) in 2.3.1. *Identification reactions of ions and functional groups* in the *European Pharmacopoeia*, and is employed in the *European Pharmacopoeia* monograph for *Risedronate Sodium*.

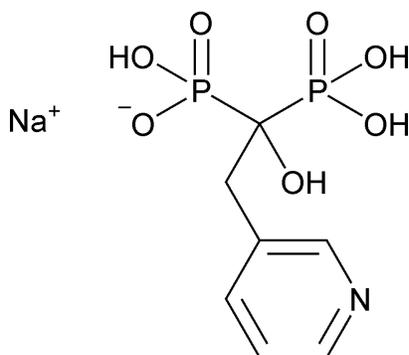
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM3: R.S. Prasad.)

Correspondence Number—C154669

Comment deadline: May 31, 2015

Risedronate Sodium



$C_7H_{10}NNaO_7P_2$	305.09
$C_7H_{10}NNaO_7P_2 \cdot H_2O$	323.12
$C_7H_{10}NNaO_7P_2 \cdot 2.5 H_2O$	350.13

Phosphonic acid, [1-hydroxy-2-(3-pyridinyl)ethylidene]bis-, monosodium salt;

Sodium trihydrogen [1-hydroxy-2-(3-pyridyl)ethylidene]diphosphonate;

Hemi-pentahydrate [329003-65-8].

Monohydrate [353228-19-0].

DEFINITION

Risedronate Sodium contains one or two and one-half molecules of hydration. The monohydrate form contains NLT 98.0% and NMT 102.0% of risedronate sodium ($C_7H_{10}NNaO_7P_2$), calculated on the dried basis. The hemi-pentahydrate form contains NLT 98.0% and NMT 102.0% of risedronate sodium ($C_7H_{10}NNaO_7P_2$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈 197 〉: The spectra of trifluorovinyl chloride polymer and mineral oil dispersions of it, separately prepared from a test specimen, exhibit maxima in the regions of $4000\text{--}1350\text{ cm}^{-1}$ and $1350\text{--}450\text{ cm}^{-1}$, respectively, only at the same wavelengths as those of similar preparations of USP Risedronate Sodium RS. [Note—If a difference appears in the infrared spectra of the analyte and the standard, dissolve equal portions of the test specimen and the USP Reference Standard in equal volumes of water containing about 50 mg/mL of potassium bromide. Evaporate the solutions to dryness at 105° for 120 min. Repeat the test on the residues.]

Change to read:

- **B. Identification Tests—General, Sodium** 〈 191 〉: Meets the requirements of the flame test ■ pyroantimonate precipitation test. [Note—Complete dissolution of the sample is achieved only after the addition of the 15% potassium carbonate.] ■ 1S (USP39)

ASSAY

● **Procedure**

Mobile phase: 1.8 g/L of edetate disodium in water. Adjust with 1 N sodium hydroxide to a pH of 9.5 ± 0.1 .

Standard solution: Dissolve USP Risedronate Sodium RS and USP Risedronate Related Compound A RS in *Mobile phase* to obtain a solution containing 1.0 mg/mL of anhydrous risedronate sodium and 0.1 mg/mL of risedronate related compound A.

Sample solution: 1.1 mg/mL of Risedronate Sodium in *Mobile phase*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 263 nm

Column: 4.0-mm \times 25-cm; 10- μ m packing L48

Flow rate: 0.8 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.3 between risedronate related compound A and risedronate

Tailing factor: NMT 1.6 for the risedronate peak

Relative standard deviation: NMT 1.0% for the risedronate peak from three replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of risedronate sodium ($\text{C}_7\text{H}_{10}\text{NNaO}_7\text{P}_2$) in the portion of Risedronate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

$C_{\bar{s}}$ concentration of USP Risedronate Sodium RS in the *Standard solution* (mg/mL)

$C_{\bar{r}}$ concentration of Risedronate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria

Monohydrate: 98.0%–102.0% on the dried basis

Hemi-pentahydrate: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Delete the following:

•

• Heavy Metals

Lead nitrate solution: Add 1 mL of nitric acid to 100 mL of water. Dissolve 100 mg of lead nitrate in it, and dilute with water to 1000 mL.

Sodium bicarbonate solution: Transfer 0.840 g of sodium bicarbonate to a 1000-mL volumetric flask containing about 950 mL of water. Dissolve in and dilute with water to volume. Adjust with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid, as necessary, to a pH of 4.40 ± 0.02 .

Hydrogen sulfide solution: Transfer 200 mL of *Sodium bicarbonate solution* to a suitable conical flask, and bubble hydrogen sulfide gas through the solution until it turns a strip of lead acetate test paper black (see *Reagents, Indicators, and Solution—Indicator and Test Papers*).

Standard solutions: Transfer 500 mg of Risedronate Sodium to each of three separate beakers. Add 41 mL of water to each beaker, and stir to dissolve. Adjust with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid, as necessary, to a pH of 4.40 ± 0.02 . Label the first beaker as *Standard solution 1*. Add 200 μ L of *Lead nitrate solution* to the second beaker (*Standard solution 2*) and 400 μ L to the third beaker (*Standard solution 3*). These solutions contain the equivalent of 0, 12.5, and 25 μ g of lead (representing 0, 10, and 20 ppm, respectively).

Sample solution: Transfer 1.75 g of Risedronate Sodium to a suitable beaker. Add 41 mL of water, and stir to dissolve. Adjust with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid, as necessary, to a pH of 4.40 ± 0.02 .

Analysis: Add 7 mL of *Hydrogen sulfide solution* to each of the beakers containing the *Standard solutions* and the *Sample solution*. Allow the solutions to stand for at least 5 min. Add 60 μ L of 1 N hydrochloric acid to each of the beakers containing the *Standard solutions*, add 200 μ L of 1 N hydrochloric acid to the beaker containing the *Sample solution*, and stir. Transfer the solutions into 50-mL color-comparison tubes, and view downward over a white surface.

Acceptance criteria: The color of the solution obtained from the *Sample solution* is not darker than that of the solution from *Standard solution 3* (NMT 20 ppm).

•(Official 1-Dec-2015)

• Organic Impurities, Procedure 1

[Note—Perform both *Procedure 1* and *Procedure 2*.]

Mobile phase, Standard solution, Sample solution, and Chromatographic system:

Proceed as directed in the *Assay*.

Diluted standard solution: Dilute a portion of the *Standard solution* with *Mobile phase* to

obtain a solution containing 5 µg/mL of anhydrous risedronate sodium and about 0.5 µg/mL of risedronate related compound A.

System suitability

Samples: *Standard solution and Diluted standard solution*

Suitability requirements

Resolution: NLT 2.3 between risedronate related compound A and risedronate, *Standard solution*

Tailing factor: NMT 1.6 for the risedronate peak, *Standard solution*

Relative standard deviation: NMT 1.0% for the risedronate peak from three replicate injections, *Standard solution*; NMT 15% for the risedronate related compound A peak from three replicate injections, *Diluted standard solution*

Analysis

Samples: *Sample solution and Diluted standard solution*

Calculate the percentage of each impurity in the portion of Risedronate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of risedronate from the *Diluted standard solution*

C_S = concentration of USP Risedronate Sodium RS in the *Diluted standard solution* (mg/mL)

C_U = concentration of Risedronate Sodium in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Table 1

Name	Relative Response Factor	Relative Retention Time
3-Pyridyl acetic acid	1.65	0.22
2-Pyridinyl isomer (USP Risedronate Related Compound A RS)	1.0	0.84
Risedronate sodium	—	1.0

Acceptance criteria

Any individual impurity: NMT 0.10%

[Note—Disregard the peak due to the sodium ion, eluting at about 1.6 min, and any peak observed in the blank. The reporting level for impurities is 0.05%.]

• Organic Impurities, Procedure 2

Mobile phase: Transfer 16.15 g of dibasic potassium phosphate and 0.46 g of edetate disodium to a 1-L beaker, and dissolve in about 400 mL of water. Add 1 vial of commercially available tetrabutylammonium dihydrogen phosphate buffered solution in methanol¹ and 1 mL of hydrochloric acid. Adjust with 1 N sodium hydroxide or 1 N hydrochloric acid, as necessary, to a pH of 7.5 ± 0.1, and dilute with water to 480 mL. Add 20 mL of methanol, mix well, pass the solution through a nylon filter of 0.45-µm pore size, and degas.

Diluent: Transfer 0.46 g of edetate disodium to a 1-L beaker, and dissolve in 500 mL of water. Adjust with 1 N sodium hydroxide to a pH of 7.5 ± 0.1.

Standard solution: 5 µg/mL of USP Risedronate Related Compound B RS in *Diluent*

Diluted standard solution: 0.5 µg/mL of USP Risedronate Related Compound B RS in *Diluent* from the *Standard solution*

Sample solution: 2 mg/mL of Risedronate Sodium in *Diluent*, using sonication if necessary

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 263 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *Diluted standard solution*

Suitability requirements

Capacity factor: Greater than 2, *Standard solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 5% from three replicate injections, *Standard solution*; NMT 10% from three replicate injections, *Diluted standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—Disregard any peak eluting before risedronate related compound B. The risedronate peak elutes unretained at the void volume.]

Calculate the percentage of each impurity in the portion of Risedronate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of risedronate related compound B from the *Standard solution*

C_S = concentration of USP Risedronate Related Compound B RS in the *Standard solution* (mg/mL)

C_U = concentration of Risedronate Sodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of risedronate related compound B as a free acid, 530.20

M_{r2} = molecular weight of risedronate related compound B as a tetrahydrate disodium salt, 646.22

Acceptance criteria

Risedronate related compound B: NMT 0.10%

Individual impurities: NMT 0.10%

Total impurities: NMT 0.50%, *Procedure 1* and *Procedure 2* being combined

[Note—Disregard any peak observed in the blank. The reporting level for impurities is 0.05%.]

SPECIFIC TESTS

- **Water Determination, Method Ic** { 921 } (where it is labeled as a hemi-pentahydrate): 11.9%–13.9%. Perform the test by direct introduction of solid sample into the titrator. Alternatively, *Method 1a* may be used.
- **Loss on Drying** (where it is labeled as a monohydrate)

(See *Thermal Analysis* 〈 891 〉.)

Analysis: Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument, using 7–15 mg of Risedronate Sodium. Heat the specimen under test at a rate of 10°/min in a stream of nitrogen at a flow rate of about 40 mL/min. Record the thermogram from ambient temperature to 250°.

Acceptance criteria: 5.5%–7.5%

ADDITIONAL REQUIREMENTS

- **Labeling:** Label to indicate whether it is the monohydrate or the hemi-pentahydrate form.
- **Packaging and Storage:** Preserve in well-closed containers. Store at room temperature.
- **USP Reference Standards** 〈 11 〉

USP Risedronate Sodium RS

USP Risedronate Related Compound A RS

2-Pyridinyl isomer [1-hydroxy-2-(2-pyridinyl)ethylidene]bis(phosphonic acid) monohydrate.

C₇H₁₁NO₇P₂ 283.12

USP Risedronate Related Compound B RS

Cyclic dimer, disodium tetrahydrate salt, [3,6-bis[(3-pyridinyl)methyl]-2,5-dihydroxy-2,5-dioxido-1,4,2,5-dioxadiphosphorinane-3,6-diyl]bis[phosphonic acid] disodium tetrahydrate salt.

C₁₄H₁₆N₂O₁₂P₄Na₂·4H₂O 646.22

¹ Available from Waters Corp. as Part #85101 (PIC A).

BRIEFING

Salicylic Acid Topical Foam, *USP 38* page 5243. As part of the USP monograph modernization effort, the following revisions are proposed:

1. Add *Identification* test *B* based on the UV spectrum match of the main peak from the proposed UHPLC procedure for the *Assay*.
2. Replace the current HPLC procedure that uses an ion-pairing reagent and an internal standard with a straightforward stability-indicating UHPLC procedure. The liquid chromatographic procedure replaces the 4-mm internal diameter column previously used with a Waters Acquity UPLC BEH-C18, 1.7-µm brand of column containing L1 packing. The typical retention time for salicylic acid is about 5.2 min.
3. Add a test for *Organic Impurities* based on the proposed UHPLC procedure in the *Assay*. This method monitors impurities that are consistent with those found in the API.
4. Add three new Reference Standards, introduced by the proposed HPLC procedure, to the *USP Reference Standards* section: USP Salicylic Acid Related Compound A RS, USP Salicylic Acid Related Compound B RS, and USP Phenol RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: C. Anthony.)

Correspondence Number—C137585

Comment deadline: May 31, 2015

Salicylic Acid Topical Foam

DEFINITION

Salicylic Acid Topical Foam contains NLT 90.0% and NMT 110.0% of the labeled amount of salicylic acid ($C_7H_6O_3$).

IDENTIFICATION

- **A.** The retention time of the salicylic acid peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

- **B.** The UV-Vis spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

ASSAY

Change to read:

- **Procedure**

~~**Mobile phase:** Dissolve 225 mg of tetramethylammonium hydroxide pentahydrate in acetonitrile, methanol, water, and glacial acetic acid (150:150:700:1).~~

~~**Internal standard solution:** 8 mg/mL of benzoic acid in methanol~~

~~**Standard solution:** 0.20 mg/mL of USP Salicylic Acid RS and 0.80 mg/mL of internal standard from *Internal standard solution* in *Mobile phase* prepared as follows. Transfer an appropriate quantity of USP Salicylic Acid RS to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution* and dilute with *Mobile phase* to volume.~~

~~**Sample solution:** Nominally 0.20 mg/mL of Salicylic Acid from a portion of Topical Foam and 0.80 mg/mL of internal standard from *Internal standard solution* in *Mobile phase* prepared as follows. Transfer a portion of Topical Foam, equivalent to 20 mg of salicylic acid, to a 100-mL volumetric flask. Add 10.0 mL of *Internal standard solution* and dilute with *Mobile phase* to volume. Cool in an ice bath to below room temperature and filter, discarding the first few mL of the filtrate.~~

~~**Chromatographic system**~~

~~(See *Chromatography* <621>, *System Suitability*.)~~

~~**Mode:** LC~~

~~**Detector:** UV 280 nm~~

~~**Column:** 4 mm × 30 cm; packing L1~~

~~**Flow rate:** 2 mL/min~~

~~**Injection volume:** 5 µL~~

~~**System suitability**~~

~~**Sample:** *Standard solution*~~

~~[Note—The retention times for salicylic acid and benzoic acid are about 2.5 and 5.5 min, respectively.]~~

~~**Suitability requirements**~~

~~**Resolution:** NLT 3.0 between salicylic acid and benzoic acid~~

~~**Tailing factor:** NMT 2.0 for salicylic acid and benzoic acid~~

~~**Relative standard deviation:** NMT 3.0% from four replicate injections~~

~~**Analysis**~~

~~**Samples:** *Standard solution* and *Sample solution*~~

~~Calculate the percentage of the labeled amount of salicylic acid ($C_7H_6O_3$) in the portion of Topical Foam taken:~~

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

~~R_S = peak response ratio of salicylic acid to the internal standard from the *Sample solution*~~

~~R_U = peak response ratio of salicylic acid to the internal standard from the *Standard solution*~~

~~C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)~~

~~C_U = nominal concentration of salicylic acid in the *Sample solution* (mg/mL)~~

~~**Acceptance criteria:** 90.0%–110.0%~~

Mobile phase: Methanol, trifluoroacetic acid, and water (35: 0.1: 65)

Standard solution: 0.04 mg/mL of USP Salicylic Acid RS in *Mobile phase*

Sample solution: Nominally 0.04 mg/mL of salicylic acid in *Mobile phase* prepared as follows. Add an appropriate quantity of Topical Foam, equivalent to 4 mg of salicylic acid, to a 100-mL volumetric flask and dissolve in 40 mL of *Mobile phase*. Sonicate to dissolve as needed and dilute with *Mobile phase* to volume. Pass through a suitable filter of 0.45- μ m pore size, discarding the first 2–3 mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 212 nm. For *Identification test B*, use a diode array detector in the range of 200–400 nm.

Column: 2.1-mm \times 5.0-cm; 1.7- μ m packing L1

Column temperature: 30 $^{\circ}$

Flow rate: 0.2 mL/min

Injection volume: 2.0 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of salicylic acid ($C_7H_6O_3$) in the portion of Topical Foam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of salicylic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% $\pm 1S$ (USP39)

IMPURITIES

Add the following:

• Organic Impurities

Mobile phase and Chromatographic system: Proceed as directed in the *Assay*.

Standard stock solution: 0.5 mg/mL of USP Salicylic Acid RS, 0.25 mg/mL of USP Salicylic Acid Related Compound A RS, 0.125 mg/mL of USP Salicylic Acid Related Compound B RS, and 0.05 mg/mL of USP Phenol RS in *Mobile phase*

Standard solution: 5 µg/mL of USP Salicylic Acid RS, 2.5 µg/mL of USP Salicylic Acid Related Compound A RS, 1.25 µg/mL of USP Salicylic Acid Related Compound B RS, and 0.5 µg/mL of USP Phenol RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Nominally 2.5 mg/mL of salicylic acid in *Mobile phase*, prepared as follows. Transfer an appropriate amount of Topical Foam equivalent to 25 mg of salicylic acid to a 10-mL volumetric flask and dissolve by sonication in 5 mL of *Mobile phase*. Dilute with *Mobile phase* to volume, pass through a suitable filter of 0.45-µm pore size, and discard the first 2–3 mL of filtrate.

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 7.0 between phenol and salicylic acid related compound B

Relative standard deviation: NMT 2% for each peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any other individual impurity in the portion of Topical Foam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*

r_S = peak response of salicylic acid from the *Standard solution*

C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of salicylic acid in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid related compound A ^a	0.2	—
Phenol ^a	0.4	—
Salicylic acid related compound B ^a	0.6	—
Salicylic acid	1.0	—
Any other individual impurity	—	0.10
Total impurities	—	1.0

^a These are process impurities controlled in the API and are included in *Table 1* for identification purposes only. They are not reported in the drug product and should not be included in the total impurities.

■ 1S (USP39)

SPECIFIC TESTS

- pH $\langle 791 \rangle$: 5.0–6.0

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

Change to read:

- **USP Reference Standards $\langle 11 \rangle$**

■ USP Phenol RS

■ 1S (USP39)

USP Salicylic Acid RS

■ USP Salicylic Acid Related Compound A RS

4-Hydroxybenzoic acid.

$C_7H_6O_3$ 138.12

USP Salicylic Acid Related Compound B RS

4-Hydroxyisophthalic acid.

$C_8H_6O_5$ 182.13

■ 1S (USP39)

BRIEFING

Sodium Thiosulfate, *USP 38* page 5338. Based on the comments received and as part of USP monograph modernization efforts, the monograph is revised as follows:

1. Replace the wet chemistry-based *Identification* tests A and C with the IR *Identification* test.
2. For *Identification* test B, it is proposed to express the *Sample solution* concentration

in g/mL to be consistent with the classic version.

3. Revise the *Assay* to allow the use of dilute acid or dilute base for pH adjustments.
4. For clarity, add the method number to the *Water Determination* test.
5. USP Sodium Thiosulfate RS is added to the *USP Reference Standards* section to support the proposed revision in the *Identification* test by IR.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: R.S. Prasad, E. Gonikberg.)

Correspondence Number—C135789

Comment deadline: May 31, 2015

Sodium Thiosulfate

Na₂S₂O₃·5H₂O 248.18

Na₂S₂O₃ 158.11

Thiosulfuric acid, disodium salt, pentahydrate;
Disodium thiosulfate pentahydrate [10102-17-7].
Anhydrous [7772-98-7].

DEFINITION

Sodium Thiosulfate contains NLT 99.0% and NMT 100.5% of sodium thiosulfate (Na₂S₂O₃), calculated on the anhydrous basis.

IDENTIFICATION

Delete the following:

■● A. Procedure

Sample solution: ~~100 mg/mL in water~~

Analysis: ~~Add a few drops of iodine TS.~~

Acceptance criteria: ~~The color is discharged.~~ ■1S (USP39)

Add the following:

■● A. Infrared Absorption < 197A > ■1S (USP39)

Change to read:

● B. Identification Tests—General, Sodium < 191 >

Sample solution: ~~100 mg/mL~~

■0.1 g/mL ■1S (USP39)

in water

Acceptance criteria: Meets the requirements

Delete the following:

■● C. Identification Tests—General, Thiosulfate < 191 >

Sample solution: ~~100 mg/mL in water~~

Acceptance criteria: ~~Meets the requirements~~ ■1S (USP39)

ASSAY

Change to read:

- **Procedure**

Sample: 800 mg

Titrimetric system

(See *Titrimetry* 〈 541 〉.)

Mode: Direct titration

Titrant: 0.1 N iodine VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 30 mL of water. Adjust with ~~3 N hydrochloric acid~~
■ a suitable solution of dilute hydrochloric acid or dilute sodium hydroxide ■1S (USP39)

to a pH of 6.2–6.7, if necessary. Titrate with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Each mL of *Titrant* is equivalent to 15.81 mg of sodium thiosulfate (Na₂S₂O₃).

Acceptance criteria: 99.0%–100.5% on the anhydrous basis

IMPURITIES

- **Calcium**

Sample solution: Dissolve 1 g in 20 mL of water.

Analysis: To the *Sample solution* add a few mL of ammonium oxalate TS.

Acceptance criteria: No turbidity is produced.

Delete the following:

-

- **Heavy Metals** 〈 231 〉

Test preparation: Dissolve 1 g in 10 mL of water.

Analysis: To the *Test preparation* slowly add 5 mL of 3 N hydrochloric acid, evaporate on a steam bath nearly to dryness, and heat the residue at 150° for 1 h. Add 15 mL of water to the residue, boil gently for 2 min, and filter. Heat the filtrate to boiling, and add sufficient bromine TS to produce a clear solution and to provide a slight excess of bromine. Boil the solution to expel the excess bromine, cool to room temperature, add 1 drop of phenolphthalein TS, and neutralize with 1 N sodium hydroxide. Dilute with water to 25 mL.

Acceptance criteria: NMT 20 ppm (Official 1-Dec-2015)

SPECIFIC TESTS

Change to read:

- **Water Determination,**

■ *Method III* ■1S (USP39)

〈 921 〉

Sample: 1.0 g

Analysis: Dry the *Sample* under vacuum at 40°–45° for 16 h.

Acceptance criteria: 32.0%–37.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

Add the following:

- **USP Reference Standards** 〈 11 〉

USP Sodium Thiosulfate RS

- **1S** (USP39)

BRIEFING

Sodium Thiosulfate Injection, *USP 38* page 5338. On the basis of comments received, the monograph is revised as follows:

1. For *Identification* tests *A*, *B*, and *C*, the *Sample solution* concentration is expressed in g/mL to be consistent with the classic version.
2. Revise the *Assay* to allow the use of dilute acid or dilute base for pH adjustments.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: R.S. Prasad.)

Correspondence Number—C154312

Comment deadline: May 31, 2015

Sodium Thiosulfate Injection

DEFINITION

Sodium Thiosulfate Injection is a sterile solution of Sodium Thiosulfate in freshly boiled Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$).

IDENTIFICATION

Change to read:

- **A. Procedure**

Sample solution: Equivalent to ~~100 mg/mL~~

■ 0.1 g/mL ■ 1S (USP39)

of sodium thiosulfate in water

Analysis: Add a few drops of iodine TS.

Acceptance criteria: The color is discharged.

Change to read:

- **B. Identification Tests—General**, *Sodium* 〈 191 〉

Sample solution: Equivalent to ~~100 mg/mL~~

■ 0.1 g/mL ■ 1S (USP39)

of sodium thiosulfate in water

Acceptance criteria: Meets the requirements

Change to read:

● **C. Identification Tests—General, Thiosulfate** 〈 191 〉

Sample solution: Equivalent to ~~100 mg/mL~~

■ 0.1 g/mL ■ 1S (USP39)

of sodium thiosulfate in water

Acceptance criteria: Meets the requirements

ASSAY

Change to read:

● **Procedure**

Sample solution: Equivalent to 1 g of sodium thiosulfate from Injection. Adjust with ~~3-N~~ hydrochloric acid

■ a suitable solution of dilute hydrochloric acid or dilute sodium hydroxide ■ 1S (USP39)

to a pH of 6.2–6.7, and dilute with water to about 20 mL.

Titrimetric system

(See *Titrimetry* 〈 541 〉.)

Mode: Direct titration

Titrant: 0.1 N iodine VS

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Each mL of *Titrant* is equivalent to 24.82 mg of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$).

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

● **Bacterial Endotoxins Test** 〈 85 〉: NMT 0.03 USP Endotoxin Units/mg of sodium thiosulfate

● **pH** 〈 791 〉: 6.0–9.5

● **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* 〈 1 〉

ADDITIONAL REQUIREMENTS

● **Packaging and Storage:** Preserve in single-dose containers of Type I glass.

● **USP Reference Standards** 〈 11 〉

USP Endotoxin RS

BRIEFING

Succinylcholine Chloride, *USP 38* page 5363. As part of the USP monograph modernization effort, it is proposed to revise the monograph as follows:

1. The liquid chromatographic procedure in the test for *Limit of Choline* is difficult to execute and, in addition, one of the *Mobile phase* components, 1-hexanesulfonic acid, is unavailable outside of the United States. The current procedure is replaced with a liquid chromatographic procedure based on validated methods of analysis performed with the Dionex IonPac CS19 brand of L## column and the Dionex IonPac CG19 brand of L## guard column. The typical retention time for choline is about 11 min.
2. The *Definition* is revised to make it less ambiguous and a *Caution* statement is added.
3. *Identification* test C is deleted because *Identification* test A and *Identification* test B are two orthogonal methods sufficient to ensure the identity of the drug substance.
4. An *Identification* test for chloride is added.
5. The particle size for the column used in the *Assay* is specified, the *Note* is deleted, and the *Standard solution* preparation is updated.
6. The test for the counter ion, *Content of Chloride*, is deleted because it is not needed.
7. Citric acid and succinic acid are replaced with USP Citric Acid RS and USP Succinic Acid RS, respectively, in the test for *Organic Impurities*.
8. The *Sterility Tests* and *Bacterial Endotoxins Test* are deleted because they are redundant as the testing is performed on the drug product, *Succinylcholine Chloride Injection*.
9. The *Packaging and Storage* section is updated to remove the reference to a storage temperature.
10. The *Labeling* statement is deleted because it is redundant as the requirements are covered in the *Definition* and in the drug product monograph, *Succinylcholine Chloride Injection*.
11. USP Potassium Chloride RS, USP Citric Acid RS, and USP Succinic Acid RS are added to the *Reference Standards* section in support of the proposed changes to the tests for *Limit of Choline* and *Organic Impurities*.
12. USP Endotoxin RS is deleted from the *USP Reference Standards* section due to the proposed omission of the *Bacterial Endotoxins Test*.

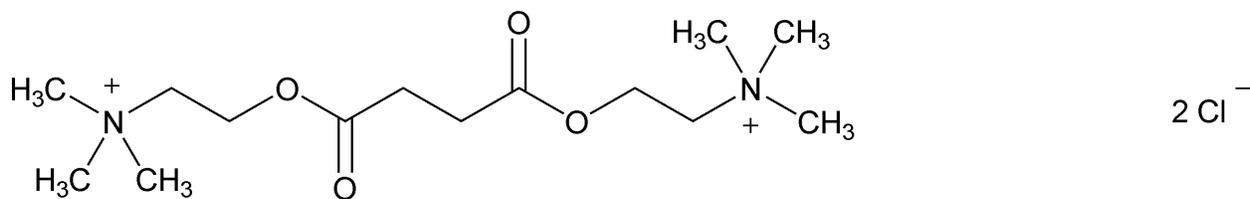
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: M. Koleček.)

Correspondence Number—C127194

Comment deadline: May 31, 2015

Succinylcholine Chloride



$C_{14}H_{30}Cl_2N_2O_4$ (anhydrous) 361.31

$C_{14}H_{30}Cl_2N_2O_4 \cdot 2H_2O$ 397.34

Ethanaminium, 2,2'-[(1,4-dioxo-1,4-butanediyl) bis(oxy)]bis[*N,N,N*-trimethyl]-, dichloride;

Choline chloride succinate (2:1) [71-27-2].
Dihydrate [6101-15-1].

DEFINITION

Change to read:

Succinylcholine Chloride ~~usually contains approximately two molecules of water of hydration. It~~

■ **1S (USP39)**

contains NLT 96.0% and NMT 102.0% of succinylcholine chloride ($C_{14}H_{30}Cl_2N_2O_4$), calculated on the anhydrous basis.

■ **[Caution—**Succinylcholine chloride is a neuromuscular blocking agent. Great care should be taken when handling to avoid inhalation of dust or contact with skin.]

■ **1S (USP39)**

IDENTIFICATION

- **A. Infrared Absorption** ~~(197K)~~
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

Delete the following:

- **C. Thin-Layer Chromatographic Identification Test** ~~(201)~~

Standard solution: ~~1 mg/mL of USP Succinylcholine Chloride RS in water~~

Sample solution: ~~1 mg/mL of succinylcholine chloride in water~~

Chromatographic system

~~See Chromatography (621) Thin-Layer Chromatography~~

Adsorbent: ~~0.25-mm layer of chromatographic silica gel~~

Application volume: ~~1 μ L~~

Developing solvent system: ~~Acetone and 1 N hydrochloric acid (1:1)~~

Analysis

Samples: ~~Standard solution and Sample solution~~

~~Proceed as directed in Thin-Layer Chromatographic Identification Test (201). To locate the spots, heat the plate at 105° for 5 min, cool, and spray with potassium bismuth iodide TS, then heat again at 105° for 5 min.~~

Acceptance criteria: ~~Meet the requirements~~ ■ **1S (USP39)**

Add the following:

- **C. Identification Tests—General, Chloride** ~~(191)~~: Meets the requirements ■ **1S (USP39)**

ASSAY

Change to read:

- **Procedure**

~~[Note—Because the *Mobile phase* used in this procedure has a fairly high concentration of chloride ion and a low pH, it is advisable to rinse the entire system with water following the use of this *Mobile phase*.]~~

■ ■ 1S (USP39)

Mobile phase: Prepare a 1 in 10 solution of 1 N aqueous tetramethylammonium chloride in methanol. Adjust with hydrochloric acid to a pH of about 3.0.

Standard solution: 8.8 mg/mL of USP Succinylcholine Chloride RS prepared as follows. Transfer a suitable amount of USP Succinylcholine Chloride RS to a suitable volumetric flask and dissolve in 40% of the total volume of water. Dilute with *Mobile phase* to volume while mixing. ~~Prepare the *Standard solution* concurrently with the *Sample solution*.~~

■ ■ 1S (USP39)

Sample solution: 8.8 mg/mL of Succinylcholine Chloride prepared as follows. Transfer a suitable amount of Succinylcholine Chloride to a suitable volumetric flask and dissolve in 40% of the total volume of water. Dilute with *Mobile phase* to volume while mixing.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4-mm × 25-cm;

■ 10- μ m ■ 1S (USP39)

packing L3

Flow rate: 0.75 mL/min

Injection volume: 10 μ L by means of a suitable microsyringe or sampling valve

■ ■ 1S (USP39)

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of succinylcholine chloride ($C_{14}H_{30}Cl_2N_2O_4$) in the portion of Succinylcholine Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Succinylcholine Chloride RS in the *Standard solution* (mg/mL)

C_U concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–102.0% on the anhydrous basis

Analysis**Samples:** *Standard solution and Sample solution*

Calculate the percentage of each impurity in the portion of Succinylcholine Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak area of each impurity from the *Sample solution* r_S = peak area of succinylmonocholine chloride from the *Standard solution* C_S = concentration of USP Succinylmonocholine Chloride RS in the *Standard solution* (mg/mL) C_U = concentration of Succinylcholine Chloride in the *Sample solution* (mg/mL) F = relative response factor (see *Table 1*)**Acceptance criteria:** See *Table 1*.**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
■ Edetate disodium ^a	0.18	—	— ■ 1S (USP39)
Succinic acid	0.22	1.6	0.1
Unidentified impurity 1 ^b	0.32	1.0	0.4
Unidentified impurity 2 ^b	0.32	1.0	
Succinylmonocholine	0.49	1.0	0.4
Succinylcholine	1.0	—	—
Any unspecified impurity	—	1.0	0.2
Total impurities ^{b,c}	—	—	1.5

■^a Included for identification purposes only. Begin integration after this peak, if present.
■ 1S (USP39)

b May occur as a doublet. Acceptance criteria is for the sum of both peaks.

c Total impurities include the sum of the results for *Organic Impurities* and *Limit of Choline*.

Change to read:● **Limit of Choline****Solution A:** ~~5% (v/v) of acetonitrile and 5% (w/v) of 0.1 M 1-hexanesulfonic acid in water~~**Solution B:** ~~Acetonitrile and water (1:1)~~**Mobile phase:** ~~See Table 2.~~**Table 2**

	Time (min)	Solution A (%)	Solution B (%)
θ		100	θ
15		100	θ
16		θ	100
25		θ	100
27		100	θ

Time (min)	Solution A (%)	Solution B (%)
40	100	0

System suitability solution: 0.05 mg/mL of USP Choline Chloride RS and 0.01 mg/mL of sodium chloride in water

Standard stock solution: 0.5 mg/mL of USP Choline Chloride RS in water

Standard solution: 0.01 mg/mL of USP Choline Chloride RS from *Standard stock solution* in water

Sample solution: 2 mg/mL of Succinylcholine Chloride in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC— Ion chromatograph is equipped with a suitable device for chemical suppression.

Detector: Conductivity detector at 30 μ S

Suppressor current setting: 50 mA

Regenerant for the suppressor: Water

Regenerant flow rate: 5–10 mL/min

Column: 4.6 mm \times 25 cm; 5 μ m packing L1

Flow rate: 1 mL/min

Injection volume: 50 μ L

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between sodium and choline

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of choline in the portion of Succinylcholine Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of choline from the *Sample solution*

r_S = peak area of choline from the *Standard solution*

C_S = concentration of USP Choline Chloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Succinylcholine Chloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of choline, 104.17

M_{r2} = molecular weight of choline chloride, 139.62

Mobile phase:

0.62 g/L of methanesulfonic acid. Alternatively, the *Mobile phase* can be generated electrolytically using an automatic eluant generator.

System suitability solution: 10 μ g/mL of USP Choline Chloride RS and 5 μ g/mL of USP Potassium Chloride RS

Standard solution: 8 μ g/mL of USP Choline Chloride RS

Sample solution: 2 mg/mL of Succinylcholine Chloride. Store at 4° immediately following

preparation.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Conductivity with suppression

Cell temperature: 35°

Columns

Guard: 2-mm × 5-cm; packing L##

Analytical: 2-mm × 25-cm; packing L##

Flow rate: 0.25 mL/min

Injection volume: 5 µL

Autosampler temperature: 4°

Run time: NLT 3 times the retention time of choline

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for potassium and choline are 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5.0 between choline and potassium

Relative standard deviation: NMT 3.0% for choline

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of choline in the portion of Succinylcholine Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U

= peak response of choline from the *Sample solution*

r_S

= peak response of choline from the *Standard solution*

C_S

= concentration of USP Choline Chloride RS in the *Standard solution* (mg/mL)

C_U

= concentration of Succinylcholine Chloride in the *Sample solution* (mg/mL)

M_{r1}

= molecular weight of choline, 104.17

M_{r2}

= molecular weight of choline chloride, 139.62

■ 1S (USP39)

Acceptance criteria: NMT 0.3%

SPECIFIC TESTS

- **Water Determination, Method I** (921): NMT 10.0%

Delete the following:

- ● **Sterility Tests** (71): Where the label states that Succinylcholine Chloride is sterile, it meets the requirements. ■ 1S (USP39)

Delete the following:

- ● **Bacterial Endotoxins Test** (85): Where the label states that Succinylcholine Chloride must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 2.0 USP Endotoxin Units/mg of succinylcholine chloride. ■ 1S (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers. Store at 25° , excursions permitted between 15° and 30° .

■ 1S (USP39)

Delete the following:

- ● **Labeling:** Label it in terms of its anhydrous equivalent. Where it is intended for use in preparing injectable or other sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. ■ 1S (USP39)

Change to read:

- **USP Reference Standards** (11)

USP Choline Chloride RS

USP Endotoxin RS

■ USP Citric Acid RS

USP Potassium Chloride RS

USP Succinic Acid RS ■ 1S (USP39)

USP Succinylcholine Chloride RS

USP Succinylmonocholine Chloride RS

Ethanaminium, 2-(carboxy-1-oxopropoxy)-*N,N,N*-trimethyl-, chloride.

C₉H₁₈ClNO₄ 239.70

BRIEFING

Sulfamethoxazole and Trimethoprim Oral Suspension, USP 38 page 5397 and PF 40(1) [Jan.-Feb. 2014]. On the basis of a comment received, the revision appearing in PF 40(1) is

canceled and replaced with the following proposal. It is proposed to modernize the monograph with the following changes:

1. Replace the *Limit of Trimethoprim Degradation Product* and *Limit of Sulfanilamide, Sulfanilic Acid, and Sulfamethoxazole N₄-Glucoside* tests based on TLC with a selective and validated HPLC method for *Organic Impurities*. The HPLC procedure in the test for *Organic Impurities* is based on analysis performed with the Sunfire C18 brand of L1 column. The typical retention times of trimethoprim and sulfamethoxazole are 14.4 and 20.4 min, respectively.
2. Revise the *Identification* test based on TLC with the retention time agreement for sulfamethoxazole and trimethoprim in the *Assay*.
3. Add additional storage requirements based on the drug package insert.
4. Add USP Diaveridine RS, USP Sulfamethoxazole Related Compound C RS, USP Trimethoprim Related Compound A RS, and USP Trimethoprim Related Compound B RS to the *USP Reference Standards* section to ensure the system suitability performance for the HPLC procedure and quantitative control of impurities in the product.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM1: D. Min.)

Correspondence Number—C145225; C120149

Comment deadline: May 31, 2015

Sulfamethoxazole and Trimethoprim Oral Suspension

DEFINITION

Sulfamethoxazole and Trimethoprim Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amounts of sulfamethoxazole (C₁₀H₁₁N₃O₃S) and trimethoprim (C₁₄H₁₈N₄O₃).

IDENTIFICATION

Delete the following:

- ~~A. The *R_f* values of the principal spots of the *Sample solution* correspond to those of the *Standard solutions* of USP Trimethoprim RS and USP Sulfamethoxazole RS, as obtained in the *Impurities* tests. ■1S (USP39)~~

Add the following:

- A. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

ASSAY

Change to read:

● Procedure

Mobile phase: Mix 1400 mL of water, 400 mL of acetonitrile, and 2.0 mL of triethylamine in a 2000-mL volumetric flask. Allow to equilibrate to room temperature, and adjust with 0.2 N sodium hydroxide or dilute glacial acetic acid (1 in 100) to a pH of 5.9 ± 0.1. Dilute with

water to volume, and pass through a filter of 0.45- μm pore size.

Standard stock solution: 0.32 mg/mL of USP Trimethoprim RS and 0.32*J* mg/mL of USP Sulfamethoxazole RS in methanol, where *J* is the ratio of the labeled amount, in mg, of sulfamethoxazole to the labeled amount, in mg, of trimethoprim in the dosage form

Standard solution: 0.032 mg/mL of USP Trimethoprim RS per mL and 0.032*J* mg/mL of USP Sulfamethoxazole RS per mL in *Mobile phase* from *Standard stock solution*

Sample stock solution: Transfer a volume of Oral Suspension, equivalent to 80 mg of sulfamethoxazole, to a 50-mL volumetric flask with the aid of 30 mL of methanol. Sonicate the mixture for 10 min with occasional shaking. Allow to equilibrate to room temperature, dilute with methanol to volume, and centrifuge. Use the filtrate in the preparation of the *Sample solution*.

Sample solution: Nominally 0.16 mg/mL of sulfamethoxazole in *Mobile phase* from *Sample stock solution*. Filter the solution.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm;

■ 10- μm ■ 1S (USP39)

packing L1

Flow rate: 2 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for trimethoprim and sulfamethoxazole are 1.0 and 1.8, respectively.]

Suitability requirements

Resolution: NLT 5.0 between sulfamethoxazole and trimethoprim

Tailing factor: NMT 2.0 for sulfamethoxazole and trimethoprim

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of trimethoprim ($\text{C}_{14}\text{H}_{18}\text{N}_4\text{O}_3$) and sulfamethoxazole ($\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_3\text{S}$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trimethoprim or sulfamethoxazole from the *Sample solution*

r_S = peak response of trimethoprim or sulfamethoxazole from the *Standard solution*

C_S = concentration of the USP Trimethoprim RS or USP Sulfamethoxazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of trimethoprim or sulfamethoxazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of sulfamethoxazole ($\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_3\text{S}$) and trimethoprim ($\text{C}_{14}\text{H}_{18}\text{N}_4\text{O}_3$)

PERFORMANCE TESTS

- **Uniformity of Dosage Units** (905): Meets the requirements for oral suspension packaged in single-unit containers
- **Deliverable Volume** (698): Meets the requirements for oral suspension packaged in multiple-unit containers

IMPURITIES**Delete the following:**

- **Limit Of Trimethoprim Degradation Product**

Diluent: Chloroform and methanol (8:2)

Standard solution A: 20 mg/mL of USP Trimethoprim RS in *Diluent*

Standard solution B: 0.1 mg/mL of USP Trimethoprim RS in *Diluent* from *Standard solution A*

Sample solution: Transfer a measured volume of Oral Suspension, equivalent to 40 mg of trimethoprim, to a separatory funnel. Extract with three 25-mL portions of *Diluent*, collecting the extracts in a 125-mL conical flask. Evaporate the combined extracts with the aid of a current of air to dryness on a steam bath. Dissolve the residue in 2.0 mL of *Diluent*, then centrifuge.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5- μ L

Developing solvent system: Chloroform, methanol, and ammonium hydroxide (80:20:3)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram until the solvent front has moved at least 15 cm. Remove the plate from the chamber, air-dry, and view under short-wavelength UV light. Trimethoprim produces a spot at about R_f 0.7, and the trimethoprim degradation product can be seen at R_f 0.3–0.5.

Acceptance criteria: Any spot from the *Sample solution* at about R_f 0.3–0.5 is not greater in size and intensity than the spot produced by *Standard solution B* at about R_f 0.7, corresponding to NMT 0.5%. ■ 1S (USP39)

Delete the following:

- **Limit of Sulfanilamide, Sulfanilic Acid, and Sulfamethoxazole N_4 -Glucoside**

Solution A: Dehydrated alcohol and methanol (95:5)

Standard solution A: Transfer 20 mg of USP Sulfamethoxazole RS into a 10-mL volumetric flask, dissolve in 1 mL of ammonium hydroxide, and dilute with methanol to volume.

Standard solution B: Transfer 10 mg of USP Sulfanilamide RS into a 50-mL volumetric flask, dissolve in 5 mL of ammonium hydroxide, and dilute with methanol to volume. Pipet 5 mL of this solution into a 100-mL volumetric flask, add 10 mL of ammonium hydroxide, and dilute with methanol to volume.

Standard solution C: Transfer 10 mg of USP Sulfanilic Acid RS into a 50-mL volumetric flask, dissolve in 5 mL of ammonium hydroxide, and dilute with methanol to volume. Pipet 3

~~mL of this solution into a 100-mL volumetric flask, add 10 mL of ammonium hydroxide, and dilute with methanol to volume.~~

~~**Standard solution D:** Transfer 3.0 mg of USP Sulfamethoxazole N_4 -Glucoside RS into a 50-mL volumetric flask, dissolve in 5 mL of ammonium hydroxide, and dilute with methanol to volume.~~

~~**Sample solution:** Using a syringe, transfer a volume of Oral Suspension, equivalent to 200 mg of sulfamethoxazole, to a 100-mL volumetric flask containing 10 mL of ammonium hydroxide, and add 50 mL of methanol. Shake for 3 min, and dilute with methanol to volume. Centrifuge a portion of the solution for 3 min.~~

Chromatographic system

~~(See Chromatography <621>, Thin-Layer Chromatography.)~~

~~**Mode:** TLC~~

~~**Adsorbent:** 0.25-mm layer of chromatographic silica gel~~

~~**Application volume:** 50 μ L~~

~~**Developing solvent system:** Solution A, heptane, chloroform, and glacial acetic acid (25:25:25:7)~~

~~**Spray reagent (Modified Ehrlich's reagent):** 100 mg of *p*-dimethylaminobenzaldehyde in 1 mL of hydrochloric acid. Dilute with alcohol to 100 mL.~~

Analysis

~~**Samples:** Standard solution A, Standard solution B, Standard solution C, Standard solution D, and Sample solution~~

~~Apply the Samples to separate points on the Adsorbent. Place the plate in an unsaturated chromatographic chamber, and develop the chromatogram until the solvent front has moved at least 12 cm. Remove the plate from the developing chamber, air-dry, spray with Spray reagent, and allow the plate to stand for 15 min.~~

~~**Acceptance criteria:** Sulfamethoxazole produces a spot at about R_f 0.7. Any spots from the Sample solution at about R_f 0.5, 0.1, and 0.3 are not greater in size and intensity than spots produced by Standard solutions B, C, and D, respectively, corresponding to NMT 0.5% of sulfanilamide, 0.3% of sulfanilic acid, and 3.0% of sulfamethoxazole N_4 -glucoside. ■1S (USP39)~~

Add the following:

■● Organic Impurities

Solution A: Triethylamine and water (2.5: 2000). Adjust with glacial acetic acid to a pH of 5.9.

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	80	20
25	60	40
30	20	80

Time (min)	Solution A (%)	Solution B (%)
31	90	10
35	90	10

Diluent: *Solution A* and *Solution B* (75:25)

System suitability solution: 1 mg/mL of USP Sulfamethoxazole RS; 0.2 mg/mL of USP Trimethoprim RS; 1 µg/mL each of USP Sulfamethoxazole *N*₄-Glucoside RS, USP Sulfamethoxazole Related Compound C RS, USP Sulfanilamide RS, and USP Sulfanilic Acid RS from *Standard stock solution A*; and 0.3 µg/mL each of USP Trimethoprim Related Compound A RS, USP Trimethoprim Related Compound B RS, and USP Diaveridine RS from *Standard stock solution B* in *Diluent*

Standard stock solution A: 0.2 mg/mL each of USP Sulfamethoxazole *N*₄-Glucoside RS, USP Sulfamethoxazole Related Compound C RS, USP Sulfanilamide RS, and USP Sulfanilic Acid RS in *Diluent*. Sonicate for 5 min to dissolve.

Standard stock solution B: 0.03 mg/mL each of USP Trimethoprim Related Compound A RS, USP Trimethoprim Related Compound B RS, and USP Diaveridine RS in *Diluent*. Sonicate for 5 min to dissolve.

Standard solution: 0.03 mg/mL of USP Sulfamethoxazole *N*₄-Glucoside RS; 5 µg/mL each of USP Sulfamethoxazole Related Compound C RS and USP Sulfanilamide RS; 3 µg/mL of USP Sulfanilic Acid RS from *Standard stock solution A*; and 1 µg/mL each of USP Trimethoprim RS, USP Trimethoprim Related Compound A RS, USP Trimethoprim Related Compound B RS, and USP Diaveridine RS from *Standard stock solution B* in *Diluent*

Sample solution: Nominally 1 mg/mL of sulfamethoxazole and 0.2 mg/mL of trimethoprim from a volume of Oral Suspension in *Diluent*. Sonicate for 15 min in *Diluent* before diluting to final volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between trimethoprim and trimethoprim related compound A; and NLT 1.5 between sulfamethoxazole and trimethoprim related compound B

Relative standard deviation: NMT 2.0% for sulfanilic acid, sulfanilamide, sulfamethoxazole related compound C, sulfamethoxazole *N*₄-glucoside, diaveridine, trimethoprim, trimethoprim related compound A, sulfamethoxazole, and trimethoprim related compound B

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sulfanilic acid, sulfanilamide, sulfamethoxazole related compound C, and sulfamethoxazole *N*₄-glucoside in the portion of Oral Suspension taken:

$$\text{Result} = (r_i/r_c) \times (C_c/C_i) \times 100$$

r_U peak response of sulfanilic acid, sulfanilamide, sulfamethoxazole related compound C, or sulfamethoxazole N_4 -glucoside from the *Sample solution*

r_S peak response of sulfanilic acid, sulfanilamide, sulfamethoxazole related compound C, or sulfamethoxazole N_4 -glucoside from the *Standard solution*

C_S concentration of USP Sulfanilic Acid RS, USP Sulfanilamide RS, USP Sulfamethoxazole Related Compound C RS, or USP Sulfamethoxazole N_4 -Glucoside RS in the *Standard solution* (mg/mL)

C_U nominal concentration of sulfamethoxazole in the *Sample solution* (mg/mL)

Calculate the percentage of diaveridine, trimethoprim related compound A, trimethoprim related compound B, and any other unspecified degradation product in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of diaveridine, trimethoprim related compound A, trimethoprim related compound B, or any other unspecified degradation product from the *Sample solution*

r_S peak response of diaveridine, trimethoprim related compound A, trimethoprim related compound B, or trimethoprim (for any other unspecified degradation product) from the *Standard solution*

C_S concentration of USP Diaveridine RS, USP Trimethoprim Related Compound A RS, USP Trimethoprim Related Compound B RS, or USP Trimethoprim RS (for any other unspecified degradation product) in the *Standard solution* (mg/mL)

C_U nominal concentration of trimethoprim in the *Sample solution* (mg/mL)

Acceptance criteria See *Table 2*. Disregard limit: 0.01%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sulfanilic acid	0.18	0.3
Sulfanilamide	0.32	0.5
Sulfamethoxazole related compound C	0.36	0.2
Sulfamethoxazole related compound F ^a	0.50	—
Sulfamethoxazole N_4 -glucoside	0.54	3.0
Diaveridine	0.62	0.5
Trimethoprim	0.71	—
Trimethoprim related compound A	0.74	0.5
Sulfamethoxazole related compound A ^a	0.81	—
Sulfamethoxazole	1.00	—
Trimethoprim related compound B	1.03	0.5
Any other unspecified degradation product	—	0.2
^a Process related impurities monitored in the drug substance.		

SPECIFIC TESTS

- **pH** 〈 791 〉: 5.0–6.5
- **Alcohol Determination, Method II** 〈 611 〉: NMT 0.5% of alcohol (C₂H₅OH)

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- Store at controlled room temperature. Protect from light. ■ 1S (USP39)

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Alcohol Determination–Acetonitrile RS

USP Alcohol Determination–Alcohol RS

- USP Diaveridine RS

5-[(3,4-Dimethoxyphenyl)methyl]-2,4-pyrimidinediamine.

C₁₃H₁₆N₄O₂ 260.29 ■ 1S (USP39)

USP Sulfamethoxazole RS

USP Sulfamethoxazole N₄-Glucoside RS

4-(Beta-d-glucopyranosylamino)-N-(5-methyl-3-isoxazolyl)-benzenesulfonamide.

C₁₆H₂₁N₃O₈S 415.42

- USP Sulfamethoxazole Related Compound C RS

5-Methyl-3-isoxamine.

C₄H₆N₂O 98.10 ■ 1S (USP39)

USP Sulfanilamide RS

p-Aminobenzenesulfonamide.

C₆H₈N₂O₂S 172.20

USP Sulfanilic Acid RS

Benzenesulfonic acid, 4-amino-

C₆H₇NO₃S 173.19

USP Trimethoprim RS

- USP Trimethoprim Related Compound A RS

4-Amino-5-(3,4,5-trimethoxybenzyl)pyrimidin-2-ol.

C₁₄H₁₇N₃O₄ 291.30

USP Trimethoprim Related Compound B RS

(2,4-Diaminopyrimidin-5-yl)(3,4,5-trimethoxyphenyl)methanone.

C₁₄H₁₆N₄O₄ 304.30

■ 1S (USP39)

BRIEFING

Tacrolimus, USP 38 page 5425. On the basis of comments received, the monograph is revised as follows:

1. The diluent in the *Assay* and *Organic Impurities, Procedure 2* is revised from an acetonitrile–water mixture to acetonitrile to minimize the formation of unknown impurity peaks. Because the solution does not contain water, the formation of the open ring and 19-epimer is minimized and the 3-h equilibration step is not required.
2. The requirement for storing solutions in low-actinic glassware is deleted, allowing flexibility to use other ways of protecting solutions from light.
3. *Organic Impurities, Procedure 1* is revised to correct the calculation formula.
4. Tacrolimus 8-epimer, a degradation product, and tacrolimus 8-propyl analog, a process impurity, elute close together and may switch positions depending on the column used. *Peak identification solution 1* and *Peak identification solution 2* are added to *Organic Impurities, Procedure 2* to facilitate the identification of these two peaks.
5. The *USP Reference Standards* section is revised to include the Reference Standards used in *Organic Impurities, Procedure 2*.

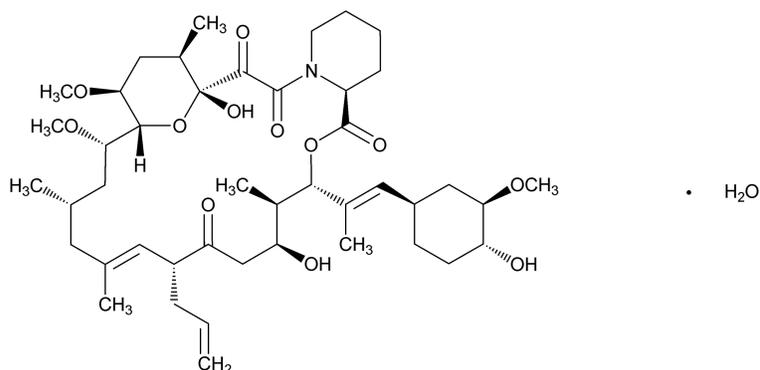
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM1: A. Wise.)

Correspondence Number—C126637

Comment deadline: May 31, 2015

Tacrolimus



$C_{44}H_{69}NO_{12} \cdot H_2O$ 822.03

15,19-Epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-, monohydrate, [3*S*-[3*R**,*E*(1*S**,3*S**,4*S**)],4*S**,5*R**,8*S**,9*E*,12*R**,14*R**,15*S**,16*R**,18*S**,19*S**,26a*R**]]-;

(-)-(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone monohydrate [109581-93-3].

DEFINITION

Tacrolimus contains NLT 98.0% and NMT 102.0% of tacrolimus ($C_{44}H_{69}NO_{12}$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. Infrared Absorption** (197M)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

- Protect solutions containing tacrolimus from light.

- 1S (USP39)

Solution A: 6 mM phosphoric acid

Solution B: Acetonitrile and *tert*-butyl methyl ether (81:19)

Solution C: *Solution A* and *Solution B* (4:1)

Solution D: *Solution A* and *Solution B* (1:4)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution C (%)	Solution D (%)
0	72	28
30	72	28
53	15	85
54	72	28
60	72	28

~~**Diluent:** Acetonitrile and water (7:3)~~

~~**System suitability solution:** 3 mg/mL of USP Tacrolimus System Suitability Mixture RS in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use. Protect from light by using low-actinic glassware.~~

~~**Standard solution:** 3 mg/mL of USP Tacrolimus RS in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use. Protect from light by using low-actinic glassware.~~

~~**Sample solution:** 3 mg/mL of Tacrolimus in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use. Protect from light by using low-actinic glassware.~~

- **System suitability solution:** 3 mg/mL of USP Tacrolimus System Suitability Mixture RS in acetonitrile

- Standard solution:** 3 mg/mL of USP Tacrolimus RS in acetonitrile

- Sample solution:** 3 mg/mL of Tacrolimus in acetonitrile ■ 1S (USP39)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 3-μm packing L1

Temperatures

Column: 60°

Autosampler: 4°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for tacrolimus open ring, tacrolimus 19-epimer, ascomycin, tacrolimus, and are 0.52, 0.63, 0.87, and 1.0, respectively.]

■ See *Table 3* for relative retention times. ■ 1S (USP39)

]

Suitability requirements

Resolution: NLT 3.0 between ascomycin and tacrolimus, *System suitability solution*

Relative standard deviation: NMT 1.0% for the sum of the responses of tacrolimus, tacrolimus open ring, and tacrolimus 19-epimer, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tacrolimus (C₄₄H₆₉NO₁₂) in the portion of Tacrolimus taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak responses of tacrolimus open ring, tacrolimus 19-epimer, and tacrolimus from the *Sample solution*

r_S = sum of the peak responses of tacrolimus open ring, tacrolimus 19-epimer, and tacrolimus from the *Standard solution*

C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)

C_U = concentration of Tacrolimus in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Delete the following:

●

- **Heavy Metals, Method II** 〈 231 〉

: NMT 10 ppm (Official 1-Dec-2015)

Change to read:

- **Organic Impurities, Procedure 1**

Use *Organic Impurities, Procedure 1* when the impurity profile includes tacrolimus methylacrylaldehyde and tacrolimus diene. It is suggested that new columns be conditioned with about 500 mL of alcohol before use to meet the resolution criterion.

Mobile phase: Hexane, *n*-butyl chloride, and acetonitrile (7:2:1). Add *n*-butyl chloride to hexane, and mix well before adding acetonitrile. After adding acetonitrile, mix the mobile phase for 2 h to get a clear solution. Any deviations from the ratio of components in the

mobile phase and the order of mixing will result in a two-phase solution.

System suitability solution: 0.1 mg/mL each of USP Tacrolimus RS and USP Tacrolimus Related Compound A RS in *Mobile phase*

Sample solution: 2.0 mg/mL of Tacrolimus in *Mobile phase*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: Two 4.6-mm × 25-cm columns; 5-µm packing L20

Column temperature: 28 ± 2°

Flow rate: 1.5 mL/min

Adjust the flow rate so that the retention time of tacrolimus is approximately 15 min.

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.1 between tacrolimus and tacrolimus related compound A

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tacrolimus taken:

$$\text{Result} = (r_U/F) \times [1/\Sigma(r_U/F)] \times 100$$

~~r_U~~ = peak response of each peak from the *Sample solution*

~~F~~ = relative response factor for the corresponding peak (see *Table 2*)

■
$$\text{Result} = (r_U/F_i) \times \{1/[r_T + \Sigma(r_U/F_i)]\} \times 100$$

r_U
= peak response of each impurity from the *Sample solution*

F_i
= relative response factor for each corresponding impurity (see *Table 2*)

r_T
= peak response of tacrolimus from the *Sample solution*

■ 1S (USP39)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tacrolimus methylacryl aldehyde ^a	0.55	16.7	0.2

Tacrolimus diene ^b	0.79	2.2	0.2
Tacrolimus impurity 1 ^c	0.96	1.0	0.2
Tacrolimus related compound A ^d	0.96	—	—
Tacrolimus	1.0	1.0	—
Tacrolimus 19-epimer ^{d,e}	1.1	—	—
Tacrolimus open ring ^{d,f}	1.3	—	—
Any individual unspecified impurity	—	1.0	0.2
Total impurities ^g	—	—	0.3

a (E)-3-[[(1R,3R,4R)-4-Hydroxy-3-methoxycyclohexyl]-2-methylacrylaldehyde.

b (14E,18E)-17-Allyl-1-hydroxy-12-[(E)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}] octacosan-14,18-diene-2,3,10,16-tetrone.

c Specified unidentified impurity.

d For informational purposes only; not to be reported.

e (3S,4R,5S,8R,9E,12S,14S,15R,16S,18R,19S,26aS)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a,hexadecahydro-5,19-dihydroxy-3-[(E)-2-[(1R,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone.

f (3S,4R,5S,8R,12S,14S,15R,16S,18R,26aS,E)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26a-tetradecahydro-5,15,20,20-tetrahydroxy-3-[(E)-2-[(1R,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-3H-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,19,21(4H,8H,20H,23H)-tetrone.

g Total impurities limit does not include tacrolimus open ring and tacrolimus 19-epimer.

Change to read:

• Organic Impurities, Procedure 2

Use *Organic Impurities, Procedure 2* when the impurity profile includes ascomycin, desmethyl tacrolimus, tacrolimus 8-epimer, and tacrolimus 8-propyl analog.

■ Protect solutions containing tacrolimus from light. ■ 1S (USP39)

Solution A, Solution B, Solution C, Solution D, Mobile phase, Diluent,

■ 1S (USP39)

System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

~~Standard solution: 30 µg/mL of USP Tacrolimus RS in Diluent. Allow the solution to stand for 3 h at ambient temperature before use. Protect from light by using low-actinic glassware.~~

~~Reporting threshold solution: 1.5 µg/mL of USP Tacrolimus RS in Diluent~~

■ Standard solution: 30 µg/mL of USP Tacrolimus RS in acetonitrile

Reporting threshold solution: 1.5 µg/mL of USP Tacrolimus RS in acetonitrile

Peak identification solution 1: 10 µg/mL of USP Tacrolimus 8-epimer RS in acetonitrile

Peak identification solution 2: 10 µg/mL of USP Tacrolimus 8-propyl Analog RS in acetonitrile ■ 1S (USP39)

System suitability

[Note—Identify the related compounds by the relative retention times provided in Table 3.]

Samples: System suitability solution and Standard solution

Suitability requirements**Resolution:** NLT 3.0 between tacrolimus and ascomycin, *System suitability solution***Relative standard deviation:** NMT 10.0% for the sum of the responses of tacrolimus and tacrolimus 19-epimer, *Standard solution***Analysis****Samples:** *Sample solution, Standard solution, Reporting threshold solution,*■ *Peak identification solution 1, and Peak identification solution 2* ■ 1S (USP39)

Calculate the percentage of each impurity in the portion of Tacrolimus taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_S = sum of the peak responses of tacrolimus 19-epimer and tacrolimus from the *Standard solution* C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL) C_U = concentration of Tacrolimus in the *Sample solution* (mg/mL)**Acceptance criteria:** See *Table 3*.■ Identify tacrolimus 8-epimer and tacrolimus 8-propyl analog using *Peak identification solution 1* and *Peak identification solution 2*. ■ 1S (USP39)Report impurity peaks with responses NLT that of the peak in the *Reporting threshold solution* (0.05%). Disregard peaks with retention times less than 3 min.**Table 3**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tacrolimus open ring ^{a,b}	0.52	—
Ascomycin 19-epimer ^c	0.54	0.1
Tacrolimus 19-epimer ^{b,d}	0.63	—
Ascomycin ^e	0.87	0.50
Desmethyl tacrolimus ^f	0.94	0.1
Tacrolimus	1.00	—
Tacrolimus 8-epimer ^g	1.28	0.15
Tacrolimus 8-propyl analog ^h	1.33	0.15
Any individual unspecified impurity	—	0.1
Total impurities ⁱ	—	1.0

- a (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26*aS*,*E*)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26*a*-tetradecahydro-5,15,20,20-tetrahydroxy-3- $\{(E)$ -2- $\{[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl\}$ -14,16-dimethoxy-4,10,12,18-tetramethyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,19,21(4*H*,8*H*,20*H*,23*H*)-tetrone.
- b Tacrolimus open ring and tacrolimus 19-epimer are isomers of tacrolimus, which are present in equilibrium with the active ingredient. They are not to be reported as degradation products.
- c (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26*aS*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3- $\{(E)$ -2- $\{[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl\}$ -14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.
- d (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26*aS*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3- $\{(E)$ -2- $\{[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl\}$ -14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
- e (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3- $\{(E)$ -2- $\{[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl\}$ -14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.
- f (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3- $\{(E)$ -2- $\{[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl\}$ -14,16-dimethoxy-4,12,18-trimethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.
- g (3*S*,4*R*,5*S*,8*S*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3- $\{(E)$ -2- $\{[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl\}$ -14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
- h (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-Hexadecahydro-5,19-dihydroxy-3- $\{(E)$ -2- $\{[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl\}$ -14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
- i Total impurities limit does not include tacrolimus open ring and tacrolimus 19-epimer.

SPECIFIC TESTS

- **Optical Rotation**, *Specific Rotation* $\langle 781S \rangle$
Sample solution: 10 mg/mL in *N,N*-dimethylformamide
Acceptance criteria: -110° to -115° on the "as is" basis
- **Water Determination**, *Method I* $\langle 921 \rangle$: NMT 4.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.

- **Labeling:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which test for *Organic Impurities* the article complies.

Change to read:

- **USP Reference Standards** (11)

USP Tacrolimus RS
 15,19-Epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-, monohydrate, [3*S*-[3*R**,*E*(1*S**,3*S**,4*S**)],4*S**,5*R**,8*S**,9*E*,12*R**,14*R**,15*S**,16*R**,18*S**,19*S**,26a*R**]]-.
 C₄₄H₆₉NO₁₂·H₂O 822.03

USP Tacrolimus Related Compound A RS
 (*E*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.
 C₄₃H₆₉NO₁₂ 792.01

- USP Tacrolimus 8-epimer RS

(3*S*,4*R*,5*S*,8*S*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
 C₄₄H₆₉NO₁₂ 804.02

USP Tacrolimus 8-propyl Analog RS
 (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
 C₄₄H₇₁NO₁₂ 806.03

- 1*S* (USP39)

USP Tacrolimus System Suitability Mixture RS
 This is a mixture of tacrolimus, ascomycin

(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.
 C₄₃H₆₉NO₁₂ 792.01

and tacrolimus 8-propyl analog

(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-

[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

C₄₄H₇₁NO₁₂ 806.03

BRIEFING

Tacrolimus Capsules, *USP 38* page 5428. On the basis of comments received, the monograph is revised as follows:

1. A test for *Identification* based on the UV spectral comparison is added to strengthen the monograph.
2. The requirement for storing solutions in low-actinic glassware is deleted, allowing flexibility to use other ways of protecting solutions from light.
3. In *Dissolution Test 3*, the *Sample solution* is revised to reflect the validated procedure.
4. *Organic Impurities, Procedure 1* is revised to correct the calculation formula.
5. Tacrolimus 8-epimer, a degradation product, and tacrolimus 8-propyl analog, a process impurity, elute close together and may switch positions depending on the column used. *Peak identification solution 1* and *Peak identification solution 2* are added to *Organic Impurities, Procedure 2* to facilitate the identification of these two peaks.
6. The *USP Reference Standards* section is revised to include the Reference Standards used in *Organic Impurities, Procedure 2*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM1: A. Wise.)

Correspondence Number—C153454

Comment deadline: May 31, 2015

Tacrolimus Capsules

DEFINITION

Tacrolimus Capsules contain NLT 93.0% and NMT 105.0% of the labeled amount of tacrolimus (C₄₄H₆₉NO₁₂).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

- **B.** The UV absorption spectrum of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*. ■ 1S (*USP39*)

ASSAY

Change to read:● **Procedure**

Allow the *Standard solution* and *Sample solution* to stand for 3 h at ambient temperature before use. ~~Protect from light by using low-actinic glassware.~~

■ Protect solutions containing tacrolimus from light. ■ 1S (USP39)

Solution A: 6 mM phosphoric acid

Solution B: 50 g/L of polyoxyethylene (23) lauryl ether. [Note—Polyoxyethylene (23) lauryl ether is also called Brij-35.]

Solution C: Acetonitrile and *Solution B* (7:3)

Mobile phase: Acetonitrile, *tert*-butyl methyl ether, and *Solution A* (335:55:600)

Standard solution: 50 µg/mL of USP Tacrolimus RS in *Solution C*

Sample solution: Equivalent to 50 µg/mL of tacrolimus from NLT 10 Capsules in *Solution C*.
[Note—Sonicate, and stir with a magnetic stirrer.]

Chromatographic system

(See *Chromatography* 〈621〉, *System Suitability*.)

Mode: LC

Detector: UV 205 nm.

■ When this procedure is used for *Identification test B*, use a diode array detector set at 200–400 nm. ■ 1S (USP39)

Column: 4.0-mm × 5.5-cm; 3-µm packing L1

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for tacrolimus 19-epimer and tacrolimus are 0.67 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0% for the sum of the tacrolimus and tacrolimus 19-epimer peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the *Sample solution*

r_S sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the *Standard solution*

C_S concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)

C_U nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–105.0%

PERFORMANCE TESTS

Change to read:

- **Dissolution** 〈 711 〉

Test 1

Medium: Hydroxypropylcellulose in water ($1:2 \times 10^4$), adjusted with 6% phosphoric acid to a pH of 4.5; 900 mL

Apparatus 2: 50 rpm with sinker (see *Dissolution* 〈 711 〉, *Figure 2a*)

Time: 90 min

Mobile phase: Acetonitrile, methanol, water, and 6% phosphoric acid (46: 18: 36: 0.1)

Standard stock solution: ($L/360$) mg/mL in acetonitrile, where L is the Capsule label claim in mg

Standard solution: To 20.0 mL of the *Standard stock solution* add 50.0 mL of *Medium*, and mix to obtain solutions with known concentrations as indicated in *Table 1*. Allow the solution to stand for NLT 6 h at 25° before use.

Sample solution: Pass 10 mL of the solution under test through a G4 glass filter. To 5.0 mL of the filtrate add 2.0 mL of acetonitrile, and mix. Allow the solution to stand for NLT 1 h at 25° before use.

Table 1

Capsule Strength (mg)	Final Concentration (µg/mL)
0.5	0.4
1	0.8
5	4

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: 210 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 50°

Flow rate: Adjust the flow rate so that the retention time of tacrolimus is approximately 14 min.

Injection volume: See *Table 2*.

Table 2

Capsule Strength (mg)	Injection Volume (µL)
0.5	800
1	400
5	80

[Note—For products with strengths other than those listed in *Table 2*, adjust the injection volume to deliver an equivalent amount of tacrolimus into the column.]

System suitability**Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 1.5 between tacrolimus 19-epimer and tacrolimus**Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 1.5%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times (100/L)$$

 r_U = peak response of tacrolimus from the *Sample solution* r_S = peak response of tacrolimus from the *Standard solution* C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL) D = dilution factor of the *Sample solution* V = volume of *Medium*, 900 mL L = label claim (mg/Capsule)**Tolerances:** NLT 80% (Q) of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.[Note—Allow the *Standard solution* to stand for 3 h at ambient temperature before use.~~Protect from light by using low-actinic glassware.~~

■ Protect solutions containing tacrolimus from light. ■ 1S (USP39)

]

Buffer: Dissolve 6 g of sodium dodecyl sulfate and 8.28 g of monobasic sodium phosphate in 6000 mL of water. Adjust with 2 N sodium hydroxide to a pH of 7.0.**Medium:** *Buffer*; 900 mL**Apparatus 2:** 50 rpm, with sinkers**Time:** 60 min**Standard stock solution:** 0.2 mg/mL of USP Tacrolimus RS in alcohol and *Medium* (3:7). [Note—Dissolve USP Tacrolimus RS in alcohol using 30% of the final volume. Sonicate until dissolved, and dilute with *Medium* to volume.]**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of 5 µg/mL.**Sample solution:** Pass a portion of the solution under test through a suitable filter.**Solution A:** 6 mM phosphoric acid**Mobile phase:** Acetonitrile, *tert*-butyl methyl ether, and *Solution A* (335:50:600)**Chromatographic system**(See *Chromatography* { 621 }, *System Suitability*.)**Mode:** LC**Detector:** UV 205 nm**Column:** 4.0-mm × 5.5-cm; 3-µm packing L1**Column temperature:** 60°**Flow rate:** 1.2 mL/min

Injection volume: 100 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times for tacrolimus 19-epimer and tacrolimus are 0.67 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 5.0% for the sum of the areas of tacrolimus and tacrolimus 19-epimer

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the *Sample solution*

r_S = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: 50 mg/L of hydroxypropyl cellulose in water. Adjust with phosphoric acid to a pH of 4.5; 900 mL.

Apparatus 2 (without sinker) and Time: and Sample solution:

■ **1S (USP39)**

Proceed as directed in *Test 1*.

Buffer: 3.6 g/L of monobasic potassium phosphate in water. Adjust with diluted phosphoric acid to a pH of 2.5.

Mobile phase: *Buffer* and acetonitrile (1:1)

Standard stock solution: 0.1 mg/mL of USP Tacrolimus RS in acetonitrile

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of $(L/900)$ mg/mL, where L is the Capsule label claim in mg.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 10-cm; 5- μ m packing L1

Column temperature: 60 $^{\circ}$

Flow rate: 1.3 mL/min

Injection volume: 100 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times for tacrolimus 19-epimer, tacrolimus open ring, and tacrolimus are 0.67, 0.79, and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = sum of the peak responses of tacrolimus, tacrolimus 19-epimer, and tacrolimus open ring from the *Sample solution*

r_S = sum of the peak responses of tacrolimus, tacrolimus 19-epimer, and tacrolimus open ring from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) is dissolved.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: Hydroxypropylcellulose in water (1 in 20,000), adjusted with phosphoric acid to a pH of 4.5. See *Table 3* for the volume.

Table 3

Capsule Strength (mg)	Volume of Medium (mL)
0.5	500
1	900
5	900

Apparatus 2: 50 rpm, with sinkers

Time: 120 min

Diluent: 1 mg/mL of hydroxypropylcellulose in water. Sonicate as needed to dissolve.

Buffer: To a solution of 1 g/L of sodium 1-hexanesulfonate in water add 0.1 mL/L of trifluoroacetic acid.

Mobile phase: Acetonitrile, methanol, and *Buffer* (550:50:400)

Standard stock solution: Dissolve USP Tacrolimus RS in acetonitrile. See *Table 4* for the concentrations (L is the Capsule label claim in mg).

Table 4

Capsule Strength (mg)	Concentration (mg/mL)
0.5	$L/25$
1	$L/45$
5	$L/45$

Standard solution: Dilute the *Standard stock solution* with *Diluent*. See *Table 5* for the concentrations (L is the Capsule label claim in mg).

Table 5

Capsule Strength (mg)	Concentration (mg/mL)
0.5	L/500
1	L/900
5	L/900

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 100 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tacrolimus (C₄₄H₆₉NO₁₂) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of *Medium* (mL) (see *Table 3*)

Tolerances: NLT 75% (Q) of the labeled amount of tacrolimus (C₄₄H₆₉NO₁₂) is dissolved.

- **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

IMPURITIES

Change to read:

- **Organic Impurities, Procedure 1**

Use *Organic Impurities, Procedure 1* when the impurity profile includes tacrolimus diene and tacrolimus regioisomer. It is suggested that new columns be conditioned with about 500 mL of ethanol before use to meet the resolution criterion.

Mobile phase: Hexane, *n*-butyl chloride, and acetonitrile (7:2:1). Add *n*-butyl chloride to hexane, and mix well before adding acetonitrile. After adding acetonitrile, mix the *Mobile phase* for 2 h to get a clear solution. Any deviations from the ratio of components in the *Mobile phase* and the order of mixing will result in a two-phase solution.

System suitability solution: 0.1 mg/mL each of USP Tacrolimus RS and USP Tacrolimus Related Compound A RS in *Mobile phase*

Sample solution: Transfer the contents of a suitable number of Capsules (equivalent to about 5 mg of tacrolimus for 0.5-mg Capsules or 10 mg of tacrolimus for 1-mg and 5-mg Capsules) into a centrifuge tube. Add 1.5 mL of a mixture of *n*-butyl chloride and acetonitrile (2:1), sonicate in an ultrasonic bath for 2 min, add 3.5 mL of *n*-hexane, and mix. Centrifuge this solution, and collect the supernatant or pass the solution through a 0.5- μ m membrane filter. Use the solution within 30 min of preparation.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Columns: Two 4.6-mm \times 25-cm columns; 5- μ m packing L20

Column temperature: 28 \pm 2 $^{\circ}$

Flow rate: 1.5 mL/min.

Adjust the flow rate so that the retention time of tacrolimus is approximately 15 min.

Injection volume: 20 μ L

Run time: 3 times the retention time of tacrolimus

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.1 between tacrolimus and tacrolimus related compound A

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/F_i) \times [1/\Sigma(r_U/F_i)] \times 100$$

■

$$\text{Result} = (r_U/F_i) \times \{1/[r_T + \Sigma(r_U/F_i)]\} \times 100 \quad \blacksquare_{1S} \text{ (USP39)}$$

r_U = peak response of each impurity from the *Sample solution*

F_i = relative response factor for each corresponding impurity (see *Table 6*)

r_T = peak response of tacrolimus from the *Sample solution*

Acceptance criteria: See *Table 6*. Disregard peaks due to the solvent.

Table 6

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tacrolimus diene ^a	0.79	2.2	0.3
Tacrolimus regioisomer ^b	0.88	1.0	0.5
Tacrolimus impurity 1 ^c	0.96	1.0	0.3

Tacrolimus related compound A ^d	0.96	—	—
Tacrolimus	1.0	—	—
Tacrolimus 19-epimer		—	—
* ^e ,*(IRA 1-Jul-2014) f	1.1		
Tacrolimus open ring		—	—
* ^e ,*(IRA 1-Jul-2014) g	1.3		
Any individual unspecified impurity	—	1.0	0.2
Total impurities ***(IRA 1-Jul-2014)	—	—	1.0
<p>a (14<i>E</i>,18<i>E</i>)-17-Allyl-1-hydroxy-12-[(<i>E</i>)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}] octacos-14,18-diene-2,3,10,16-tetrone.</p> <p>b (4<i>E</i>,11<i>E</i>)-10-Allyl-7,8,10,13,14,15,16,17,18,19,20,21,26,22,28,28a-hexadecahydro-7,21-dihydroxy-3-(4-hydroxy-3-methoxycyclohexyl)-16,18-dimethoxy-4,6,12,14,20-pentamethyl-17,21-epoxy-3<i>H</i>-pyrido[2,1-<i>c</i>][1,4]oxaazacyclopentacosine-1,9,22,23(6<i>H</i>,25<i>H</i>)-tetrone.</p> <p>c Tacrolimus impurity 1 is a specified, unidentified impurity.</p> <p>d</p> <p>* Tacrolimus related compound A is listed here to indicate the relative retention time of this compound. It is used in the procedure to evaluate system suitability and is not to be reported. It is not to be included in total impurities. *(IRA 1-Jul-2014)</p> <p>*</p> <p>e Tacrolimus open ring and tacrolimus 19-epimer are isomers of tacrolimus, which are present in equilibrium with the active ingredient. They are not to be reported as degradation products and are not included in total impurities.</p> <p>*(IRA 1-Jul-2014)</p> <p>f (3<i>S</i>,4<i>R</i>,5<i>S</i>,8<i>R</i>,9<i>E</i>,12<i>S</i>,14<i>S</i>,15<i>R</i>,16<i>S</i>,18<i>R</i>,19<i>S</i>,26<i>aS</i>)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26<i>a</i>-hexadecahydro-5,19-dihydroxy-3-{(<i>E</i>)-2-[(1<i>R</i>,3<i>R</i>,4<i>R</i>)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3<i>H</i>-pyrido[2,1-<i>c</i>][1,4]oxaazacyclotricosine-1,7,20,21(4<i>H</i>,23<i>H</i>)-tetrone.</p> <p>g (3<i>S</i>,4<i>R</i>,5<i>S</i>,8<i>R</i>,12<i>S</i>,14<i>S</i>,15<i>R</i>,16<i>S</i>,18<i>R</i>,26<i>aS</i>,<i>E</i>)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26<i>a</i>-tetradecahydro-5,15,20,20-tetrahydroxy-3-{(<i>E</i>)-2-[(1<i>R</i>,3<i>R</i>,4<i>R</i>)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-3<i>H</i>-pyrido[2,1-<i>c</i>][1,4]oxaazacyclotricosine-1,7,19,21(4<i>H</i>,8<i>H</i>,20<i>H</i>,23<i>H</i>)-tetrone.</p> <p>***(IRA 1-Jul-2014)</p>			

Change to read:

- **Organic Impurities, Procedure 2**

Use *Organic Impurities, Procedure 2* when the impurity profile includes

- *tacrolimus hydroxy acid*(IRA 1-Jul-2014)

and tacrolimus 8-epimer. It is suggested to equilibrate the column overnight with a mixture of *Solution C* and *Solution D* (17:3) before performing this procedure. Allow the *System suitability solution*, *Standard solution*, and *Sample solution* to stand for 3 h at ambient temperature before use. ~~Protect from light by using low-actinic glassware.~~

■ Protect solutions containing tacrolimus from light. ■ 1S (USP39)

Solution A: 6 mM phosphoric acid

Solution B: Acetonitrile and *tert*-butyl methyl ether (81:19). [Note—The ratio of acetonitrile to *tert*-butyl methyl ether is critical.]

Solution C: *Solution A* and *Solution B* (4:1)

Solution D: *Solution A* and *Solution B* (1:4)

Mobile phase: See *Table 7*.

Table 7

Time (min)	Solution C (%)	Solution D (%)
0	74	26
45	74	26
60	15	85
75	15	85
76	74	26
85	74	26

Solution E: 50 g/L of polyoxyethylene (23) lauryl ether in *Solution A*. [Note—Polyoxyethylene (23) lauryl ether is also called Brij-35.]

Diluent: Acetonitrile and *Solution E* (7:3)

System suitability solution: 1.5 mg/mL of USP Tacrolimus System Suitability Mixture RS in *Diluent*

Standard solution: 7.5 µg/mL of USP Tacrolimus RS in *Diluent*

Sensitivity solution: 1.5 µg/mL of USP Tacrolimus RS in *Diluent* from *Standard solution*

■ **Peak identification solution 1:** 10 µg/mL of USP Tacrolimus 8-epimer RS in *Diluent*

■ **Peak identification solution 2:** 10 µg/mL of USP Tacrolimus 8-propyl Analog RS in *Diluent*

■ 1S (USP39)

Sample solution: Equivalent to 1.5 mg/mL of tacrolimus in *Diluent*. [Note—Shake the mixture on a mechanical shaker for 30 min, and pass through a suitable filter.]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Column temperature: 60°

Flow rate: 1.5 mL/min

Injection volume: 40 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 3.0 between tacrolimus and ascomycin, *System suitability solution*

Relative standard deviation: NMT 10.0% for the sum of the responses of tacrolimus and tacrolimus 19-epimer, *Standard solution*

Signal-to-noise ratio: NLT 10.0, *Sensitivity solution*

Analysis

Samples: *Standard solution*,

■ *Peak identification solution 1*, *Peak identification solution 2*, ■ 1S (USP39)

and *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

★ $\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$

★(IRA 1-Jul-2014)

r_U = peak response of each impurity from the *Sample solution*

r_S = sum of the peak responses of tacrolimus 19-epimer and tacrolimus from the *Standard solution*

C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of tacrolimus in the *Sample solution* (mg/mL)

P = potency of tacrolimus in USP Tacrolimus RS (mg/mg)

F = relative response factor (see *Table 8*) ★(IRA 1-Jul-2014)

Acceptance criteria: See *Table 8*.

■ Identify tacrolimus 8-epimer and tacrolimus 8-propyl analog using *Peak identification solution 1* and *Peak identification solution 2*. ■ 1S (USP39)

Disregard peaks that are smaller than the tacrolimus peak in the *Sensitivity solution*.

Table 8

Name	Relative Retention Time	★Relative Response Factor ★(IRA 1-Jul-2014)	Acceptance Criteria, NMT (%)
★Tacrolimus hydroxy acid ^a ★(IRA 1-Jul-2014)	0.18	★1.5★(IRA 1-Jul-2014)	0.5
Tacrolimus open ring ^{b,c}	0.49	—	—
Ascomycin 19-epimer ^d , ★ ^e ★(IRA 1-Jul-2014)	0.52	—	—
Tacrolimus 19-epimer ^{b,f}	0.62	—	—
Ascomycin ^{e,g}	0.84	—	—
Desmethyl tacrolimus ^{e,h}	0.91	—	—
Tacrolimus	1.0	—	—
Tacrolimus 8-epimer ⁱ	1.28	★1.0★(IRA 1-Jul-2014)	0.5
Tacrolimus 8-propyl analog ^{e,j}	1.30	—	—
Any individual unspecified impurity	—	★1.0★(IRA 1-Jul-2014)	0.2

Total impurities	—	—	1.5
a			
<p>•(3<i>S</i>,4<i>R</i>,5<i>S</i>,8<i>R</i>,12<i>S</i>,14<i>S</i>,15<i>R</i>,16<i>S</i>,18<i>R</i>,25<i>aS</i>,<i>E</i>)-8-Allyl-5,15,19-trihydroxy-3-{(<i>E</i>)-1-[(1<i>R</i>,3<i>R</i>,4<i>R</i>)-4-hydroxy-3-methoxycyclohexyl]prop-1-en-2-yl}-14,16-dimethoxy-4,10,12,18-tetramethyl-1,7,20-trioxo-1,3,4,5,6,7,8,11,12,13,14,15,16,17,18,19,20,22,23,24,25,25<i>a</i>-docosahydropyrido[2,1-<i>c</i>][1]oxa[4]azacyclodocosine-19-carboxylic acid. •(IRA 1-Jul-2014)</p>			
b Tacrolimus open ring and tacrolimus 19-epimer are isomers of tacrolimus, which are present in equilibrium with the active ingredient. They are not to be reported as degradation products •and are not included in total impurities. •(IRA 1-Jul-2014)			
c (3 <i>S</i> ,4 <i>R</i> ,5 <i>S</i> ,8 <i>R</i> ,12 <i>S</i> ,14 <i>S</i> ,15 <i>R</i> ,16 <i>S</i> ,18 <i>R</i> ,26 <i>aS</i> , <i>E</i>)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26 <i>a</i> -tetradecahydro-5,15,20,20-tetrahydroxy-3-{(<i>E</i>)-2-[(1 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-3 <i>H</i> -pyrido[2,1- <i>c</i>][1,4]oxaazacyclotricosine-1,7,19,21(4 <i>H</i> ,8 <i>H</i> ,20 <i>H</i> ,23 <i>H</i>)-tetrone.			
d (3 <i>S</i> ,4 <i>R</i> ,5 <i>S</i> ,8 <i>R</i> ,9 <i>E</i> ,12 <i>S</i> ,14 <i>S</i> ,15 <i>R</i> ,16 <i>S</i> ,18 <i>R</i> ,19 <i>S</i> ,26 <i>aS</i>)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26 <i>a</i> -hexadecahydro-5,19-dihydroxy-3-[(<i>E</i>)-2-[(1 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3 <i>H</i> -pyrido[2,1- <i>c</i>][1,4]oxaazacyclotricosine-1,7,20,21-(4 <i>H</i> ,23 <i>H</i>)-tetrone.			
e These are process impurities that are controlled in the drug substance. They are not to be reported in the drug product.			
f (3 <i>S</i> ,4 <i>R</i> ,5 <i>S</i> ,8 <i>R</i> ,9 <i>E</i> ,12 <i>S</i> ,14 <i>S</i> ,15 <i>R</i> ,16 <i>S</i> ,18 <i>R</i> ,19 <i>S</i> ,26 <i>aS</i>)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26 <i>a</i> -hexadecahydro-5,19-dihydroxy-3-{(<i>E</i>)-2-[(1 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3 <i>H</i> -pyrido[2,1- <i>c</i>][1,4]oxaazacyclotricosine-1,7,20,21(4 <i>H</i> ,23 <i>H</i>)-tetrone.			
g (3 <i>S</i> ,4 <i>R</i> ,5 <i>S</i> ,8 <i>R</i> ,9 <i>E</i> ,12 <i>S</i> ,14 <i>S</i> ,15 <i>R</i> ,16 <i>S</i> ,18 <i>R</i> ,19 <i>R</i> ,26 <i>aS</i>)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26 <i>a</i> -hexadecahydro-5,19-dihydroxy-3-[(<i>E</i>)-2-[(1 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3 <i>H</i> -pyrido[2,1- <i>c</i>][1,4]oxaazacyclotricosine-1,7,20,21-(4 <i>H</i> ,23 <i>H</i>)-tetrone.			
h (3 <i>S</i> ,4 <i>R</i> ,5 <i>S</i> ,8 <i>R</i> ,9 <i>E</i> ,12 <i>S</i> ,14 <i>S</i> ,15 <i>R</i> ,16 <i>S</i> ,18 <i>R</i> ,19 <i>R</i> ,26 <i>aS</i>)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26 <i>a</i> -hexadecahydro-5,19-dihydroxy-3-[(<i>E</i>)-2-[(1 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,12,18-trimethyl-15,19-epoxy-3 <i>H</i> -pyrido[2,1- <i>c</i>][1,4]oxaazacyclotricosine-1,7,20,21-(4 <i>H</i> ,23 <i>H</i>)-tetrone.			
i (3 <i>S</i> ,4 <i>R</i> ,5 <i>S</i> ,8 <i>S</i> ,9 <i>E</i> ,12 <i>S</i> ,14 <i>S</i> ,15 <i>R</i> ,16 <i>S</i> ,18 <i>R</i> ,19 <i>R</i> ,26 <i>aS</i>)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26 <i>a</i> -hexadecahydro-5,19-dihydroxy-3-{(<i>E</i>)-2-[(1 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3 <i>H</i> -pyrido[2,1- <i>c</i>][1,4]oxaazacyclotricosine-1,7,20,21(4 <i>H</i> ,23 <i>H</i>)-tetrone.			
j (3 <i>S</i> ,4 <i>R</i> ,5 <i>S</i> ,8 <i>R</i> ,9 <i>E</i> ,12 <i>S</i> ,14 <i>S</i> ,15 <i>R</i> ,16 <i>S</i> ,18 <i>R</i> ,19 <i>R</i> ,26 <i>aS</i>)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26 <i>a</i> -Hexadecahydro-5,19-dihydroxy-3-{(<i>E</i>)-2-[(1 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3 <i>H</i> -pyrido[2,1- <i>c</i>][1,4]oxaazacyclotricosine-1,7,20,21(4 <i>H</i> ,23 <i>H</i>)-tetrone.			

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.
- **Labeling:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which test for *Organic Impurities* the article complies. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:● **USP Reference Standards** { 11 }USP Tacrolimus RS USP Tacrolimus Related Compound A RS

(*E*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.
 $C_{43}H_{69}NO_{12}$ 792.01

■ USP Tacrolimus 8-epimer RS

(3*S*,4*R*,5*S*,8*S*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
 $C_{44}H_{69}NO_{12}$ 804.02

USP Tacrolimus 8-propyl Analog RS

(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
 $C_{44}H_{71}NO_{12}$ 806.03

■ 1*S* (USP39)

USP Tacrolimus System Suitability Mixture RS

It contains tacrolimus, ascomycin

(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.

 $C_{43}H_{69}NO_{12}$ 792.01

and tacrolimus 8-propyl analog

(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.

 $C_{44}H_{71}NO_{12}$ 806.03

BRIEFING

Teniposide. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods of analysis, is proposed.

1. The liquid chromatographic procedure in the *Assay* and the test for *Organic Impurities* is based on analyses performed with the Synergi Polar-RP brand of L11 column manufactured by Phenomenex. The typical retention time for teniposide is about 30 min.

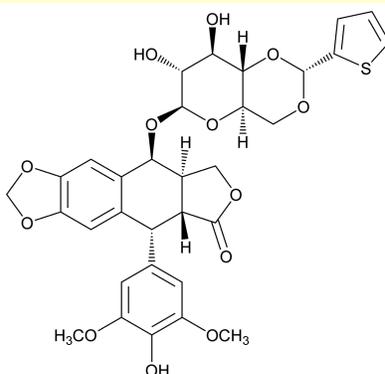
(SM3: F. Mao.)

Correspondence Number—C93995

Comment deadline: May 31, 2015

Add the following:

■ Teniposide



$C_{32}H_{32}O_{13}S$ 656.65

Furo[3',4':6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5*aH*)-one, 5,8,8*a*,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)-9-[4,6-*O*-(2-thienylmethylene)- β -*D*-glucopyranosyloxy]-, [5*R*-[5 α ,5 β ,8 α ,9 β (*R**)]]-;

4'-Demethylepipodophyllotoxin 9-[4,6-*O*-(*R*)-2-thenylidene- β -*D*-glucopyranoside]; (5*S*,5*aR*,8*aR*,9*R*)-9-(4-Hydroxy-3,5-dimethoxyphenyl)-8-oxo-5,5*a*,6,8,8*a*,9-

hexahydrofuro[3',4':6,7]naphtho[2,3-*d*][1,3]dioxol-5-yl 4,6-*O*-(thiophen-2-ylmethylidene)- β -*D*-glucopyranoside [29767-20-2].

DEFINITION

Teniposide contains NLT 97.0% and NMT 103.0% of teniposide ($C_{32}H_{32}O_{13}S$), calculated on the anhydrous and solvent-free basis.

[**Caution**—Great care should be taken in handling Teniposide, because it is a potentially cytotoxic agent.]

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY● **Procedure**

Buffer: 5 mM monobasic potassium phosphate in water

Solution A: Acetonitrile and *Buffer* (36:64). Adjust with dibasic potassium phosphate to an apparent pH of 7.0.

Solution B: Acetonitrile and water (90:10)

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for 15 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
25	100	0
60	0	100
66 ^a	0	100

^a This time may be adjusted if needed to be at least 2.2 times of the retention time of the teniposide peak.

Diluent: Acetonitrile and *Buffer* (1:1)

Standard solution: 0.4 mg/mL of USP Teniposide RS, prepared as follows. Transfer USP Teniposide RS to a suitable volumetric flask, add acetonitrile equivalent to 10% of the final volume, and sonicate to dissolve. Dilute with *Diluent* to volume.

Sample solution: 0.4 mg/mL of Teniposide, prepared as follows. Transfer Teniposide to a suitable volumetric flask, add acetonitrile equivalent to 10% of the final volume, and sonicate to dissolve. Dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 4-μm packing L11

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 0.85%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of teniposide (C₃₂H₃₂O₁₃S) in the portion of Teniposide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Teniposide RS in the *Standard solution* (mg/mL)

C_U concentration of Teniposide in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the anhydrous and solvent-free basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉

Analysis: Ignite the sample at 700°–800°.

Acceptance criteria: NMT 0.2%

- **Organic Impurities**

Solution A, Solution B, Mobile phase, Diluent, and Sample solution: Proceed as directed in the *Assay*.

System suitability solution: Use the *Standard solution* in the *Assay*. [Note—USP Teniposide RS contains a small amount of *R*-3-thienylidene regioisomer.]

Chromatographic system: Proceed as directed in the *Assay*, except for *Detectors*.

Detectors

UV 242 nm: For thiophenealdehyde

UV 220 nm: For all other impurities

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.0 between *R*-3-thienylidene regioisomer and teniposide peaks

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Teniposide taken:

$$\text{Result} = r_U / \{ \sum [r_U \times (1/F)] + r_T \} \times (1/F) \times 100$$

r_U peak area of each impurity from the *Sample solution* at 242 nm for thiophenealdehyde and 220 nm for all other impurities

F = relative response factor for each individual impurity (see *Table 2*)

r_T peak area of Teniposide from the *Sample solution* at 220 nm

Acceptance criteria

Individual impurities: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lignan P ^a	0.12	1.0	0.1
Teniposide related compound A ^b	0.29	1.4	0.1
Thiophenealdehyde ^c	0.30	1.0	0.1
S-3-Thienylidene regioisomer ^d	0.91	1.0	0.1
R-3-Thienylidene regioisomer ^e	0.92	1.0	0.5
Teniposide	1.0	—	—

^a 4'-Demethylepipodophyllotoxin 9-β-d-glucopyranoside.

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Picroteniposide ^f	1.09	1.0	0.1
Demethylepipodophyllotoxin dimer ^g	1.39	1.3	0.1
Tetrabenzyl benzoyl lignan P ^h	2.11	0.81	0.1
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	3

a 4'-Demethylepipodophyllotoxin 9- β -d-glucopyranoside.

b 4'-Demethylepipodophyllotoxin.

c Thiophene-2-carbaldehyde. It is quantitated at 242 nm.

d 4'-Demethylepipodophyllotoxin 9-[4,6-*O*-(*S*)-3-thenylidene- β -d-glucopyranoside].

e 4'-Demethylepipodophyllotoxin 9-[4,6-*O*-(*R*)-3-thenylidene- β -d-glucopyranoside].

f (5*R*,5*aS*,8*aR*,9*S*)-9-[4,6-*O*-(*R*)-2-Thenylidene- β -d-glucopyranosyloxy]-5,8,8*a*,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)furo[3',4':6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5*aH*)-one.

g (5*R*,5*aR*,8*aR*,9*S*,5'*R*,5*a'R*,8*a'R*,9'*S*)-9,9'-Oxybis(5,8,8*a*,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)furo[3',4':6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5*aH*)-one).

h (5*R*,5*aR*,8*aR*,9*S*)-9-[(*O*²,*O*³,*O*⁴,*O*⁶-Tetrabenzyl)- β -d-glucopyranosyloxy]-5,8,8*a*,9-tetrahydro-5-(4-benzoyloxy-3,5-dimethoxyphenyl)furo[3',4':6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5*aH*)-one.

SPECIFIC TESTS

- **Water Determination, Method I** (921): NMT 1%
- **Optical Rotation, Specific Rotation** (781S)
Sample solution: 5 mg/mL in chloroform and methanol (9:1)
Acceptance criteria: -101.0° to -113.0° , at 20° on the anhydrous and solvent-free basis

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at room temperature.
- **USP Reference Standards** (11)
 USP Teniposide RS
 ■ 1S (USP39)

BRIEFING

Teniposide Injection. Because there is no existing *USP* monograph for this drug product, a

new monograph, based on validated methods of analysis, is proposed.

1. The liquid chromatographic procedure for the *Assay* and the test for *Organic Impurities* is based on analyses performed with the Luna Phenyl-Hexyl brand of L11 column manufactured by Phenomenex. The typical retention time for teniposide is about 35 min.

(SM3: F. Mao, R. Tirumalai.)
Correspondence Number—C94377

Comment deadline: May 31, 2015

Add the following:

■ Teniposide Injection

DEFINITION

Teniposide Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of teniposide ($C_{32}H_{32}O_{13}S$). It is a sterile solution in a nonaqueous medium and may contain benzyl alcohol or other suitable preservatives.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Solution A: Acetonitrile and water (25:75)

Solution B: Acetonitrile and water (56:44)

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for 10 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
16	64.5	35.5
36	64.5	35.5
60	0	100
90	0	100

Diluent: Acetonitrile and 5 μ M monobasic potassium phosphate (1:1)

Standard solution: 0.4 mg/mL of USP Teniposide RS in *Diluent*, prepared as follows.

Transfer a known amount of USP Teniposide RS into a suitable volumetric flask and add acetonitrile equivalent to 10% of the final volume. Dissolve with the aid of sonication and dilute with *Diluent* to volume.

Sample solution: Nominally equivalent to 0.4 mg/mL of teniposide in *Diluent*, prepared as follows. Dilute a portion of the Injection with *Diluent* to obtain a solution containing 0.4 mg/mL of teniposide.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-μm packing L11

Flow rate: 1.3 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of teniposide ($C_{32}H_{32}O_{13}S$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Teniposide RS in the *Standard solution* (mg/mL)

C_U nominal concentration of teniposide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- Organic Impurities**

Solution A, Solution B, Diluent, and Sample solution: Proceed as directed in the *Assay*.

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for 10 min. [Note—The duration of initial isocratic hold may be adjusted from 4 min to up to 10 min to meet the resolution requirements and to achieve the retention time of the teniposide peak to about 35 min.]

Benzaldehyde stock solution: 0.15 mg/mL of USP Benzaldehyde RS in *Diluent*

Teniposide related compound A stock solution: 0.15 mg/mL of USP Teniposide Related Compound A RS, prepared as follows. Transfer USP Teniposide Related Compound A RS to a suitable volumetric flask, add acetonitrile equivalent to 20% of the final volume, and sonicate to dissolve. Dilute with *Diluent* to volume.

System suitability solution 1: 15 μg/mL each of USP Teniposide Related Compound A RS and USP Benzaldehyde RS in *Diluent* from *Teniposide related compound A stock solution* and *Benzaldehyde stock solution*

System suitability solution 2: Use the *Standard solution* in the *Assay*. [Note—USP Teniposide RS contains a small amount of *R*-3-thienylidene regioisomer.]

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Proceed as directed in the *Assay*, except for the *Detectors*.

Detectors

UV 242 nm: For thiophenealdehyde

UV 220 nm: For all other impurities

System suitability

Samples: *System suitability solution 1* and *System suitability solution 2*

[Note—The relative retention times for benzaldehyde, teniposide related compound A, *R*-3-thienylidene regioisomer, and teniposide are about 0.42, 0.43, 0.97, and 1.0, respectively.]

Suitability requirements

Resolution 1: NLT 0.9 between benzaldehyde and teniposide related compound A, *System suitability solution 1*

Resolution 2: NLT 1.2 between *R*-3-thienylidene regioisomer and teniposide peaks, *System suitability solution 2*

Analysis

Sample: *Sample solution*

Calculate the percentage of each specified degradation product in the portion of Injection taken:

$$\text{Result} = r_U / \{ \Sigma [r_U \times (1/F)] + r_T \} \times (1/F) \times 100$$

r_U peak area of each specified degradation product from the *Sample solution* at 242 nm for thiophenealdehyde and 220 nm for all other impurities

F = relative response factor for each individual impurity (see *Table 2*)

r_T peak area of teniposide from the *Sample solution* at 220 nm

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lignan P ^a	0.16	1.0	2.5
Thiophenealdehyde ^b	0.28	1.2	0.5
Teniposide	1.0	—	—
Picroteniposide ^c	1.11	1.0	0.25
Total specified degradation products	—	—	3.0

^a 4'-Demethylepipodophyllotoxin 9- β -d-glucopyranoside.

^b Thiophene-2-carbaldehyde. It is quantitated at 242 nm.

^c (5*R*,5*aS*,8*aR*,9*S*)-9-[4,6-*O*-(*R*)-2-Thienylidene- β -d-glucopyranosyloxy]-5,8,8*a*,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)furo[3',4':6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5*aH*)-one.

SPECIFIC TESTS

- **Bacterial Endotoxins Test** (85): It contains NMT 1.5 USP Endotoxin Units/mg of teniposide.
- **Sterility Tests** (71): Meets the requirements

- **pH** 〈 791 〉
Sample solution: Dilute 5 mL of Injection with 45 mL of water.
Acceptance criteria: 4.0–6.5
- **Particulate Matter in Injections** 〈 788 〉: It meets the requirements for small-volume injections.
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store under refrigeration and protect from light.
- **Labeling:** Label it to indicate that the Injection is to be diluted with a suitable parenteral vehicle prior to intravenous infusion.
- **USP Reference Standards** 〈 11 〉
 USP Benzaldehyde RS
 USP Endotoxin RS
 USP Teniposide RS
 USP Teniposide Related Compound A RS
 4'-Demethylepipodophyllotoxin.
 $C_{21}H_{20}O_8$ 400.38

■ 1S (USP39)

BRIEFING

Timolol Maleate Tablets, *USP 38* page 5585. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Replace the current HPLC procedure in the *Assay* with the stability-indicating HPLC method as used in the proposed revision for the *Timolol Maleate* monograph. The proposed liquid chromatographic procedure is based on analysis performed with the Kinetix XB-C18 brand of L1 column manufactured by Phenomenex in which timolol elutes at about 2.8 min.
2. Replace the TLC method in *Identification* test A with the HPLC retention time agreement from the proposed *Assay*.
3. Add *Identification* test B in which the UV (or UV-Vis) spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the proposed *Assay*.
4. Add an HPLC procedure for *Organic Impurities* consistent with the proposed HPLC procedure in the *Assay*.
5. Add the following Reference Standards, introduced by the proposed HPLC procedure in the *Assay* and *Organic Impurities*, to the *USP Reference Standards* section: USP Timolol Related Compound B RS and USP Timolol Related Compound D RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: D.A. Porter.)

Correspondence Number—C131114

Comment deadline: May 31, 2015

Timolol Maleate Tablets

DEFINITION

Timolol Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$).

IDENTIFICATION**Delete the following:**

- ● **A. Thin-Layer Chromatography**

Standard solution: ~~6 mg/mL of USP Timolol Maleate RS prepared as follows. Transfer 30 mg of USP Timolol Maleate RS into a 50 mL volumetric flask. Add 2 mL of 0.1 N hydrochloric acid, and shake gently. Add 30 mL of methanol, agitate for 20 min, add methanol to volume, mix, and centrifuge.~~

Sample solution: ~~Nominally 6 mg/mL of timolol maleate prepared as follows. Transfer 30 mg of timolol maleate, from a portion of powdered Tablets, into a 50 mL volumetric flask. Add 2 mL of 0.1 N hydrochloric acid, and shake gently. Add 30 mL of methanol, agitate for 20 min, add methanol to volume, mix, and centrifuge.~~

Chromatographic system

Adsorbent: ~~0.25 mm layer of chromatographic silica gel mixture~~

Application volume: ~~10 μ L~~

Developing solvent system: ~~Chloroform, methanol, and ammonium hydroxide (80:20:1)~~

Analysis

Samples: ~~Standard solution and Sample solution~~

~~Separately apply the Sample solution and the Standard solution to a thin-layer chromatographic plate (see Chromatography <621>). Develop the chromatogram using the Developing solvent system, until the solvent front has moved three-fourths of the length of the plate. Air-dry, and examine under short-wavelength UV light.~~

Acceptance criteria: ~~The R_f values of the principal spots of the Sample solution correspond to those of the Standard solution. ■ 1S (USP39)~~

Add the following:

- ● **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

Add the following:

- ● **B.** The UV (or UV-Vis) spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

ASSAY**Change to read:**

- **Procedure**

Solution A: ~~11.04 mg/mL of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 2.8 ± 0.05 .~~

Mobile phase: ~~Methanol and Solution A (2:3), degassed and filtered~~

Standard solution: ~~0.1 mg/mL of USP Timolol Maleate RS prepared as follows. Transfer 50 mg of USP Timolol Maleate RS into a 500-mL volumetric flask. Add 50 mL of *Solution A*. Sonicate until the standard is dissolved, add 100 mL of acetonitrile, shake, and dilute with water to volume.~~

Sample solution: ~~Nominally 0.1 mg/mL of timolol maleate prepared as follows. Transfer 10 mg of timolol maleate, from finely powdered Tablets (NLT 20), to a 100-mL volumetric flask. Add 10 mL of *Solution A*, sonicate for 5 min, and add 20 mL of acetonitrile. Sonicate for 5 min, add 20 mL of water, shake for 10 min, and dilute with water to volume.~~

Chromatographic system

~~(See *Chromatography* (621), *System Suitability*.)~~

Mode: ~~LC~~

Detector: ~~UV 295 nm~~

Column: ~~3.9 mm × 30 cm; packing L1~~

Flow rate: ~~1.8 mL/min~~

Injection size: ~~15 µL~~

System suitability

Sample: ~~Standard solution~~

Suitability requirements

Tailing factor: ~~NMT 2.0, main peak~~

Relative standard deviation: ~~NMT 2.0~~

■ **Solution A:** Transfer 0.5 mL of trifluoroacetic acid to a 1-L volumetric flask and dilute with water to volume.

Solution B: Transfer 0.5 mL of trifluoroacetic acid to a 1-L volumetric flask and dilute with acetonitrile to volume.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	84	16
2.4	84	16
8	20	80
8.1	84	16
11	84	16

Buffer: Transfer 11.04 g of monobasic sodium phosphate to a 1-L volumetric flask and dilute with water to volume. Adjust with phosphoric acid to a pH of 2.8 ± 0.05.

Diluent: Methanol and *Buffer* (20:80)

System suitability solution: 0.1 mg/mL of USP Timolol Maleate RS and 10.0 µg/mL of USP Timolol Related Compound D RS in *Diluent* with sonication if necessary

Standard solution: 0.1 mg/mL of USP Timolol Maleate RS in *Diluent* with sonication if necessary

Sample solution: 0.1 mg/mL of timolol maleate from NLT 20 finely ground Tablets (ground with a mortar and pestle) in *Diluent* with sonication if necessary, and filtration with 0.2-µm syringe filters, discarding the first 2 mL

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 295 nm. For *Identification* test B, use a diode array detector in the range of 210–400 nm.

Column: 2.1-mm × 10-cm; 2.6-μm packing L1

Flow rate: 0.4 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times are listed in *Table 2*.]

Suitability requirements

Resolution: NLT 2.5 between timolol and timolol related compound D, *System suitability solution*

Tailing factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

■ 1S (USP39)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Timolol Maleate RS in the *Standard solution* (mg/mL)

C_U nominal concentration of timolol maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

● **Dissolution** 〈 711 〉

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 1: 100 rpm

Time: 20 min

Standard solution: USP Timolol Maleate RS in *Medium*

Sample solution: Sample per the chapter. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Analysis: Determine the amount of timolol maleate in solution in filtered portions of the *Sample solution*, in comparison with the *Standard solution*, using the procedure in the *Assay*.

Tolerances: NLT 80% (Q) of the labeled amount of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$) is dissolved.

● **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

IMPURITIES**Add the following:****Organic Impurities**

Mobile phase, Buffer, Diluent, and Chromatographic system: Proceed as directed in the Assay.

System suitability solution: 0.1 mg/mL of USP Timolol Maleate RS and 10 µg/mL of USP Timolol Related Compound D RS in *Diluent* with sonication if necessary

Standard solution: 2.0 µg/mL each of USP Timolol Maleate RS, USP Timolol Related Compound B RS, and USP Timolol Related Compound D RS in *Diluent*

Sample solution: 1.0 mg/mL of timolol maleate from NLT 20 finely ground Tablets (ground with a mortar and pestle) in *Diluent* with sonication if necessary, and filtration with 0.2-µm syringe filters, discarding the first 2 mL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times are listed in *Table 2*.]

Suitability requirements

Resolution: NLT 3 between timolol and timolol related compound D, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of timolol maleate related compound B and timolol maleate related compound D the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_S peak response of each impurity from the *Standard solution*

C_S concentration of each impurity in the *Standard solution* (mg/mL)

C_U nominal concentration of timolol maleate in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any impurity from the *Sample solution*

r_S peak response of timolol from the *Standard solution*

C_S concentration of USP Timolol Maleate RS in the *Standard solution*

C_U nominal concentration of timolol maleate in the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Timolol related compound B	0.5	0.4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Timolol related compound D	0.8	0.4
Timolol	1.0	—
Any individual impurity	—	0.2
Total impurities	—	1.0

■ 1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** < 11 >

USP Timolol Maleate RS

■ USP Timolol Related Compound B RS

3-(*tert*-Butylamino)-2-(4-morpholino-1,2,5-thiadiazol-3-yloxy)propan-1-ol.

C₁₃H₂₄N₄O₃S 316.42

USP Timolol Related Compound D RS

4-Morpholino-1,2,5-thiadiazol-3-ol.

C₆H₉N₇O₄S 187.22

■ 1S (USP39)

BRIEFING

Triamcinolone Acetonide Nasal Spray. Because there is no existing *USP* monograph for this drug product, a new monograph is being proposed based on validated methods.

1. The procedures for the *Assay* and *Organic Impurities* were validated using the Phenomenex Luna C18(2) brand of L1 column, in which triamcinolone acetonide elutes at about 27 min. A Phenomenex Luna C18 column may also be suitable for these tests.
2. The procedure for *Content of Edetate Disodium* was validated using the Hamilton PRP-1 brand of L21 column manufactured by the Hamilton Company, from which EDTA elutes at about 6 min.
3. The procedure for *Content of Benzalkonium Chloride* was validated using the Kinetex C18 brand of L1 column manufactured by Phenomenex, in which the C₁₀, C₁₂, C₁₄, and C₁₆ homologs of benzalkonium elute at about 1.1, 1.7, 2.3, and 2.7 min, respectively.
4. The procedures for *Delivered Dose Uniformity* were validated using the Ultracarb 5 ODS 30 brand of L1 column, in which triamcinolone acetonide elutes at about 3 min.

(SM4: R. Ravichandran.)

Correspondence Number—C85166

Comment deadline: May 31, 2015

Add the following:**■ Triamcinolone Acetonide Nasal Spray****DEFINITION**

Triamcinolone Acetonide Nasal Spray is an aqueous suspension of Triamcinolone Acetonide. It is supplied in a form suitable for nasal administration. It contains NLT 90.0% and NMT 110.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer A: 3.4 g/L of monobasic potassium phosphate prepared as follows. Dissolve 3.4 g of monobasic potassium phosphate in 900 mL of water, adjust with 5 M sodium hydroxide to a pH of 7.0, and dilute with water to 1000 mL.

Buffer B: 3.4 g/L of monobasic potassium phosphate prepared as follows. Dissolve 3.4 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 3.0, and dilute with water to 1000 mL.

Solution A: Acetonitrile and *Buffer A* (27.5: 72.5)

Solution B: Acetonitrile and *Buffer A* (60:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	60	40
30.1	0	100
44	0	100
44.1	100	0
52	100	0

Diluent: Acetonitrile and *Buffer B* (27.5: 72.5)

Standard stock solution: 0.4 mg/mL of USP Triamcinolone Acetonide RS in acetonitrile. Sonication for 15 min may be used to affect the complete dissolution.

System suitability stock solution: 0.04 mg/mL of USP Triamcinolone Acetonide Related Compound B RS and USP Triamcinolone Acetonide Related Compound C RS in *Diluent*

System suitability solution: 40 µg/mL of USP Triamcinolone Acetonide RS and 0.8 µg/mL each of USP Triamcinolone Acetonide Related Compound B RS and USP Triamcinolone Acetonide Related Compound C RS in suitable volumes of *Standard stock solution* and *System suitability stock solution* in *Diluent*

Standard solution: 40 µg/mL of USP Triamcinolone Acetonide RS from *Standard stock solution* in *Diluent*

Sample solution: Nominally 40 µg/mL of triamcinolone acetonide prepared as follows. Transfer a portion of the Nasal Spray, equivalent to 4 mg of triamcinolone acetonide, to a

100-mL volumetric flask. Dissolve in 28 mL of acetonitrile with the aid of sonication. Allow to cool to room temperature and dilute with *Buffer B* to volume. Centrifuge and use the clear supernatant.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 0.75 mL/min

Injection volume: 100 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 4* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between triamcinolone acetonide related compound C and triamcinolone acetonide related compound B; NLT 3.0 between triamcinolone acetonide related compound B and triamcinolone acetonide, *System suitability solution*

Tailing factor: NMT 1.3 for triamcinolone acetonide, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of triamcinolone acetonide (C₂₄H₃₁FO₆) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Triamcinolone Acetonide RS in the *Standard solution* (μg/mL)

C_U nominal concentration of triamcinolone acetonide in the *Sample solution* (μg/mL)

Acceptance criteria: 90.0%–110.0 %

OTHER COMPONENTS

• Content of Edetate Disodium

Perform this test if edetate disodium is a known component in the Nasal Spray.

Buffer: Add 990 mL of water into a 1000-mL beaker, followed by 10.0 mL of 1.0 M tetrabutylammonium hydroxide in methanol. Adjust with phosphoric acid to a pH of 7.0.

Mobile phase: Acetonitrile and *Buffer* (15:85)

Solution A: 40 g/L of sodium chloride and 2 g/L of sodium acetate. Adjust with glacial acetic acid to a pH of 5.5.

Solution B: 1.0 g/L of cupric sulfate in water

Diluent: Acetonitrile and water (50:50)

Standard stock solution: 0.5 mg/mL of edetate disodium in water. Sonication may be used to aid dissolution.

Standard solution: 0.05 mg/mL of edetate disodium. Transfer 5.0 mL of *Standard stock*

solution to a 50-mL volumetric flask. Next add 10 mL of *Solution A* and then add 5.0 mL of acetonitrile. Mix the resulting solution and then add 20.0 mL of *Solution B*, dilute with *Diluent* to volume, and mix.

Sample solution: Combine the contents of NLT 5 bottles of Nasal Spray and mix the contents to obtain a composite suspension. Transfer a 5.0-g portion of the Nasal Spray to a 50-mL volumetric flask. Add 10 mL of *Solution A* and 5.0 mL of acetonitrile. Mix and sonicate for 10 min and allow the solution to equilibrate to room temperature. Add 20.0 mL of *Solution B*, and sonicate for 10 min. Allow the sample to equilibrate to room temperature and dilute with *Diluent* to volume. Centrifuge a portion for 15 min, and use the supernatant. [Note—Centrifuging at 4000 rpm for 15 min may be suitable.]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.1-mm × 15-cm; 5-μm packing L21

Flow rate: 1 mL/min

Injection volume: 25 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of edetate disodium ($C_{10}H_{14}N_2Na_2O_8$) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of edetate disodium in the *Standard solution* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Nasal Spray in the *Sample solution* (mg)

Acceptance criteria: 0.045%–0.055%

● Content of Benzalkonium Chloride

Perform this test if benzalkonium chloride is a known component in the Nasal Spray.

Buffer: Dissolve 10.8 g of monobasic sodium phosphate dihydrate in 90 mL of water, and adjust with phosphoric acid a pH of 2.5. Dilute with water to 100 mL.

Solution A: Mix 50 mL of *Buffer*, 750 mL of water, and 200 mL of methanol. Add 5 mL of triethylamine. Mix and adjust with phosphoric acid to a pH of 2.5.

Solution B: Methanol and phosphoric acid prepared as follows. Mix 1 L of methanol with 50 mL of phosphoric acid.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	55	45
3.0	5	95
3.2	55	45
5.0	55	45

Diluent: 1% (v/v) hydrochloric acid in methanol

System suitability solution: 0.4 mg/mL of USP Benzalkonium Chloride RS prepared as follows. Transfer the required quantity of USP Benzalkonium Chloride RS to a suitable volumetric flask, and add 30% of the flask volume of water. Dilute with *Diluent* to volume.

Standard stock solution: 0.2 mg/mL of USP Benzalkonium Bromide RS in water. [Note—A few drops of methanol may be used to resolve the formation of foam prior to dilution.]

Standard solution: 0.04 mg/mL of USP Benzalkonium Bromide RS. Transfer an aliquot of *Standard stock solution* to a suitable volumetric flask, and add water equal to 30% of the flask volume. Dilute with *Diluent* to volume.

Sample solution: Combine the contents of NLT 5 bottles of Nasal Spray and mix the contents to obtain a composite suspension. Transfer a 5.0-g portion of the Nasal Spray to a 10-mL volumetric flask. Dilute with *Diluent* to volume. Centrifuge and use the supernatant. [Note—Centrifuging at 4000 rpm for 15 min may be suitable. Supernatant may be passed through a suitable filter with a pore size of NMT 0.2 μm .]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 3.0-cm; 2.6- μm packing L1

Column temperature: 50 $^{\circ}$

Flow rate: 2 mL/min

Injection volume: 100 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 3* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.5 between the pairs of C12 and C14 homologs and C14 and C16 homologs of benzalkonium, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the sum of each corrected benzalkonium peak response (r_U) as follows:

$$\text{Result} = \sum(r_U) \times (1/F)$$

$r_{\bar{r}}$ peak response of each benzalkonium homolog

F relative response factor of the corresponding benzalkonium homolog relative to benzalkonium bromide (see *Table 3*)

Table 3

Benzalkonium Chloride Analog	Relative Retention Time	Relative Response Factor
C10	0.65	1.3
C12	1.0	1.2
C14	1.35	1.0
C16	1.59	0.98

Calculate the percentage of benzalkonium chloride in the portion of Nasal Spray taken:

$$\text{Result} = (\Sigma r_U / r_S) \times C_S \times (V/W) \times 100$$

Σr_U = sum of the corrected peak responses for benzalkonium homologs from the *Sample solution*

r_S = response for benzalkonium from the *Standard solution*

C_S = concentration of USP Benzalkonium Bromide RS in the *Standard solution* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Nasal Spray in the *Sample solution* (mg)

Acceptance criteria: 0.0135%–0.0165%

PERFORMANCE TESTS

• Delivered Dose Uniformity (Between Containers)

Buffer: 7.0 g/L of sodium perchlorate prepared as follows. Dissolve 7.0 g of sodium perchlorate in 900 mL of water, adjust with perchloric acid to a pH of 3.0, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (50:50)

Standard solution: 40 µg/mL of USP Triamcinolone Acetonide RS in *Mobile phase*

Sample solution: Nominally 40 µg/mL of triamcinolone acetonide, prepared as follows.

Transfer a portion of Nasal Spray equivalent to 4.0 mg of triamcinolone acetonide to a suitable volumetric flask. Add 80% of the flask volume of *Mobile phase* to the flask.

Sonicate for 15 min. Allow to equilibrate to room temperature. Dilute with *Mobile phase* to volume. Centrifuge and pass the supernatant through a filter of 0.45-µm pore size. [Note—Centrifuging at 4000 rpm for 45 min may be suitable.]

Repeat this procedure with 9 additional units.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 40 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of triamcinolone acetonide ($C_{24}H_{31}FO_6$) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Triamcinolone Acetonide RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U nominal concentration of triamcinolone acetonide in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria

Tier 1: The content of each of the 10 units is within 90.0%–110.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

If the criterion in *Tier 1* cannot be met, proceed to *Tier 2*.

Tier 2: If the content of 1 unit is outside 90.0%–110.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$) and the content of none of the units is outside 85.0%–115.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$), test an additional 20 units. All the 30 results (including the results from *Tier 1*) meet the following acceptance criteria.

1.The content of each of 29 of 30 units is within 90.0%–110.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

2.The content of each of the 30 units is within 85.0%–115.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

- Delivered Dose Uniformity (Within Container)**

Buffer: 7.0 g/L of sodium perchlorate prepared as follows. Dissolve 7.0 g of sodium perchlorate in 900 mL of water, adjust with perchloric acid to a pH of 3.0, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (60:40)

Standard solution: 4.8 $\mu\text{g/mL}$ of USP Triamcinolone Acetonide RS in *Mobile phase*

Beginning sample solution (BOU): Hold the pump upright, actuate five times, and wipe the nosepiece dry. Hold a 25-mL volumetric flask in an inverted position and actuate the pump. Quickly turn the flask upright, wait 10 s, and repeat the process. Add 15 mL of *Mobile phase* while rinsing the neck of the flask and sonicate for 15 min. Allow the flask to equilibrate to room temperature, and dilute with *Mobile phase* to volume. Centrifuge and use the clear supernatant. [Note—Centrifuging at 4000 rpm for 15 min may be suitable.]

Middle sample solution (MOU): Using the same pump as above, discharge an appropriate number of actuations to arrive at 50% of the labeled number of actuations to waste and wipe the nosepiece dry. Hold a 25-mL volumetric flask in an inverted position and actuate the pump to collect the next actuation. Quickly turn the flask upright, wait 10 s, and repeat the process to collect the next actuation. Add 15 mL of *Mobile phase* while rinsing the neck of the flask and sonicate for 15 min. Allow the flask to equilibrate to room temperature, and dilute with *Mobile phase* to volume. Centrifuge and use the clear supernatant. This is the MOU sample. [Note—Centrifuging at 4000 rpm for 15 min may be suitable.]

End sample solution (EOU): Using the same pump as above, discharge the next

appropriate number of actuations to arrive at 100% of the labeled number of actuations to waste, and wipe the nosepiece dry. Hold a 25-mL volumetric flask in an inverted position and actuate the pump to collect the next actuation. Quickly turn the flask upright, wait 10 s, and repeat the process to collect the next actuation. Add 15 mL of *Mobile phase* while rinsing the neck of the flask and sonicate for 15 min. Allow the flask to equilibrate to room temperature, and dilute with *Mobile phase* to volume. Centrifuge and use the clear supernatant. This is the EOU sample. [Note—Centrifuging at 4000 rpm for 15 min may be suitable.]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 200 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution, Beginning sample solution, Middle sample solution, and End sample solution*

Calculate the percentage of the labeled amount of the triamcinolone acetonide ($C_{24}H_{31}FO_6$) delivered dose:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U response from the appropriate *Sample solution*

r_S response from the *Standard solution*

C_S concentration of USP Triamcinolone Acetonide RS in the *Standard solution* (μg/mL)

C_U nominal concentration of triamcinolone acetonide in the *Sample solution* (μg/mL)

Acceptance criteria

Calculate the mean dose delivered from the BOU results from all 10 containers.

Calculate the mean dose delivered from the MOU results from all 10 containers.

Calculate the mean dose delivered from the EOU results from all 10 containers.

Calculate mean dose delivered from the BOU, MOU, and EOU results from each of the 10 containers.

Tier 1

1. Mean delivered dose of BOU samples from all 10 units is within 85.0%–115.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).
2. Mean delivered dose of MOU samples from all 10 units is within 85.0%–115.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).
3. Mean delivered dose of EOU samples from all 10 units is within 85.0%–115.0% of

the labeled amount of triamcinolone acetonide (C₂₄H₃₁FO₆).

4.NMT 1 of 10 mean delivered doses is outside 80.0%–120.0% of the labeled amount of triamcinolone acetonide (C₂₄H₃₁FO₆).

5.None of the 10 mean delivered doses is outside 75.0%–125.0% of the labeled amount of triamcinolone acetonide (C₂₄H₃₁FO₆).

If the criterion in *Tier 1* cannot be met, proceed to *Tier 2*.

Tier 2: If NMT 3 of 10 mean of BOU, MOU, and EOU delivered doses for each unit are outside 80.0%–120.0% of the labeled amount of triamcinolone acetonide (C₂₄H₃₁FO₆) and none of the mean delivered doses is outside 75.0%–125.0% of the labeled amount of triamcinolone acetonide (C₂₄H₃₁FO₆), test an additional 20 units. All 30 results (including the results from *Tier 1*) meet the following acceptance criteria.

1.NMT 3 of 30 mean delivered doses are outside 80.0%–120.0% of the labeled amount of triamcinolone acetonide (C₂₄H₃₁FO₆).

2.None of the 30 mean delivered doses is outside 75.0%–125.0% of the labeled amount of triamcinolone acetonide (C₂₄H₃₁FO₆).

IMPURITIES

• Organic Impurities

Buffer A, Buffer B, Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each degradation product in the portion of Nasal Spray taken:

$$\text{Result} = (r_I/r_U) \times 100$$

r_I = peak response of each degradation product from the *Sample solution*

r_U = peak response of triamcinolone acetonide from the *Sample solution*

Acceptance criteria: See *Table 4*. Disregard any peak below 0.05%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Triamcinolone acetonide ketoacid derivative ^a	0.4	0.2
Triamcinolone acetonide related compound C	0.83	2.0
Triamcinolone acetonide related compound B	0.91	0.4
Triamcinolone acetonide	1.0	—
Any other individual degradation product	—	0.1

^a 9-Fluoro-11-hydroxy-16,17-[(1-methylethylidene)bis(oxy)]-(11 β ,16 α)-3,20-dioxopregna-1,4-diene-21-oic acid.

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total degradation products	—	2.5
^a 9-Fluoro-11-hydroxy-16,17-[(1-methylethylidene)bis(oxy)]-(11 β ,16 α)-3,20-dioxopregna-1,4-diene-21-oic acid.		

SPECIFIC TESTS

- **pH** 〈 791 〉: 4.5–6.0
- **Osmolality and Osmolarity** 〈 785 〉: 280–380 mOsmol/kg
- **Microbial Enumeration Tests** 〈 61 〉 and **Tests for Specified Microorganisms** 〈 62 〉: It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* species, and *Pseudomonas aeruginosa*. The total aerobic microbial count is NMT 10¹ cfu/mL and the total combined molds and yeasts count is NMT 10¹ cfu/mL.
- **Particle Size**
Analysis: Shake the Nasal Spray and prime the pump by spraying 3–4 times. Actuate the spray and collect the sample on a glass microscope slide held in above the nozzle, and repeat to prepare a second slide. Using light microscopy, determine the dimension of NLT 200 particles of triamcinolone acetonide by measuring NLT 100 particles from 20 random fields of view for each slide prepared. Repeat the procedure using a second container of Nasal Spray.

Acceptance criteria: See Table 5.

Table 5

Particle Size (μm)	Acceptance Criteria (%)
<1	NMT 3
1–6	70–95
>9	NMT 4

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in a tight, light-resistant container, and store at controlled room temperature.
- **USP Reference Standards** 〈 11 〉
 USP Benzalkonium Bromide RS
 USP Benzalkonium Chloride RS
 USP Triamcinolone Acetonide RS
 USP Triamcinolone Acetonide Related Compound B RS
 9-Fluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-(11 β ,16 α)-pregna-1,4,14-triene-3,20-dione.
 C₂₄H₂₉FO₆ 432.48
 USP Triamcinolone Acetonide Related Compound C RS
 9-Fluoro-11,21,21-trihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-(11 β ,16 α)-pregna-1,4-diene-3,20-dione.

C₂₄H₃₁FO₇ 450.50

■ 1S (USP39)

BRIEFING

Valproic Acid Capsules, USP 38 page 5737. In an effort to modernize the monograph, it is proposed to revise the monograph as follows:

1. Replace the non-specific visual colorimetric test in *Identification* test B with a spectral match based on the diode array spectra of the major peaks in the *Assay*.
2. Replace the existing packed column gas chromatographic procedure within the *Dissolution* section with a liquid chromatographic procedure that is similar to the *Assay*. The proposed procedure is based on analyses performed with a Zorbax Eclipse XDB-C8 column in which valproic acid elutes at about 6 min.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: R. Ravichandran.)

Correspondence Number—C155158

Comment deadline: May 31, 2015

Valproic Acid Capsules**DEFINITION**

Valproic Acid Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of valproic acid (C₈H₁₆O₂).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B.**

Sample: Equivalent to 250 mg of valproic acid

Analysis: Place the *Sample* in a separator, add 20 mL of 1 N sodium hydroxide, shake, and allow the layers to separate. Transfer the aqueous layer to a second separator, add 4 mL of hydrochloric acid, mix, and extract with 40 mL of *n*-heptane. Filter the *n*-heptane layer through glass wool into a beaker, and evaporate the solvent completely on a steam bath with the aid of a current of air. Transfer 2 drops of the residue to a test tube containing 0.5 mL each of potassium iodide solution (1 in 50) and potassium iodate solution (1 in 25), and mix.

Acceptance criteria: A yellow color is produced.

- The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

ASSAY

Change to read:● **Procedure**

Buffer: 3.5 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

Mobile phase: Acetonitrile and *Buffer* (45:55)

Diluent: Acetonitrile and water (45:55)

System suitability solution: 0.5 mg/mL of USP Valproic Acid RS and 50 µg/mL of USP Valproic Acid Related Compound B RS in *Diluent*

Standard solution: 0.5 mg/mL of USP Valproic Acid RS in *Diluent*

Sample solution: Nominally 0.5 mg/mL of valproic acid from the contents of the Capsules in *Diluent*, prepared as follows. Transfer a suitable portion of the contents of NLT 20 Capsules to an appropriate volumetric flask, and dilute with *Diluent* to volume. Sonicate the resulting solution for 5 min. Alternatively, stir the resulting solution for 1 h. Centrifuge a portion of the solution for about 10 min.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 215 nm.

■ For *Identification* test *B*, use a diode array detector in the wavelength range of 200–300 nm. ■ 1S (USP39)

Column: 4.6-mm × 15.0-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for valproic acid related compound B and valproic acid are 0.90 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between valproic acid related compound B and valproic acid, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of valproic acid (C₈H₁₆O₂) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)

C_U nominal concentration of valproic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS**Change to read:**● **Dissolution** (711)

Medium: 5 mg/mL of sodium lauryl sulfate in simulated intestinal fluid TS (prepared without the enzyme and with monobasic sodium phosphate instead of monobasic potassium phosphate), adjusted with 5 M sodium hydroxide to a pH of 7.5; 900 mL

Apparatus 2: 50 rpm

Time: 60 min

~~**Internal standard solution:** 5 mg/mL of biphenyl in *n*-heptane~~

~~**Standard stock solution:** $(L/900)$ mg/mL of USP Valproic Acid RS in *Medium*, where *L* is the label claim, in mg/Capsule~~

~~**Standard solution:** $(L/450)$ mg/mL of USP Valproic Acid RS from the *Standard stock solution*, where *L* is the label claim, in mg/Capsule, prepared as follows. Transfer 10.0 mL of the *Standard stock solution* to a suitable container, add 3.0 g of sodium chloride, and mix on a vortex mixer for 5 min. Add 1 mL of 6 N hydrochloric acid and 5.0 mL of the *Internal standard solution*, and shake for 2 min. Allow the phases to separate, remove the *n*-heptane layer, and filter.~~

Sample solution: Nominally $(L/450)$ mg/mL of valproic acid, where *L* is the label claim, in mg/Capsule, prepared as follows. Transfer 10.0 mL of the solution under test to a suitable container, add 3.0 g of sodium chloride, and mix on a vortex mixer for 5 min. Add 1 mL of 6 N hydrochloric acid and 5.0 mL of the *Internal standard solution*, and shake for 2 min. Allow the phases to separate, remove the *n*-heptane layer, and filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

~~**Mode:** GC~~

~~**Detector:** Flame ionization~~

~~**Column:** 2-mm \times 1.8-m glass; packed with 10% phase G34 on 80 to 100-mesh support S1A~~

~~**Temperatures**~~

~~**Column:** 150^o~~

~~**Injection port:** 250^o~~

~~**Detector:** 250^o~~

~~**Carrier gas:** Dry helium~~

~~**Flow rate:** 40 mL/min~~

~~**Injection volume:** 2 μ L~~

~~**System suitability**~~

~~[Note—The relative retention times for valproic acid and biphenyl are 0.5 and 1.0, respectively.]~~

~~**Sample:** *Standard solution*~~

~~**Suitability requirements**~~

~~**Resolution:** NLT 3.0 between valproic acid and biphenyl~~

~~**Relative standard deviation:** NMT 2%~~

~~**Analysis**~~

~~**Samples:** *Standard solution* and *Sample solution*~~

Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved:

$$\text{Result} = (R_U/R_S) \times C_S \times (V/L) \times 100$$

R_U peak response ratio of valproic acid to the internal standard from the *Sample solution*

R_S peak response ratio of valproic acid to the internal standard from the *Standard solution*

C_S concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Capsule)

■ Buffer, Mobile phase, Diluent, System suitability solution, Chromatographic system, and System suitability:

Proceed as directed in the *Assay*.

Standard stock solution: 1.5 mg/mL of USP Valproic Acid RS in *Diluent*, prepared as follows. Transfer NLT 150 mg of USP Valproic Acid RS to a suitable volumetric flask. Add 10% of the flask volume of *Diluent* and dissolve the valproic acid. Dilute with *Diluent* to volume.

Standard solution: 0.3 mg/mL of USP Valproic Acid RS from the *Standard stock solution* and *Medium*

Sample solution: Pass a portion of the solution through suitable filter of 0.45- μ m pore size and use the filtrate.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/L) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)

V

= volume of *Medium*, 900 mL

L

= label claim (mg/Capsule)

■ 1S (USP39)

Tolerances: NLT 85% (Q) of valproic acid ($C_8H_{16}O_2$) is dissolved.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
- **USP Reference Standards** { 11 }
 - USP Valproic Acid RS
 - USP Valproic Acid Related Compound B RS
(2*RS*)-2-(1-Methylethyl)pentanoic acid.
C₈H₁₆O₂ 144.21

BRIEFING

Warfarin Sodium, *USP 38* page 5801 and *PF 39(2)* [Mar.–Apr. 2013]. On the basis of comments received, the previously published proposal for the test for *Organic Impurities*, which appeared in *PF 39(2)* [Mar.–Apr. 2013], is canceled and replaced with the following revised proposal:

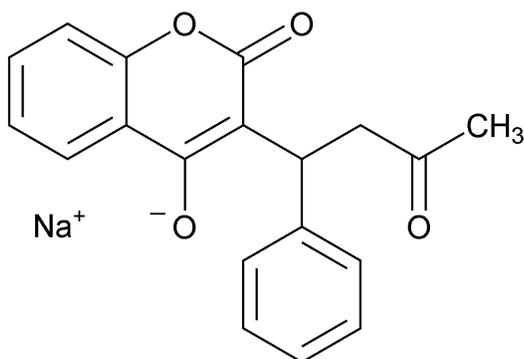
1. The flame test for *Identification test C* is replaced with *Sodium Identification test B* in *2.3.1 Identification Reactions of Ions and Functional Groups* in the *European Pharmacopoeia*. This test is also used in the *European Pharmacopoeia* monograph *Warfarin Sodium* for sodium identification.
2. The specified impurities and limits are proposed based on the FDA-approved specification.
3. The *Column temperature* and *Run time* are added for clarification.
4. The *System suitability solution* is added and the *Suitability requirements* are revised to assure that Alice's ketone is adequately resolved from warfarin at the *Sample solution* concentration.
5. A filtration step is added in the *Sample* preparation in the *Absorbance in Alkaline Solution*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: F. Mao.)

Correspondence Number—C133829

Comment deadline: May 31, 2015

Warfarin Sodium

C₁₉H₁₅NaO₄ 330.31

2*H*-1-Benzopyran-2-one, 4-hydroxy-3-(3-oxo-1-phenylbutyl)-, sodium salt;

3-(~~α~~-Acetonylbenzyl)-4-hydroxycoumarin sodium salt [129-06-6].

DEFINITION

Warfarin Sodium is an amorphous solid or a crystalline clathrate. The crystalline form consists principally of warfarin sodium and isopropyl alcohol in a 2:1 molecular ratio. It contains NLT 8.0% and NMT 8.5% of isopropyl alcohol. Warfarin Sodium contains NLT 97.0% and NMT 102.0% of warfarin sodium ($C_{19}H_{15}NaO_4$), calculated on the anhydrous basis for the amorphous form or on the anhydrous and isopropyl alcohol-free basis for the crystalline form.

IDENTIFICATION

Change to read:

- **A. Infrared Absorption** ~~(197K)~~

Sample: Dissolve 100 mg in 25 mL of water, and adjust with hydrochloric acid to a pH of less than 3, using short-range pH indicator paper. Stir the mixture, and allow the precipitate to coagulate. Filter the mixture, ~~and retain the filtrate for Identification test C.~~
Wash

■ wash ■_{1S} (USP39)

the precipitate with four, 5-mL portions of water, and dry under vacuum over phosphorus pentoxide for 4 h. Use the warfarin obtained.

Standard: Use USP Warfarin RS.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Delete the following:

- **C. Identification Tests—General, Sodium** ~~(191)~~

Sample: The filtrate obtained in *Identification test A*

Acceptance criteria: Meets the requirements of the flame test ■_{1S} (USP39)

Add the following:

- **C.**

Methoxyphenylacetic reagent: Dissolve 2.7 g of methoxyphenylacetic acid in 6 mL of 10% tetramethylammonium hydroxide aqueous solution, and add 20 mL of absolute alcohol.

Diluted ammonia: Dilute 41 g of ammonium hydroxide with water to 100 mL.

Ammonium carbonate solution: 158 mg/mL of ammonium carbonate in water

Sample solution: Dissolve 30 mg of warfarin sodium in 0.5 mL of water.

Analysis: Add 1.5 mL of *Methoxyphenylacetic reagent* in the *Sample solution*, and cool in ice water for 30 min.

Acceptance criteria: A voluminous, white, crystalline precipitate is formed. Place in water at 20° and stir for 5 min. The precipitate does not disappear. Add 1 mL of *Diluted ammonia*. The precipitate dissolves completely. Add 1 mL of *Ammonium carbonate solution*. No precipitate is formed. ■_{1S} (USP39)

ASSAY● **Procedure**

Buffer: Transfer 1.36 g of monobasic potassium phosphate to a 200-mL volumetric flask, and dissolve in 50 mL of water. Add 39.1 mL of 0.2 N sodium hydroxide, and dilute with water to volume. Adjust with sodium hydroxide or phosphoric acid to a pH of 7.4 ± 0.1 .

Mobile phase: Methanol, glacial acetic acid, and water (64:1:36)

Standard stock solution: 0.376 mg/mL of USP Warfarin RS prepared as follows. Transfer USP Warfarin RS to a suitable volumetric flask, and dissolve in 0.1 N sodium hydroxide equivalent to 39% of the final volume. Add 0.2 M monobasic potassium phosphate, equivalent to 25% of the final volume, and dilute with water to volume.

Standard solution: Transfer 5 mL of *Standard stock solution* and 15 mL of *Buffer* into a conical flask, and mix.

Sample stock solution: 0.4 mg/mL of Warfarin Sodium prepared as follows. Transfer 100 mg of Warfarin Sodium, accurately weighed, to a 250-mL volumetric flask, and dissolve in 97.8 mL of 0.1 N sodium hydroxide. Add 62.5 mL of 0.2 M monobasic potassium phosphate, and dilute with water to volume.

Sample solution: Transfer 5 mL of *Sample stock solution* and 15 mL of *Buffer* into a conical flask, and mix.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm \times 25-cm; packing L7

Flow rate: 1.4 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of warfarin sodium ($C_{19}H_{15}NaO_4$) in the portion of Warfarin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of warfarin from the *Sample solution*

r_S = peak response of warfarin from the *Standard solution*

C_S = concentration of USP Warfarin RS in the *Standard solution* (mg/mL)

C_U = concentration of Warfarin Sodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of warfarin sodium, 330.31

M_{r2} = molecular weight of warfarin, 308.33

Acceptance criteria: 97.0%–102.0% on the anhydrous basis for the amorphous form or on the anhydrous and isopropyl alcohol-free basis for the crystalline form

IMPURITIES**Delete the following:**

•

• **Heavy Metals** 〈 231 〉

Sample: Dissolve 4.0 g in 45 mL of water, add 5 mL of glacial acetic acid, stir until the precipitate agglomerates, filter, and use 25 mL of the filtrate, using glacial acetic acid, if necessary, to make the pH adjustment.

Acceptance criteria: NMT 10 ppm (Official 1-Dec-2015)

Change to read:• **Organic Impurities**

Diluent: Methanol and water (25:75)

Mobile phase: Acetonitrile, glacial acetic acid, and water (32:1:68)

Standard stock solution: 0.12 mg/mL each of USP Warfarin RS and USP Warfarin Related Compound A RS prepared as follows. Transfer USP Warfarin RS and USP Warfarin Related Compound A RS to a suitable volumetric flask, and add 0.1 N sodium hydroxide and methanol equivalent to 2% and 25% of the final volume, respectively. Dilute with water to volume.

Standard solution: 2.4 µg/mL each of USP Warfarin RS and USP Warfarin Related Compound A RS in *Diluent*, from *Standard stock solution*

Sample solution: 0.8 mg/mL of Warfarin Sodium in *Diluent*

■ **System suitability solution:** 2.4 µg/mL of USP Warfarin Related Compound A RS and 0.8 mg/mL of Warfarin Sodium in *Diluent* prepared as follows. Transfer 0.5 mL of *Standard stock solution* to a 25-mL volumetric flask and dilute with *Sample solution* to volume.

■ 1S (USP39)

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 25-cm; packing L10

■ **Column temperature:** 35 °C ■ 1S (USP39)

Flow rate: 1.5 mL/min

Injection volume: 50 µL

■ **Run time:** 20 min ■ 1S (USP39)

System suitability

Samples: *Standard solution*

■ and *System suitability solution* ■ 1S (USP39)

[Note—The relative retention times for warfarin and warfarin-related compound A are 1.0 and about 1.2, respectively.]

■ ■ 1S (USP39)

Suitability requirements**Resolution:** NLT 3 between warfarin and warfarin related compound A peaks,■ *System suitability solution* ■ 1S (USP39)**Relative standard deviation:** NMT 5.0%,■ *Standard solution* ■ 1S (USP39)**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Warfarin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 ~~r_U~~ = peak response for any individual impurity from the *Sample solution* ~~r_S~~ = peak response for warfarin from the *Standard solution* ~~C_S~~ = concentration of USP Warfarin RS in the *Standard solution* (mg/mL) ~~C_U~~ = concentration of the *Sample solution* (mg/mL)

■ Calculate the percentage of Alice's ketone in the portion of Warfarin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of warfarin related compound A from the *Sample solution* r_S = peak response of warfarin related compound A from the *Standard solution* C_S = concentration of USP Warfarin Related Compound A RS in the *Standard solution* (mg/mL) C_U = concentration of Warfarin Sodium in the *Sample solution* (mg/mL) M_{r1}

= molecular weight of Alice's ketone, 286.30

 M_{r2}

= molecular weight of warfarin related compound A, 264.33

Calculate the percentage of any other individual impurity in the portion of Warfarin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution* r_S = peak response of warfarin from the *Standard solution* C_S = concentration of USP Warfarin RS in the *Standard solution* (mg/mL) C_U = concentration of Warfarin Sodium in the *Sample solution* (mg/mL) M_{r1}

= molecular weight of warfarin sodium, 330.31

 M_{r2}

= molecular weight of warfarin, 308.33

 F = relative response factor for each individual impurity (see *Table 1*)

■ 1S (USP39)

Acceptance criteria**Individual impurity:** NMT 0.3%**Total impurities:** NMT 1.0%■ **Acceptance criteria****Individual impurities:** See *Table 1*. Disregard any impurity peaks less than 0.06%.**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
4-Hydroxycoumarin ^a	0.4	2.0	0.3
Benzalacetone ^b	0.6	2.0	0.3
Warfarin	1.0	—	—
Alice's ketone ^c	1.2	1.0	0.3
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

^a 4-Hydroxy-2H-chromen-2-one.

^b (E)-4-Phenylbut-3-en-2-one.^c 3-(o-Hydroxyphenyl)-5-phenyl-2-cyclohexen-1-one sodium salt. It is sodium salt of warfarin

related compound A.

■ 1S (USP39)

SPECIFIC TESTS

• Isopropyl Alcohol Content (crystalline clathrate form)

Internal standard solution: Transfer 1.0 mL of *n*-propyl alcohol into a 100-mL volumetric flask, and dilute with water to volume.

Standard stock solution: 15.7 mg/mL of isopropyl alcohol in water

Standard solution: 0.157 mg/mL of isopropyl alcohol in water prepared as follows. Transfer 1 mL of *Standard stock solution* and 2.0 mL of *Internal standard solution* to a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: 1.85 mg/mL of Warfarin Sodium in water prepared as follows. Dissolve 185 mg of Warfarin Sodium in a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, and dilute with water to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: GC

Detector: Flame-ionization

Column: 30-m × 0.32-mm; 1.8-μm coating of G43

Temperatures

Injection port: 210°

Detector: 280°

Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	4
50	25	200	4

Carrier gas: Helium

Flow rate: 5.0 mL/min

Injection volume: 1 μL

Injection type: Split ratio 5:1

System suitability

Sample: *Standard solution*

[Note—The relative retention times for isopropyl alcohol and *n*-propyl alcohol are about 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 2.0 between *n*-propyl alcohol and isopropyl alcohol

Tailing factor: NMT 2.0, isopropyl alcohol peak

Relative standard deviation: NMT 2.0%, peak response ratio of isopropyl alcohol to *n*-propyl alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of isopropyl alcohol in the portion of Warfarin Sodium taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\overline{U}}$ peak response ratio of isopropyl alcohol to *n*-propyl alcohol from the *Sample solution*

$R_{\overline{S}}$ peak response ratio of isopropyl alcohol to *n*-propyl alcohol from the *Standard solution*

$C_{\overline{S}}$ concentration of isopropyl alcohol in the *Standard solution* (mg/mL)

$C_{\overline{U}}$ concentration of Warfarin Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 8.0%–8.5%

- **pH** 〈 791 〉

Sample solution: 10 mg/mL

Acceptance criteria: 7.2–8.3

- **Water Determination, Method I** 〈 921 〉: NMT 4.5% for the amorphous form; NMT 0.3% for the crystalline clathrate form

Change to read:

- **Absorbance in Alkaline Solution**

Sample: 125 mg/mL in sodium hydroxide solution (1 in 20).

■ Pass through a membrane filter. ■ 1S (USP39)

Blank: Sodium hydroxide solution (1 in 20)

Analysis: Determine the absorbance of the solution in a 1-cm cell at 385 nm, with a suitable spectrometer, within 15 min.

Acceptance criteria: NMT 0.1

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
- **Labeling:** Label it to indicate whether it is the amorphous or the crystalline form.
- **USP Reference Standards** 〈 11 〉

USP Warfarin RS

USP Warfarin Related Compound A RS

3-(*o*-Hydroxyphenyl)-5-phenyl-2-cyclohexen-1-one.

C₁₈H₁₆O₂ 264.33

BRIEFING

meso-Zeaxanthin Preparation, USP 38 page 6481. On the basis of comments received, it is proposed to modify the *Stereoisomeric Composition* test to improve the resolution of three zeaxanthin isomers: (3*S*,3'*S*)-zeaxanthin, (3*R*,3'*S*-*meso*)-zeaxanthin, and (3*R*,3'*R*)-zeaxanthin.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(DS: H. Dinh.)

Correspondence Number—C155286

Comment deadline: May 31, 2015

meso-Zeaxanthin Preparation

DEFINITION

meso-Zeaxanthin Preparation is a combination of *meso*-Zeaxanthin with one or more inert substances. It may be in a solid or a liquid form. It contains NLT 95.0% and NMT 130.0% of the labeled amount of total carotenoids, calculated as zeaxanthin ($C_{40}H_{56}O_2$) on the anhydrous basis. It contains NLT 85.0% of zeaxanthin and NMT 9.0% of lutein of the total carotenoid content.

IDENTIFICATION● **A.**

Sample solution: Prepare as directed for *Sample solution A* or *Sample solution B* in the test for *Content of Total Carotenoids*.

Analysis: Record the UV-Vis spectrum from 300 to 600 nm.

Acceptance criteria: The *Sample solution* shows a shoulder at about 427 nm, an absorption maximum at about 453 nm, and another maximum at about 480 nm.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Content of Zeaxanthin*.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of (3*R*,3'*S*)-*meso*-zeaxanthin from the *Standard solution*, as obtained in the test for *Stereoisomeric Composition*.

COMPOSITION**Change to read:**● **Content of Total Carotenoids**

Sample stock solution A (for solid Preparations): Transfer an amount of Preparation equivalent to 7.5 mg of zeaxanthin to a 100-mL low-actinic volumetric flask, add 5 mL of water, and sonicate at 60° for 5 min. Add 50 mL of alcohol, and dilute with methylene chloride to volume. Shake thoroughly to obtain a fine dispersion, transfer 10 mL of this dispersion to a centrifuge tube, and centrifuge. Discard the foremost 2 mL of the supernatant. Use the clear solution.

Sample solution A: Transfer 1.0 mL of *Sample stock solution A* to a 50-mL low-actinic flask, and dilute with a mixture of cyclohexane and alcohol (9:1) to volume.

Sample stock solution B (for liquid *meso*-zeaxanthin suspensions in oil Preparations): ■ 1S (USP39)

Transfer an amount of Preparation equivalent to 7.5 mg of *meso*-zeaxanthin to a 100-mL low-actinic volumetric flask, add 20 mL of chloroform, and sonicate for 5 min. Cool the solution to room temperature, and dilute with cyclohexane to volume. Shake thoroughly to obtain a fine dispersion, transfer 10 mL of this dispersion to a centrifuge tube, and centrifuge.

Sample solution B: Transfer 1.0 mL of *Sample stock solution B* to a 50-mL low-actinic flask, and dilute with cyclohexane to volume.

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* { 851 } .)

Analytical wavelength: 453 nm

Cell: 1 cm

Blank: Cyclohexane

Analysis**Sample:** *Sample solution*Calculate the percentage of total carotenoids (*T*) as ~~meso-zeaxanthin~~

■ zeaxanthin ■ 1S (USP39)

(C₄₀H₅₆O₂) in the portion of Preparation taken:

$$\text{Result} = A/(C \times F)$$

A = absorbance of the *Sample solution**C* = concentration of Preparation in *Sample solution A* or *Sample solution B* (g/mL)*F* = coefficient of extinction (E^{1%}) of zeaxanthin in cyclohexane (100 mL·g⁻¹·cm⁻¹), 2540

Calculate the percentage of the labeled amount of total carotenoids:

$$\text{Result} = (T/L) \times 100$$

T = percentage of total carotenoids found as calculated above*L* = labeled amount of total carotenoids

■ (%) ■ 1S (USP39)

Acceptance criteria: 95.0%–130.0% of the labeled amount of total carotenoids as zeaxanthin (C₄₀H₅₆O₂) on the anhydrous basis● **Content of Zeaxanthin**

[Note—Use low-actinic glassware.]

Mobile phase: Hexane and ethyl acetate (75:25)**Standard solution:** 75 µg/mL of USP *meso*-Zeaxanthin RS in *Mobile phase***Sample solution:** Proceed as directed in *Sample stock solution A* or *Sample stock solution B* in the test for *Content of Total Carotenoids*. Pass through a membrane filter of 0.45-µm pore size.**Chromatographic system**(See *Chromatography* { 621 }, *System Suitability*.)**Mode:** LC**Detector:** UV 453 nm**Column:** 4.6-mm × 25-cm; 3-µm packing L3**Flow rate:** 1.5 mL/min**Injection volume:** 10 µL**System suitability****Sample:** *Standard solution*

[Note—The relative retention times for zeaxanthin and lutein are about 1.0 and 0.95, respectively.]

Suitability requirements**Resolution:** NLT 1.0 between zeaxanthin and lutein**Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Sample:** *Sample solution*

Calculate the percentage of zeaxanthin relative to total carotenoids in the portion of

Preparation taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U individual peak response of zeaxanthin

r_T sum of all the peak responses

Calculate the percentage of zeaxanthin in the portion of Preparation taken:

$$\text{Result} = (r_U/r_T) \times T$$

r_U individual peak response of *meso*-zeaxanthin in the *Sample solution*

r_T sum of all the peak responses

T = percentage of total carotenoids as determined in the test for *Content of Total Carotenoids*

Acceptance criteria: NLT 85.0% of zeaxanthin in the total carotenoid content

- **Lutein and Other Related Compounds**

Mobile phase, Standard solution, Sample solution, and Chromatographic system:

Proceed as directed in the test for *Content of Zeaxanthin*.

Injection volume: 10 μ L

Analysis

Sample: *Sample solution*

Calculate the percentage of lutein and other related compounds relative to total carotenoids in the portion of Preparation taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of lutein or other individual related compounds

r_T sum of all the peak areas

Acceptance criteria

Lutein: NMT 9.0%

Any other individual related compound: NMT 1.0%

Total related compounds (including lutein): NMT 15.0%

Change to read:

- **Stereoisomeric Composition**

■ [Note—Use low-actinic glassware.]

■ 1S (USP39)

Mobile phase: Hexane, alcohol, and isopropanol (80:5:5)

■ Gradient elution (see *Table 1*)

Table 1

Time (min)	<i>n</i> -Hexane (%)	2-Propanol (%)
0.0	95	5
50	95	5
55	50	50
63	50	50

Time (min)	<i>n</i> -Hexane (%)	2-Propanol (%)
65	95	5
75	95	5

■ 1S (USP39)

Standard solution: ~~0.075 mg/mL of USP meso-Zeaxanthin RS in alcohol and hexane (1:1).~~

~~Dissolve in 50% of the final volume with alcohol in an ultrasonic bath at 60° for 2–5 min, cool the flask, and dilute with hexane to volume. Pass through a 0.45- μ m pore size membrane filter.~~

■ Transfer 2.5 mg of USP *meso*-Zeaxanthin RS to a 50-mL volumetric flask, add 25 mL of dehydrated alcohol, and sonicate at 60° for 2–5 min to dissolve the substance. Cool the flask to room temperature and dilute with *n*-hexane to volume. Transfer 1.0 mL of the resultant solution to a 15-mL test tube, and evaporate with a stream of nitrogen to dryness. Dissolve the residue in a 10.0-mL mixture of 2-propanol and *n*-hexane (5:95). Pass through a membrane filter of 0.45- μ m pore size. ■ 1S (USP39)

Sample solution: Proceed as directed for *Sample stock solution A* or *Sample stock solution B* in the test for *Content of Total Carotenoids*. ~~Pass through a 0.45- μ m pore size membrane filter.~~

■ Transfer 1.0 mL of the *Sample stock solution A* or *Sample stock solution B* to a 15-mL test tube, and evaporate with a stream of nitrogen to dryness. Dissolve the residue in a 10.0-mL mixture of 2-propanol and *n*-hexane (5:95). Pass through a membrane filter of 0.45- μ m pore size. ■ 1S (USP39)

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: HPLC

Detector: 453 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L51

Column temperature: 35°

■ 30° ■ 1S (USP39)

Flow rate: 0.5 mL/min

■ 0.8 mL/min ■ 1S (USP39)

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[Note—The approximate relative retention times for (3*S*,3'*S*)-zeaxanthin, (3*R*,3'*S*-*meso*)-zeaxanthin, (3*R*,3'*R*)-zeaxanthin, and (3*R*,3'*R*,6'*R*)-lutein are 0.94, 1.00, 1.06, and 1.11, respectively.]

Suitability requirements

Resolution: NLT 1.5 between each pair of peaks due to (3*S*,3'*S*)-zeaxanthin, (3*R*,3'*S*-*meso*)-zeaxanthin, (3*R*,3'*R*)-zeaxanthin, and (3*R*,3'*R*,6'*R*)-lutein

Chromatogram similarity: The chromatogram from the *Standard solution* is similar to the reference chromatogram provided with the lot of USP *meso*-Zeaxanthin RS being used.

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the peaks of the relevant analytes in the chromatogram of the *Standard solution* by comparison with the reference chromatogram provided with the USP Reference Standard being used.

Calculate the percentage of (3*S*,3'*S*)-zeaxanthin, (3*R*,3'*S*-*meso*)-zeaxanthin, and (3*R*,3'*R*)-zeaxanthin:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of the corresponding analyte

r_T sum of all the peak responses

Acceptance criteria

(3*R*,3'*S*-*meso*)-Zeaxanthin: NLT 85.0%

(3*R*,3'*R*)-Zeaxanthin: NMT 15.0%

(3*S*,3'*S*)-Zeaxanthin: NMT 1.0%

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 2.0%

Delete the following:

-

• **Heavy Metals, Method II** 〈 231 〉

: NMT 10 ppm •(Official 1-Dec-2015)

SPECIFIC TESTS

- **Water Determination, Method I** 〈 921 〉: NMT 8.0% for solid Preparations; NMT 1.0% for liquid Preparations.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tightly sealed, light- and oxygen-resistant containers. Store in a cool place.
- **Labeling:** The label states the name and content of any carriers and antioxidants added to the formulation, and the content of total carotenoids as zeaxanthin.
- **USP Reference Standards** 〈 11 〉
USP *meso*-Zeaxanthin RS
β,β-Carotene-3,3'-diol (3*R*,3'*S*)-;
(3*R*,3'*S*-*meso*)-Zeaxanthin.
C₄₀H₅₆O₂ 568.88

BRIEFING

Zinc Sulfate Compounded Injection. Because of a need for a zinc sulfate compounded injection in the event of manufactured product unavailability or drug shortage, a new

compounded preparation monograph is proposed based on a validated method used to assess stability. The liquid chromatographic procedure in the *Assay* is based on analyses validated using the Metrosep C4-250 brand of L76 column. The typical retention time for zinc sulfate is about 7.0 min.

Please submit comments to CompoundingSL@usp.org.

(CMP: J. Sun.)

Correspondence Number—C154508

Comment deadline: May 31, 2015

Add the following:

■ Zinc Sulfate Compounded Injection

DEFINITION

Zinc Sulfate Compounded Injection contains NLT 90.0% and NMT 110.0% of the labeled content of zinc (Zn). Prepare Zinc Sulfate Compounded Injection containing 1 mg/mL or 5 mg/mL of zinc as follows (see *Pharmaceutical Compounding—Sterile Preparations* 〈 797 〉).

For 1-mg/mL Zinc Sulfate Compounded Injection

Zinc (as Zinc Sulfate Heptahydrate)	100 mg (440 mg)
Sulfuric Acid or Sodium Hydroxide	To adjust pH to 2.0–4.0
Sterile Water for Injection, a sufficient quantity to make	100 mL

For 5-mg/mL Zinc Sulfate Compounded Injection

Zinc (as Zinc Sulfate Heptahydrate)	500 mg (2199 mg)
Sulfuric Acid or Sodium Hydroxide	To adjust pH to 2.0–4.0
Sterile Water for Injection, a sufficient quantity to make	100 mL

Dissolve the *Zinc Sulfate Heptahydrate* in 95 mL of *Sterile Water for Injection*. Adjust with *Sulfuric Acid or Sodium Hydroxide* to a pH between 2.0 and 4.0. Add sufficient *Sterile Water for Injection* to bring to final volume, and mix well. Pass through a compatible filter of 1.2- μ m pore size to remove particulate matter and sterilize by autoclave.

ASSAY

• Procedure

Mobile phase: 3 mM nitric acid

Standard stock solution: 1 mg/mL of zinc prepared from USP Zinc Sulfate RS in water

Standard solution: 0.01 mg/mL of zinc prepared from *Standard stock solution* and water

Sample solution: For a 1-mg/mL Injection, transfer 1.0 mL of the compounded preparation to a 100-mL volumetric flask, dilute with water to volume, and mix well. For a 5-mg/mL Injection, transfer 0.2 mL of the compounded preparation to a 100-mL volumetric flask, dilute with water to volume, and mix well.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: Conductivity

Column: 4.0-mm × 10-cm; packing L76

Column temperature: 40°

Flow rate: 0.9 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[Note—The retention time for zinc is about 7.0 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zinc (Zn) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zinc from the *Sample solution*

r_S = peak response of zinc from the *Standard solution*

C_S = concentration of zinc from USP Zinc Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of zinc in *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** 〈 791 〉: 2.0–4.0
- **Sterility Tests** 〈 71 〉: Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **Bacterial Endotoxins Test** 〈 85 〉: NMT 25.0 USP Endotoxin Units/mg of zinc
- **Particulate Matter in Injections** 〈 788 〉: Meets the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in single-dose sterile containers, preferably Type I or Type II glass, and store at controlled room temperature.
- **Beyond-Use Date:** In the absence of performing and completing a sterility test, the storage conditions for *High-Risk Level CSPs in Pharmaceutical Compounding—Sterile Preparations* 〈 797 〉 apply. After successful completion of sterility testing, NMT 90 days after the date on which it was compounded when stored at controlled room temperature.
- **Labeling:** Label to state the *Beyond-Use Date*. Label the Injection in terms of its content of elemental zinc. Label it to state that it is for intravenous use after dilution.
- **USP Reference Standards** 〈 11 〉
 - USP Endotoxin RS
 - USP Zinc Sulfate RS

■ 1S (USP39)

BRIEFING

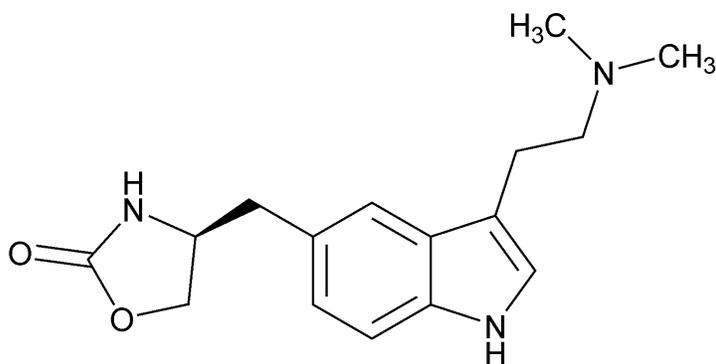
Zolmitriptan. Because there is no *USP* monograph for this drug substance, a new monograph based on validated methods of analysis is proposed.

1. The liquid chromatographic procedures in the *Assay* and the test for *Organic Impurities* are based on analyses performed with the Spherisorb ODS2 brand of L1 column manufactured by Waters Corporation. The typical retention time for zolmitriptan is about 5 min in both of the tests.
2. The test for *Limit of Zolmitriptan R-isomer and Other Impurities* is based on a validated capillary electrophoresis procedure with UV detection performed with a fused silica capillary column and a run buffer containing cyclodextrin. The migration time for zolmitriptan is about 31 min.
3. The gas chromatographic procedure in the *Limit of Zolmitriptan Related Compound H* test is based on analyses performed with the CP-Sil 5 CB brand of G1 column manufactured by Varian-Chrompack. The typical retention time for zolmitriptan related compound H is about 6 min.

(SM4: R. Ravichandran.)

Correspondence Number—C111969

Comment deadline: May 31, 2015

Add the following:**■ Zolmitriptan**

$C_{16}H_{21}N_3O_2$ 287.36

2-Oxazolidinone, 4-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-, (*S*)-;
(*S*)-4-[[3-[2-(Dimethylamino)ethyl]indol-5-yl]methyl]-2-oxazolidinone [139264-17-8].

DEFINITION

Zolmitriptan contains NLT 97.0% and NMT 102.0% of zolmitriptan ($C_{16}H_{21}N_3O_2$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The migration time of the major peak in the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Limit of Zolmitriptan R-isomer and*

*Other Impurities.***ASSAY**• **Procedure**

Mobile phase: Acetonitrile, water, trifluoroacetic acid, and triethylamine (135: 865: 1: 0.25)

System suitability solution: 0.12 µg/mL of USP Zolmitriptan Related Compound E RS and 25 µg/mL of USP Zolmitriptan RS in *Mobile phase*

Standard solution: 0.025 mg/mL of USP Zolmitriptan RS in *Mobile phase*

Sample solution: 0.025 mg/mL of Zolmitriptan in *Mobile phase*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability.*)

Mode: LC

Detector: UV 225 nm

Column: 4.0-mm × 12.5-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: About 3 times the retention time of zolmitriptan

System suitability

Sample: *System suitability solution*

[Note—Relative retention times for zolmitriptan and zolmitriptan related compound E are 1.0 and 1.6, respectively.]

Suitability requirements

Resolution: NLT 5 between zolmitriptan and zolmitriptan related compound E

Tailing factor: NMT 2.0 for zolmitriptan

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zolmitriptan (C₁₆H₂₁N₃O₂) in the portion of Zolmitriptan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U concentration of Zolmitriptan in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0%, on the anhydrous and solvent-free basis

IMPURITIES

• **Residue on Ignition** 〈 281 〉: NMT 0.1%

• **Limit of Zolmitriptan Related Compound H**

Perform this test only if zolmitriptan related compound H is a known process impurity. If this test is performed, then this is to be included in total impurities.

Standard solution: 0.1 mg/mL of USP Zolmitriptan Related Compound H RS in methanol

Sample solution: 200 mg/mL of Zolmitriptan in methanol

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Carrier gas: Helium at 6 mL/min

Column: 0.32-mm × 15-m; 1-μm coating of dimethylpolysiloxane phase, G1

Temperatures

Injection port: 200°

Column: 130°

Detector: 250°

Injection volume: 3 μL

Split ratio: 10:1

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zolmitriptan related compound H in the portion of Zolmitriptan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of zolmitriptan related compound H from the *Sample solution*

r_S peak response of zolmitriptan related compound H from the *Standard solution*

C_S concentration of USP Zolmitriptan Related Compound H RS in the *Standard solution* (mg/mL)

C_U concentration of Zolmitriptan in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1% of zolmitriptan related compound H

- **Limit of Zolmitriptan R-isomer and Other Impurities**

Buffer: 19.1 g/L of sodium borate decahydrate in water. Adjust with phosphoric acid to a pH of 2.2.

Run buffer: 50 mg/mL of hydroxypropylcyclodextrin in *Buffer*

Diluent: 0.02 M hydrochloric acid

Internal standard solution: 0.2 mg/mL of tryptamine hydrochloride in *Diluent*

System suitability solution: 0.01 mg/mL of tryptamine hydrochloride from the *Internal standard solution*, 1 mg/mL of USP Zolmitriptan RS, and 0.01 mg/mL each of USP Zolmitriptan Related Compound F RS, USP Zolmitriptan Related Compound G RS, and USP Zolmitriptan R-isomer RS, in *Diluent*

Standard solution: 0.01 mg/mL of tryptamine hydrochloride from *Internal standard solution* and 0.001 mg/mL of USP Zolmitriptan RS in *Diluent*

Sample solution: 0.01 mg/mL of tryptamine hydrochloride from *Internal standard solution* and 1 mg/mL of Zolmitriptan in *Diluent*. Filter the solution and protect from light.

Electrophoretic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: CE

Detector: UV 200 nm

Column: 75- μ m \times 0.5-cm uncoated fused-silica

Applied voltage: 15 kV

Run time: 1.5 times the migration time of zolmitriptan

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for relative migration times.]

Suitability requirements

Resolution: NLT 1.5 between zolmitriptan and zolmitriptan R-isomer peaks, *System suitability solution*

Relative standard deviation: NMT 5% for the peak response ratio of zolmitriptan and tryptamine peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the corrected peak responses:

$$\text{Result} = (r/m)$$

r = peak response

m = migration time of the peak (min)

Calculate the percentage of each impurity in the portion of Zolmitriptan taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (1/F) \times 100$$

R_U = corrected peak response ratio of the impurity to the internal standard from the *Sample solution*

R_S = corrected peak response ratio of zolmitriptan to the internal standard from the *Standard solution*

C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U = concentration of Zolmitriptan in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard peak due to zolmitriptan related compound E. Disregard peaks less than 0.10% of the area of the principal peak from the *Sample solution*.

Table 1

Name	Relative Migration Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zolmitriptan related compound F	0.68	0.39	1.2
Zolmitriptan related compound G	0.71	0.63	0.1
Tryptamine	0.78	1.0	—
Zolmitriptan	1.0	—	—
Zolmitriptan R-isomer	1.07	1.0	0.2
Any individual unspecified impurity	—	1.0	0.1

• Organic Impurities

Mobile phase: Acetonitrile, water, trifluoroacetic acid, and triethylamine (135: 865: 1: 0.25)

System suitability solution: 0.5 µg/mL of USP Zolmitriptan Related Compound E RS and 0.1 mg/mL of USP Zolmitriptan RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Zolmitriptan in *Mobile phase*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.0-mm × 12.5-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 6 times the retention time of zolmitriptan

System suitability [Note—See *Table 2* for relative retention times.]

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 5 between zolmitriptan and zolmitriptan related compound E peaks

Tailing factor: NMT 3.0 for zolmitriptan

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Zolmitriptan taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_T sum of the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05% of the area of the principal peak from the *Sample solution*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Zolmitriptan related compound B ^a	0.36	0.2
Zolmitriptan	1.0	—
Zolmitriptan related compound E	1.6	0.2
Zolmitriptan related compound F ^b	2.3	—
Any individual unspecified impurity	—	0.1
Total impurities ^c	—	0.5

- a (S)-2-Amino-3-{3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl}propan-1-ol.
- b Included for identification purposes only. Not reported here as it is monitored under *Limit of Zolmitriptan R-isomer and Other Impurities*. Not to be included in total impurities.
- c Includes all impurities except zolmitriptan related compound F. Also includes zolmitriptan related compound H from the test for *Limit of Zolmitriptan Related Compound H* if it is a known process impurity, as well as impurities from the test for *Limit of Zolmitriptan R-isomer and Other Impurities* with the exception of zolmitriptan related compound F.

SPECIFIC TESTS

- **Water Determination, Method Ia** 〈 921 〉: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at room temperature.
- **USP Reference Standards** 〈 11 〉
 - USP Zolmitriptan RS
 - USP Zolmitriptan *R*-isomer RS
 - (*R*)-4-[[3-[2-(Dimethylamino)ethyl]indol-5-yl]methyl]-2-oxazolidinone.
 - C₁₆H₂₁N₃O₂ 287.36
 - USP Zolmitriptan Related Compound E RS
 - (*S*)-*N,N*-Dimethyl-2-{5-[(2-oxooxazolidin-4-yl)methyl]-1*H*-indol-3-yl}ethanamine oxide.
 - C₁₆H₂₁N₃O₃ 303.36
 - USP Zolmitriptan Related Compound F RS
 - 2,2'-[4-(Dimethylamino)butane-1,1-diyl]bis{5-[(*S*)-(2-oxooxazolidin-4-yl)methyl]-3-(2-dimethylaminoethyl)indole}.
 - C₃₈H₅₃N₇O₄ 671.87
 - USP Zolmitriptan Related Compound G RS
 - (*S*)-4-(4-Aminobenzyl)oxazolidin-2-one.
 - C₁₀H₁₂N₂O₂ 192.21
 - USP Zolmitriptan Related Compound H RS
 - 4,4-Diethoxy-*N,N*-dimethylbutan-1-amine.
 - C₁₀H₂₃NO₂ 189.30

■ 1S (USP39)

BRIEFING

Zolmitriptan Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph based on validated methods of analysis is proposed.

1. The liquid chromatographic procedures in the *Assay* and the *Dissolution* test are based on analyses performed with the Unisphere C18 brand of L1 column manufactured by Unichrome Associates. The typical retention time for zolmitriptan is about 3 min.
2. The liquid chromatographic procedure proposed in the test for *Organic Impurities* is based on analyses performed with the YMC Pack ODS-A brand of L1 column manufactured by YMC Corporation in which the typical retention time for zolmitriptan

is about 20 min.

(SM4: R. Ravichandran.)

Correspondence Number—C142336

Comment deadline: May 31, 2015

Add the following:

■ **Zolmitriptan Tablets**

DEFINITION

Zolmitriptan Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$).

IDENTIFICATION

- **A.** The UV spectrum of the zolmitriptan peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

● **Procedure**

Buffer: Dissolve 2 mL of triethylamine in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (15:85)

Standard stock solution: 0.25 mg/mL of USP Zolmitriptan RS in methanol. Sonicate if necessary to aid dissolution.

Standard solution: 0.025 mg/mL of USP Zolmitriptan RS in *Mobile phase* from *Standard stock solution*

Sample stock solution: Nominally 0.25 mg/mL of zolmitriptan from Tablets in methanol prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask. Add 70% of the flask volume of methanol. Sonicate for 30 min. Cool to room temperature, and dilute with methanol to volume.

Sample solution: Nominally 0.025 mg/mL of zolmitriptan in *Mobile phase* from *Sample stock solution* passed through a suitable membrane filter of 0.45- μ m pore size

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 225 nm; for *Identification* test A, a diode-array detector can be used in the wavelength range of 200–300 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Temperature: 30 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: NLT 2.5 times the retention time of zolmitriptan

System suitability

Sample: *Standard solution*

Suitability requirements**Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of zolmitriptan from the *Sample solution*

r_S peak response of zolmitriptan from the *Standard solution*

C_S concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U nominal concentration of zolmitriptan in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%**PERFORMANCE TESTS**● **Dissolution** 〈 711 〉**Medium:** 0.1 N hydrochloric acid; 500 mL**Apparatus 2:** 50 rpm**Time:** 15 min**Buffer, Mobile phase, Standard stock solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.**Standard solution:** ($L/500$) mg/mL of USP Zolmitriptan RS in *Medium* from *Standard stock solution*, where L is the Tablet label claim in mg**Chromatographic procedure****Sample solution:** Pass a portion of the solution under test through a suitable membrane filter of 0.45- μ m pore size.**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U peak response of zolmitriptan from the *Sample solution*

r_S peak response of zolmitriptan from the *Standard solution*

C_S concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim of zolmitriptan (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) is dissolved.● **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements**IMPURITIES**● **Organic Impurities**

Solution A: 2.7 g/L of monobasic potassium phosphate in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
10	95	5
45	86	14
55	55	45
60	55	45
62	95	5
75	95	5

Diluent: Methanol and water (25:75)

System suitability stock solution: 0.2 mg/mL each of USP Zolmitriptan Related Compound E RS and USP Zolmitriptan Related Compound G RS in methanol

System suitability solution: 0.25 mg/mL of USP Zolmitriptan RS and 0.002 mg/mL each of USP Zolmitriptan Related Compound E RS and USP Zolmitriptan Related Compound G RS from *System suitability stock solution* in *Diluent* prepared as follows. Dissolve a suitable quantity of USP Zolmitriptan RS in a suitable volumetric flask containing 50% of the flask volume of *Diluent*. Sonicate to dissolve. Transfer a suitable volume of *System suitability stock solution* to the flask. Dilute with *Diluent* to volume.

Standard stock solution: 0.25 mg/mL of USP Zolmitriptan RS in methanol. Sonicate if necessary to aid dissolution.

Standard solution: 0.001 mg/mL of USP Zolmitriptan RS in *Diluent* from a suitable volume of *Standard stock solution*

Sample solution: Nominally 0.25 mg/mL of zolmitriptan from NLT 5 Tablets prepared as follows. Transfer the required number of Tablets to a suitable volumetric flask. Add 25% of the flask volume of methanol. Sonicate for 30 min with intermittent shaking. Cool to room temperature. Dilute with water to volume. Pass through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector

For zolmitriptan and zolmitriptan related compound E and any other unspecified degradation product: UV 223 nm

For zolmitriptan and zolmitriptan related compound G: UV 235 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Temperature: 30^o

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Use 223 nm for system suitability evaluation.

Resolution: NLT 5.0 between zolmitriptan and zolmitriptan related compound E, *System suitability solution*

Tailing factor: NMT 2.0 for the zolmitriptan peak, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of zolmitriptan related compound G in the portion of the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of zolmitriptan related compound G at 235 nm from the *Sample solution*

r_S peak response of zolmitriptan at 235 nm from the *Standard solution*

C_S concentration of the USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U nominal concentration of zolmitriptan in the *Sample solution* (mg/mL)

F = relative response factor for zolmitriptan related compound G (see *Table 2*)

Calculate the percentage of zolmitriptan related compound E and any other unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of zolmitriptan related compound E or any other unspecified degradation product at 223 nm from the *Sample solution*

r_S peak response of zolmitriptan at 223 nm from the *Standard solution*

C_S concentration of the USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U nominal concentration of zolmitriptan in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurity less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zolmitriptan related compound G	0.50	1.2	0.2
Zolmitriptan	1.0	—	—
Zolmitriptan related compound E	1.28	1.0	0.5
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	0.7

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.
- **USP Reference Standards** { 11 }
USP Zolmitriptan RS

USP Zolmitriptan Related Compound E RS

(S)-N,N-Dimethyl-2- {5- [(2-oxooxazolidin-4-yl)methyl]-1H-indol-3-yl}ethanamine oxide.

C₁₆H₂₁N₃O₃ 303.36

USP Zolmitriptan Related Compound G RS

(S)-4-(4-Aminobenzyl)oxazolidin-2-one.

C₁₀H₁₂N₂O₂ 192.21

■ 1S (USP39)

BRIEFING

Zolmitriptan Orally Disintegrating Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph based on validated methods of analysis is proposed.

1. The liquid chromatographic procedure in the *Assay* and the test for *Dissolution* is based on analyses performed with the Unisphere C18 brand of L1 column manufactured by Unichrom Associates. The typical retention time for zolmitriptan is about 4 min.
2. The liquid chromatographic procedure proposed in the test for *Organic Impurities* is based on analyses performed with the Inertsil ODS-3V brand of L1 column manufactured by GL Sciences. The typical retention time for zolmitriptan is about 23 min.

(SM4: R. Ravichandran.)

Correspondence Number—C142335

Comment deadline: May 31, 2015

Add the following:**■ Zolmitriptan Orally Disintegrating Tablets****DEFINITION**

Zolmitriptan Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of zolmitriptan (C₁₆H₂₁N₃O₂).

IDENTIFICATION

- **A.** The UV spectrum of the zolmitriptan peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**● Procedure**

Buffer: Dissolve 2 mL of triethylamine in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (15:85)

Standard stock solution: 0.25 mg/mL of USP Zolmitriptan RS in methanol

Standard solution: 0.025 mg/mL of USP Zolmitriptan RS in *Mobile phase* from a suitable volume of *Standard stock solution*

Sample stock solution: 0.25 mg/mL of zolmitriptan in methanol, prepared as follows.

Transfer NLT 20 Tablets to a suitable volumetric flask. Add 75% of the flask volume of methanol. Sonicate for 30 min. Allow to cool to room temperature and dilute with methanol to volume.

Sample solution: Nominally 0.025 mg/mL of zolmitriptan in *Mobile phase* from a suitable volume of *Sample stock solution*. Pass a portion of the solution under test through a suitable membrane filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 225 nm. For *Identification* test A, use a diode-array detector in the wavelength range of 200–300 nm.

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Column temperature: 30 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: NLT 2.5 times the retention time of zolmitriptan

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of zolmitriptan from the *Sample solution*

r_S peak response of zolmitriptan from the *Standard solution*

C_S concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U nominal concentration of zolmitriptan in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Disintegration** { 701 } : NMT 30 s

• **Dissolution** { 711 }

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 15 min

Buffer, Mobile phase, Standard stock solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Standard solution: 0.005 mg/mL of USP Zolmitriptan RS from *Standard stock solution* in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 0.45- μm pore size.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zolmitriptan ($\text{C}_{16}\text{H}_{21}\text{N}_3\text{O}_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of zolmitriptan from the *Sample solution*

r_S = peak response of zolmitriptan from the *Standard solution*

C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim of zolmitriptan (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of zolmitriptan ($\text{C}_{16}\text{H}_{21}\text{N}_3\text{O}_2$) is dissolved.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

- **Organic Impurities**

Diluent: Methanol and water (25:75)

Solution A: 2.7 g/L of monobasic potassium phosphate in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
15	92	8
45	86	14
55	55	45
60	55	45
62	95	5
75	95	5

Impurities stock solution: 0.2 mg/mL each of USP Zolmitriptan Related Compound E RS and USP Zolmitriptan Related Compound G RS in methanol

System suitability solution: 0.25 mg/mL of USP Zolmitriptan RS and 0.002 mg/mL each of USP Zolmitriptan Related Compound E RS and USP Zolmitriptan Related Compound G RS in *Diluent* prepared as follows. Dissolve a suitable quantity of USP Zolmitriptan RS in a suitable volumetric flask containing 50% of the flask volume of *Diluent*. Sonicate to dissolve. Transfer a suitable volume of *Impurities stock solution* to the flask. Dilute with *Diluent* to volume.

Standard stock solution: 0.25 mg/mL of USP Zolmitriptan RS in methanol

Standard solution: 0.001 mg/mL of USP Zolmitriptan RS from *Standard stock solution* in *Diluent*

Sample solution: Nominally 0.25 mg/mL of zolmitriptan from NLT 5 Tablets prepared as follows. Transfer the required number of Tablets to a suitable volumetric flask. Add 25% of

the flask volume of methanol. Sonicate for 30 min with intermittent shaking. Cool to room temperature. Dilute with water to volume. Pass through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector

For zolmitriptan and zolmitriptan related compound E and any other unspecified degradation products: UV 223 nm

For zolmitriptan and zolmitriptan related compound G: UV 235 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 30 $^{\circ}$

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Use 223 nm for system suitability evaluation

Resolution: NLT 5.0 between zolmitriptan and zolmitriptan related compound E, *System suitability solution*

Tailing factor: NMT 2.0 for zolmitriptan, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zolmitriptan related compound G in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of zolmitriptan related compound G at 235 nm from the *Sample solution*

r_S peak response of zolmitriptan at 235 nm from the *Standard solution*

C_S concentration of the USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U nominal concentration of zolmitriptan in the *Sample solution* (mg/mL)

F = relative response factor for zolmitriptan related compound G (see *Table 2*)

Calculate the percentage of zolmitriptan related compound E and any other unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of zolmitriptan related compound E or any other unspecified degradation product at 223 nm from the *Sample solution*

r_S peak response of zolmitriptan at 223 nm from the *Standard solution*

C_S concentration of the USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U nominal concentration of zolmitriptan in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See Table 2. Disregard any impurity less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zolmitriptan related compound G	0.66	1.2	0.2
Zolmitriptan	1.0	—	—
Zolmitriptan related compound E	1.30	1.0	0.5
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.5

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.

- **USP Reference Standards** (11)

USP Zolmitriptan RS

USP Zolmitriptan Related Compound E RS

(*S*)-*N,N*-Dimethyl-2- $\{5-[(2\text{-oxooxazolidin-4-yl)methyl]-1H-indol-3-yl}\}$ ethanamine oxide.

$C_{16}H_{21}N_3O_3$ 303.36

USP Zolmitriptan Related Compound G RS

(*S*)-4-(4-Aminobenzyl)oxazolidin-2-one.

$C_{10}H_{12}N_2O_2$ 192.21

■ 1S (USP39)

Stage 4 Harmonization

This section contains monographs or chapters undergoing harmonization by the Pharmacopoeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (**Stages 1** through **7**, as defined below). Stage 4 drafts are available for comments. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF*, this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

BRIEFING

Propylene Glycol, *USP 38* page 5065. The *European Pharmacopoeia (EP)* is the coordinating pharmacopeia (CP) for the international harmonization of the compendial standards for the *Propylene Glycol* monograph, as part of the process of international harmonization of monographs and general analytical methods of the *European, Japanese, and U.S. Pharmacopeias*. The following monograph, which is the **OFFICIAL INQUIRY STAGE 4, REVISION 1** document, is based on the **OFFICIAL INQUIRY STAGE 4** draft published in *PF 33(2)* [Mar.–Apr. 2007] as well as comments from all pharmacopeias. Changes from the current *Propylene Glycol* monograph include the following:

1. *Definition*. Increased the limit to NLT 99.7% of propylene glycol (C₃H₈O₂) according to Stage 4 PDG global text.
2. *Identification test B*. No changes. *Limit of Diethylene Glycol and Ethylene Glycol* remains as an identification test in the *USP–NF* monograph upon FDA's request. It is an impurity test in the Stage 4 PDG global text.
3. *Assay*. A new *Assay* using a capillary GC column replaced the previous method using a packed GC column. A suitable column is the Agilent Technologies HP-5, 0.32-mm × 30-m, 0.5-μm, G27 column. The retention time for propylene glycol is about 2 min.
4. *Heavy Metals*. Delete the test according to *General Notices and Requirements 5.60.30* in *USP 38*. For further details, see *Elemental Impurities* on the Key Issues page on the USP website.
5. *Color and Clarity of Solution*. New test was added to detect the color and the clarity of the sample.
6. *Oxidizing Substances*. New test was added by titration with sodium thiosulfate.
7. *Aldehydes*. New test was added by detection at 655 nm after reaction with methylbenzothiazolone hydrazone hydrochloride and ferric chloride. The proposed limit is 20 ppm, expressed as formaldehyde (HCHO).
8. *Acidity*. Modified the procedure and acceptance criteria based on Stage 4 PDG global text.
9. *Water Determination*. Added sample amount of 5.00 g.
10. *Identification test A and Packaging and Storage*. No changes.
11. *Identification test C, Residue on Ignition, Chloride and Sulfate, and Specific Gravity*. No changes from existing *USP–NF* text. These attributes are not part of the Stage 4 PDG global text. Future decision on inclusion as nonharmonized attributes or local requirements will be made at Stage 6.

[Note—USP maintains a separate section of *Description and Solubility*, where the “description” and “solubility” statements pertaining to an article are general in nature. The properties are not in themselves standards or tests for purity even though they may indirectly assist in the preliminary evaluation of the integrity of an article. *EP* as CP has proposed the following as “Characters” to be included as part of the Stage 4: “Appearance: viscous, clear, colorless, hygroscopic liquid; Solubility: miscible with water and with ethanol (96%).” USP will maintain *Description and Solubility/Characters* as a nonharmonized attribute.]

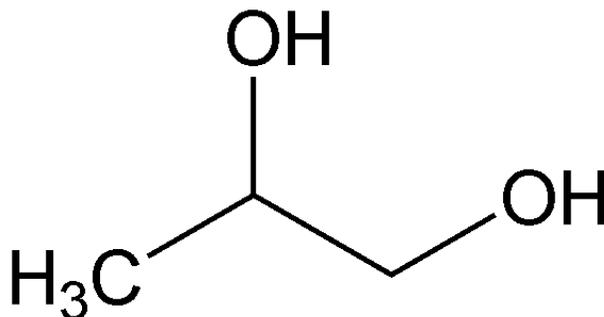
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(EXC: Jenny Liu.)

Correspondence Number—C155495

Comment deadline: May 31, 2015

Propylene Glycol



$C_3H_8O_2$ 76.09

1,2-Propanediol;
Propane-1,2-diol [57-55-6].

DEFINITION

Change to read:

Propylene Glycol contains NLT ~~99.5%~~
99.7%

of propylene glycol ($C_3H_8O_2$).

IDENTIFICATION

[Note—Compliance is determined by meeting the requirements of *Identification* tests *A*, *B*, and *C*.]

- **A. Infrared Absorption** 〈 197F 〉

[Note—Undried specimen.]

- **B. Limit of Diethylene Glycol and Ethylene Glycol**

Diluent: Methanol

Standard solution: 2.0 mg/mL of USP Propylene Glycol RS, 0.050 mg/mL of USP Ethylene Glycol RS, 0.050 mg/mL of USP Diethylene Glycol RS, and 0.10 mg/mL of 2,2,2-trichloroethanol (internal standard) in methanol

Sample solution: 50 mg/mL of Propylene Glycol and 0.10 mg/mL of 2,2,2-trichloroethanol (internal standard) in methanol

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 30-m fused-silica coated with 3.0- μ m G43 stationary phase, and a

deactivated split liner with glass wool

Temperatures

Injector: 220°

Detector: 250°

Column: See the temperature program in *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	—	100	4
100	50	120	10
120	50	220	6

Carrier gas: Helium

Flow rate: 4.5 mL/min

Injection volume: 1.0 µL

Injection type: The split flow ratio is about 10:1.

System suitability

Sample: *Standard solution*

[Note—For informational purposes only. See *Table 2* for relative retention times for ethylene glycol, internal standard, and diethylene glycol. The retention time for propylene glycol is 4 min.]

Table 2

Component	Relative Retention Time
Ethylene glycol	0.8
Propylene glycol	1.0
Internal standard	1.7
Diethylene glycol	2.4

Suitability requirements

Resolution: NLT 5 between ethylene glycol and propylene glycol

Analysis

Sample: *Sample solution*

Acceptance criteria

Diethylene glycol: If a peak at the retention time for diethylene glycol is present in the *Sample solution*, the peak response ratio relative to 2,2,2-trichloroethanol is NMT the peak response ratio for diethylene glycol relative to 2,2,2-trichloroethanol in the *Standard solution*: NMT 0.10% for diethylene glycol.

Ethylene glycol: If a peak at the retention times for ethylene glycol is present in the *Sample solution*, the peak response ratio relative to 2,2,2-trichloroethanol is NMT the peak response ratio for ethylene glycol relative to 2,2,2-trichloroethanol in the *Standard solution*: NMT 0.10% for ethylene glycol.

- **C.**

Analysis: Examine the chromatograms obtained in *Identification test B*.

Acceptance criteria: The retention time of the propylene glycol peak of the *Sample*

solution corresponds to that of the *Standard solution*.

ASSAY

Change to read:

• Procedure

~~**Sample:** Propylene Glycol~~

~~**Chromatographic system**~~

~~(See *Chromatography* \langle 621 \rangle , *System Suitability*.)~~

~~**Mode:** GC~~

~~**Detector:** Thermal conductivity~~

~~**Column:** 1-m \times 4-mm; 5% phase G16; support S5~~

~~**Temperature**~~

~~**Injector:** 240 $^{\circ}$~~

~~**Detector:** 250 $^{\circ}$~~

~~**Column:** Increase from 120 $^{\circ}$ to 200 $^{\circ}$ at a rate of 5 $^{\circ}$ /min.~~

~~**Carrier gas:** Helium~~

~~**Injection size:** 10 μ L~~

~~[Note—The approximate retention time for propylene glycol is 5.7 min, and the approximate retention times for the three isomers of dipropylene glycol, when present, are 8.2, 9.0, and 10.2 min, respectively.]~~

~~**Analysis:** Calculate the percentage of $C_3H_8O_2$ in the *Sample* by dividing the area under the propylene glycol peak by the sum of the areas under all of the peaks, excluding those due to air and water, and multiplying by 100:~~

$$\text{Result} = [r_U / (r_U + \sum r_I)] \times 100$$

~~r_U = peak response for Propylene Glycol from the *Sample*~~

~~$\sum r_I$ = sum of individual impurity peak responses, excluding those due to air and water, from the *Sample*~~

~~**Acceptance criteria:** NLT 99.5%~~

Use the gas chromatography normalization procedure.

Diluent: Anhydrous ethanol

Sample solution: To 1 g of Propylene Glycol and 1 g of diethylene glycol, add *Diluent* and dilute with the same solvent to 20 mL. Dilute 1 mL of this solution with *Diluent* to 50 mL.

Chromatographic system

(See *Chromatography* \langle 621 \rangle , *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm \times 30-m fused-silica coated with 0.5- μ m G27 stationary phase

Temperatures

Injector: 250 $^{\circ}$

Detector: 250 $^{\circ}$

Column: 150 $^{\circ}$

Carrier gas: Helium

Flow rate: 1.4 mL/min

Injection volume: 1.0 μ L

Injection type: Split flow ratio, about 70:1

Run time: 2.5 times the retention time of propylene glycol.

[Note—The retention time for propylene glycol is about 2 min. The relative retention time for diethylene glycol is about 1.2.]

System suitability

Sample: *Sample solution*

Suitability requirements

Resolution: NLT 5.0 between propylene glycol and diethylene glycol

Analysis

Sample: *Sample solution*

Calculate the percentage of propylene glycol ($C_3H_8O_2$) in the portion of Propylene Glycol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U

= peak area of propylene glycol from the *Sample solution*

r_T

= sum of all the peak areas except those due to air and water from the *Sample solution*

Acceptance criteria: NLT 99.7% of propylene glycol ($C_3H_8O_2$)

IMPURITIES

- **Residue on Ignition** 〈 281 〉

Sample: 50 g

Analysis: Heat the *Sample* in a tared 100-mL shallow dish until it ignites, and allow it to burn without further application of heat in a place free from drafts. Cool, moisten the residue with 0.5 mL of sulfuric acid, and ignite to constant weight.

Acceptance criteria: NMT 3.5 mg

- **Chloride and Sulfate, Chloride** 〈 221 〉: A 1-mL portion shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (70 ppm).

- **Chloride and Sulfate, Sulfate** 〈 221 〉: A 5.0-mL portion shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid (60 ppm).

Delete the following:

-

- **Heavy Metals** 〈 231 〉

Analysis: Mix 4.0 mL with water to make 25 mL.

Acceptance criteria: NMT 5 ppm (Official 1-Dec-2015)

SPECIFIC TESTS**Add the following:**

- **Color and Clarity of Solution**

Reference solution: Immediately before use, mix 3.0 mL of ferric chloride CS, 3.0 mL of cobaltous chloride CS, and 2.4 mL of cupric sulfate CS with 1.6 mL of hydrochloric acid [10 g/L of hydrochloride (HCl)]. Dilute 1.0 mL of this solution with hydrochloric acid [10 g/L of hydrochloride (HCl)] to 100.0 mL.

Hydrazine sulfate solution: Dissolve 1.0 g of hydrazine sulfate in water and dilute with the same solvent to 100.0 mL. Allow to stand for 4–6 h.

Hexamethylenetetramine solution: In a 100-mL ground-glass-stoppered flask, dissolve 2.5 g of hexamethylenetetramine in 25.0 mL of water.

Primary opalescent suspension (formazin suspension): To the *Hexamethylenetetramine solution* in the flask, add 25.0 mL of the *Hydrazine sulfate solution*. Mix and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Standard of opalescence: Dilute 15.0 mL of the *Primary opalescent suspension* with water to 1000.0 mL. This suspension is freshly prepared and may be stored for up to 24 h.

Reference suspension: To 5.0 mL of *Standard of opalescence* add 95.0 mL of water. Mix and shake before use.

Sample: Propylene Glycol

Analysis: Make the comparison by viewing the solutions downward in matched color-comparison tubes against a white surface (see *Spectrophotometry and Light-Scattering* 〈 851 〉, *Visual Comparison*).

Acceptance criteria: The *Sample* is clear (its clarity is the same as that of water or its opalescence is not more pronounced than that of the *Reference suspension*) and colorless as water or not more intensely colored than the *Reference solution*.

Add the following:

- **Aldehydes**

Formaldehyde methanol solution: A solution containing respectively 37% (m/m) of formaldehyde and 10%–15% (m/m) of methanol in water.¹

Determine the percentage of formaldehyde in the solution before using in the *Standard stock solution*. To 2.0 g of *Formaldehyde methanol solution*, add 100 mL of a freshly prepared 100-g/L solution of sodium sulfite in deionized distilled water. Add 0.1 mL of phenolphthalein TS. Titrate with 0.25 M sulfuric acid until the color changes from pink to colorless. Carry out a blank titration.

Calculate the percentage of formaldehyde in *Formaldehyde methanol solution*:

$$\text{Result} = (V - B) \times 2M \times (3.0/m)$$

V = volume of 0.25 M sulfuric acid used in the sample titration (mL)

B = volume of 0.25 M sulfuric acid used in the blank titration (mL)

M = molarity of the titrant

m = mass of sample (g)

Standard stock solution: To 0.300 g of *Formaldehyde methanol solution* add deionized distilled water and dilute with the same solvent to 100.0 mL. Dilute 10.0 mL

of this solution with deionized distilled water to 1000.0 mL. Dilute 20.0 mL of this solution with deionized distilled water to 500.0 mL.

Standard solutions: In six volumetric flasks add, respectively, 1.0, 3.0, 5.0, 10.0, 15.0, and 25.0 mL of *Standard stock solution*. To each flask, add 2 mL of a freshly prepared 5-g/L solution of methylbenzothiazolone hydrazone hydrochloride adjusted with 0.02 M sodium hydroxide to a pH of 4.0. Allow the solutions to stand for 30 min. Add 5 mL of a freshly prepared 7-g/L solution of ferric chloride. Cap and swirl the flasks. Allow to stand for 5 min. Add methanol to each flask and dilute with the same solvent to 50.0 mL. Mix thoroughly, then allow to stand for 1 min.

Sample solution: Add 1.00 g of Propylene Glycol into a volumetric flask and add about 5 mL of deionized distilled water. To the sample flask, add 2 mL of a freshly prepared 5-g/L solution of methylbenzothiazolone hydrazone hydrochloride adjusted with 0.02 M sodium hydroxide to a pH of 4.0. Allow the solutions to stand for 30 min. Add 5 mL of a freshly prepared 7-g/L solution of ferric chloride. Cap and swirl the flasks. Allow to stand for 5 min. Add methanol to each flask and dilute with the same solvent to 50.0 mL. Mix thoroughly, then allow to stand for 1 min.

Blank solution: Prepare in the same manner as for the *Standard solutions* but omit the *Standard stock solution*.

Analysis: Measure the absorbance of the *Standard solutions* and the *Sample solution* at 655 nm, using *Blank solution* as the blank. Calculate the content of aldehydes expressed as formaldehyde (HCHO) in the substance to be examined from the calibration curve obtained by using the *Standard solutions*.

Acceptance criteria: NMT 20 ppm, expressed as formaldehyde (HCHO)

Add the following:

- **Oxidizing Substances**

Sample: 10 mL of Propylene Glycol

Titrimetric system

(See *Titrimetry* 〈 541 〉.)

Mode: Direct titration

Titrant: 0.05 M sodium thiosulfate

Endpoint detection: Visual

Analysis: To the *Sample* add 5 mL of water, 2 mL of potassium iodide TS, and 2 mL of dilute sulfuric acid and allow to stand in a ground-glass-stoppered flask protected from light for 15 min. Titrate with *Titrant*, using 1 mL of starch TS as an indicator.

Acceptance criteria: NMT 0.2 mL of *Titrant* is required [170 ppm expressed as hydrogen peroxide (H₂O₂)].

- **Specific Gravity** 〈 841 〉: 1.035–1.037

Change to read:

- **Acidity**

Sample: ±0

50.0

mL of Propylene Glycol

Analysis: Add 1 mL of phenolphthalein TS to 50 mL of water, then add 0.1 N

0.01 N

sodium hydroxide until the solution remains pink for 30 s. Add the *Sample*, and titrate with ~~0.10~~
N

0.01 N

sodium hydroxide until the original pink color returns and remains for 30 s.

1 mL of 0.01 N sodium hydroxide is equivalent to 0.6 mg of acetic acid (CH₃COOH).

Acceptance criteria: ~~NMT 0.20 mL of 0.10 N sodium hydroxide~~
NMT 100 mg/L, calculated as acetic acid (CH₃COOH)

Change to read:

- **Water Determination, Method I** 〈 921 〉

Sample: 5.00 g

Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

- **USP Reference Standards** 〈 11 〉

USP Diethylene Glycol RS

USP Ethylene Glycol RS

USP Propylene Glycol RS

¹ Available from Fisher Scientific.

STIMULI TO THE REVISION PROCESS

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Modernization of Identification Tests in *USP-NF*

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ABSTRACT For consistency with the overall USP effort to modernize monographs and general chapters, *Identification Tests—General* 〈 191 〉 was identified for revision to address safety and environmental concerns, clarity and accuracy of the description of wet-chemistry tests, harmonization with other pharmacopeias, and provision of an option to use instrumental methods. This *Stimuli* article accompanies the proposed revision of general chapter 〈 191 〉, also published in this issue of *Pharmacopeial Forum*, and describes the rationale and strategy used to revise the chapter.

INTRODUCTION

Identity is a critical quality attribute of a pharmaceutical, and identification tests are critical for any pharmacopeial monograph. In *USP-NF*, some procedures for identification testing are included in individual monographs, and two general chapters containing identification procedures are referenced frequently in monographs. General chapter *Identification Tests—General* 〈 191 〉 is applied for the identification of ions or counterions of drug salts. Infrared (IR) and ultraviolet (UV) spectrophotometric tests, described in general chapter *Spectrophotometric Identification Tests* 〈 197 〉, are used frequently for identification of active ingredients or their salts.

For consistency with the overall USP effort to modernize monographs and general chapters, chapter 〈 191 〉 was identified for revision to address safety and environmental concerns, clarity and accuracy of the description of wet-chemistry tests, harmonization with other pharmacopeias, and the option to use instrumental methods. Existing flame tests in chapter 〈 191 〉 will be replaced; affected monographs will show other complementary tests (e.g., wet-chemistry tests from other pharmacopeias) or introduction of a specific flame test to a specific monograph. The proposed revision of chapter 〈 191 〉 is included in this issue of *Pharmacopeial Forum*.

The potential impact of changes to 〈 191 〉 is significant. Wet-chemistry tests have been used for many years and may still provide valid options; however, a revised chapter 〈 191 〉 will provide greater flexibility by providing options for wet-chemical and/or instrumental approaches to identification tests. Although qualitative wet-chemistry tests may continue to be used, the instrumental options in the proposed revision of the chapter can provide results that are less subjective, and in many cases, quantitative. If a quantitative test is required for a given ion in a monograph, an additional qualitative identification test is usually not necessary. In cases where multiple identification tests are required in a monograph, different (non-redundant) methods must be used. For example, if an IR identification test with reference to

chapter $\langle 197 \rangle$ is included in a monograph and an additional identification test is included in the monograph with reference to chapter $\langle 191 \rangle$, a test option from chapter $\langle 191 \rangle$ other than IR must be used.

RATIONALE FOR MODERNIZATION—SURVEY RESULTS

In August 2011, USP staff conducted an international survey to obtain industry feedback regarding chapter $\langle 191 \rangle$ and the potential need to modernize the chapter. A total of 392 responses to the online survey were received. The survey was designed to determine industry practices for identification testing and preferences regarding chapter $\langle 191 \rangle$. Questions in the survey addressed demographics, use of chapter $\langle 191 \rangle$, use of alternatives to chapter $\langle 191 \rangle$ tests, level of satisfaction with chapter $\langle 191 \rangle$, and suggestions for modernization of chapter $\langle 191 \rangle$.

The survey revealed that although many of the wet-chemistry tests in chapter $\langle 191 \rangle$ are used (because they are the official tests), almost 65% of the respondents use, or would like to use, instrumental tests to fulfill identification test requirements. Some alternative tests are already being used to quantify counterions, to provide process information, and to meet regulatory requirements. Other survey results indicated a strong interest in harmonizing tests with other pharmacopeias and in clarifying the procedures as well as the acceptance criteria. There has also been interest from the Food and Drug Administration in more rigorous identification tests that could help in the fight against adulteration and counterfeiting. *Table 1* lists the top 10 ions tested by survey respondents. Also included in *Table 1* is the number of monographs referencing chapter $\langle 191 \rangle$ for each ion. Not surprisingly, the listing of the top ions identified by survey respondents overlaps significantly with the most frequently referenced ions in monographs.

Table 1. Top 10 Ions Tested by Survey Respondents and Frequency of Reference to Chapter $\langle 191 \rangle$ in USP Monographs (Second Supplement to USP 36–NF 31)

Ion	Percentage of Survey Respondents Testing for Ion (in rank order)	Number of USP Monographs Referencing Chapter $\langle 191 \rangle$ for Ion Identification (rank)
Chloride	86%	286 (1)
Sodium	75%	195 (2)
Calcium	63%	60 (5)
Sulfate	61%	103 (3)
Potassium	60%	79 (4)
Phosphate	57%	28 (9)
Magnesium	55%	50 (6)
Bicarbonate/Carbonate	52%	16 (12)
Iron/Ferrous salts	51%	<12
Acetate	50%	17 (11)

The top two suggestions for improving chapter $\langle 191 \rangle$ were to include instrumental procedures and to clarify existing procedures (*Figure 1*). One of seven respondents indicated a preference

for not changing the wet-chemistry procedures.

The most frequent survey comments concerning replacement of wet-chemistry procedures are shown in *Figure 2*. The main conclusion from these comments is that wet-chemistry procedures should be maintained, but alternatives should be available.

Suggestions for Improving or Modernizing USP-NF Chapter < 191 >

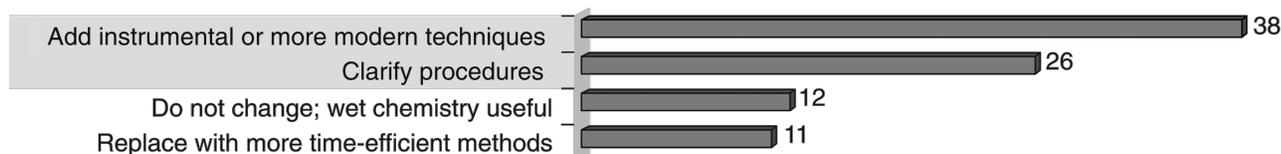
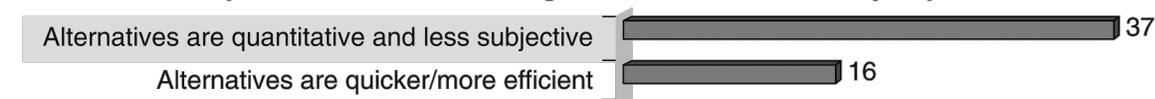


Figure 1. Top suggestions for improving or modernizing general chapter <191>. **Explanations Given for Being in Favor of Wet-Chemistry Replacement**



Explanations Given for Opposing Complete Replacement of Wet-Chemistry Tests

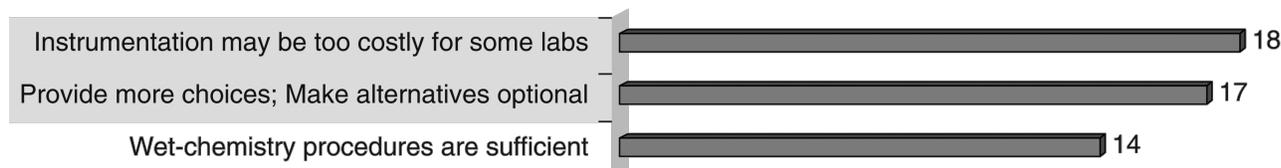


Figure 2. Survey comments concerning replacement of wet-chemistry tests.

Survey respondents also indicated which methods they use for testing various ions. *Table 2* shows that although wet-chemistry is used widely (probably because it is currently required in < 191 >), additional instrumental tests are used for many ions.

Table 2. Identification Testing Methods Used for Top Ions^a

Ion	Wet Chemistry (WC)	Atomic Absorption (AA)	Spectrophotometric Methods	Ion Chromatography (IC)	Inductively Coupled Plasma (ICP)	Other Method
	(Percentage of only those who test the ion)					
Chloride	93%	3%	4%	14%	2%	2%
Sodium	84%	26%	4%	7%	10%	4%
Calcium	79%	28%	5%	5%	12%	1%
Sulfate	91%	1%	7%	10%	2%	0%
Potassium	80%	29%	3%	7%	7%	2%
Phosphate	91%	4%	9%	9%	4%	1%
Magnesium	76%	28%	2%	5%	16%	1%
Bicarbonate/Carbonate	90%	2%	7%	8%	1%	2%
Iron/Ferrous salts	77%	26%	7%	4%	16%	1%
Acetate	89%	1%	12%	12%	1%	2%
Citrate	88%	0%	9%	9%	2%	3%
Nitrate	95%	2%	6%	9%	2%	1%
Ammonium	92%	1%	5%	11%	2%	1%
Zinc	66%	35%	3%	7%	19%	0%

^a Source: 2011 USP Identification Testing Methods Survey.

Ion	Wet Chemistry (WC)	Atomic Absorption (AA)	Spectrophotometric Methods	Ion Chromatography (IC)	Inductively Coupled Plasma (ICP)	Other Method
	(Percentage of only those who test the ion)					
Bromide	85%	0%	3%	13%	1%	5%
Aluminum	66%	33%	6%	1%	25%	1%
Iodide	91%	0%	3%	13%	1%	3%
Copper	59%	30%	3%	5%	26%	1%

^a Source: 2011 USP Identification Testing Methods Survey.

PROCESS FOR ADDRESSING COMMENTS ARISING FROM THE SURVEY

An Expert Panel was formed to address issues with wet-chemistry tests in chapter < 191 > and to consider including the option of instrumental identification tests in the chapter. The panel's goals included minimizing ambiguity in procedures and subjectivity in the interpretation of results. The panel sought to provide flexibility in the chapter by allowing the use of instrumental tests that have been validated appropriately by the user.

Wet-Chemistry Tests

Safety and environmental issues have been associated with some existing tests. Flame tests and toxic reagents pose hazards, and their use is discouraged or prohibited in many laboratories. Another concern is country-specific environmental regulations, which may also preclude the use of some reagents prescribed in chapter < 191 >. The Expert Panel evaluated and revised several tests that involve safety concerns. For example, the proposed revision of the chapter will eliminate all flame tests, which will remove a safety concern as well as the potential for ambiguous results with flame tests.

Additionally, the Expert Panel focused on some of the more frequently used or referenced tests: aluminum, calcium, magnesium, potassium, sodium, zinc, halides (chloride, bromide, iodide), sulfate, acetate, carbonate, citrate, hypophosphite, phosphate, and tartrate. For these tests, the panel attempted to harmonize with the procedures of the *European Pharmacopoeia*, clarify procedures, and clarify interpretation of the results. The potential impact of changes to the procedures was also evaluated. USP laboratory staff worked to assess the validity of the revised procedures as well as the clarity of instructions and consistency of results.

The laboratory evaluation of revised identification testing methods was performed in USP laboratories by executing the test procedure and documenting the results and observations (e.g., photographing the solution/precipitate). The samples utilized were three USP Reference Standards along with a blank, each evaluated by two scientists. The preparation of reagents was performed independently. There were some sample preparation issues, and adjustments were made to the techniques, as appropriate, by the scientists overseeing the project. Despite the adjustments, the results showed that the revised procedures still produced subjective results. Thus, many of the revisions initially considered will not be made.

Instrumental Techniques

On the basis of survey feedback and procedures currently used in various monographs, a number of instrumental techniques were identified as having potential for inclusion in chapter <

191). In addition to providing qualitative identification information regarding various ions, many instrumental tests also provide quantitative information. A number of instrumental techniques are useful for identification of the drug substance moiety or salt, but the Expert Panel focused on techniques used for counterion identification. Counterions can often be identified using several techniques, and the proposed revision to chapter 191 allows the analyst to choose the technique. This novel concept is consistent with the survey results, which indicated a desire for options beyond the wet-chemistry tests. The inclusion of instrumental techniques provides a variety of alternatives to help ensure compliance. As described in the revised chapter, any alternative technique must be validated by the user for counterion specificity to be suitable for counterion identification.

In considering instrumental techniques, the Expert Panel included X-ray fluorescence (XRF), atomic spectroscopy [atomic absorption (AA); inductively coupled plasma (ICP)–optical emission spectroscopy (OES); ICP–mass spectrometry (MS)], mid-IR, Raman, ion chromatography (IC), and other chromatographic techniques. A list of these techniques and the ions to which they might be applied is given in *Table 3*. This listing is not exhaustive, and other techniques, such as nuclear magnetic resonance, ion-selective electrodes, and near-IR, may be used, provided they meet validation requirements for specificity (for additional information, see the general chapter *Validation of Compendial Procedures* 1225).

Table 3. Alternative Instrumental Techniques for Counterion Identification

Ion	Atomic Absorption	Mid IR	Raman Spectroscopy	Ion Chromatography	Inductively Coupled Plasma	X-Ray Fluorescence
Chloride				•	•(MS)	•
Bromide				•	•(MS)	•
Iodide				•	•(MS)	•
Acetate		•	•	•		
Bi/Carbonate		•	•	•		
Citrate		•	•	•		
Phosphate		•	•	•		
Sulfate		•	•	•		
Tartrate		•	•	•		
Aluminum	•			•	•(OES/MS)	•
Calcium	•			•	•(OES/MS)	•
Magnesium	•			•	•(OES/MS)	•
Potassium	•			•	•(OES/MS)	•
Sodium	•			•	•(OES/MS)	
Zinc	•			•	•(OES/MS)	•

Although other general chapters address some of the above-mentioned instrumental techniques, they do not specifically address the use of those techniques for identification.

Chapter 197 does describe the use of UV and IR for identification purposes, but this chapter is primarily used for identification of the drug moiety or salt as a whole, rather than for counterions. In chapter 191, the use of IR will reference procedures in chapter 197, but demonstration of the counterion specificity will still be needed. USP plans to develop a general chapter on Raman spectroscopy and to include Raman as an identification test in chapter 197. Validation of specificity for the counterion of interest is also required when using Raman to satisfy the chapter 191 identification requirements.

IMPLEMENTATION

Removal of the flame tests from chapter 〈 191 〉 was done in coordination with the affected monographs to provide a replacement test that would complement the set of tests in each monograph. In some cases, a new test was proposed for the monograph. If the flame test was considered suitable, it was incorporated into the specific monograph.

This article describes the first step in achieving modernization of the identification tests in *USP-NF*. USP will continue exploring different approaches to address the safety and environmental concerns, to improve the clarity and accuracy of the description of wet-chemistry tests, and to work toward harmonizing with other pharmacopeias.

Although not directly related to chapter 〈 191 〉 revisions, another initiative of the USP monograph development committees is the removal or omission of counterion identification tests for drug product monographs. In many cases, the potential for interference by excipients in different formulations of a product prevents identification of a counterion because of the drug substance salt. These monographs will be handled on a case-by-case basis.

The Expert Panel is hopeful that USP will receive feedback regarding the removal of the flame tests and the addition of instrumental approaches for identification testing. In particular, any problems encountered when testing with existing monographs using the revised chapter 〈 191 〉 should be referred to the liaison for that monograph.

^a Chairperson of the Modernization of Identification Tests Expert Panel.

^b Member of the Modernization of Identification Tests Expert Panel.

^c Senior Scientific Liaison, USP.

^d Correspondence should be addressed to: Antonio Hernandez-Cardoso, MSc, Senior Scientific Liaison, US Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel +1.301.816.8308; e-mail ahc@usp.org.

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The United States Pharmacopeial Convention comprises representatives from colleges and national and state organizations of medicine and pharmacy. It publishes the U.S. Pharmacopeia and National Formulary, the legally recognized compendia of standards for drugs and products of other health care technologies. The USP and NF include assays and tests for the determination of strength, quality, and purity and requirements for packaging and labeling. **Pharmacopeial Forum's Public Review and Comment Process**

This section presents an overview of the public review and comment process, conducted through *Pharmacopeial Forum (PF)*, for the development of official standards in the *United States Pharmacopeia* and the *National Formulary (USP–NF)*.

USP publishes *PF* on a bimonthly basis to provide an opportunity to review and comment on new or revised standards.

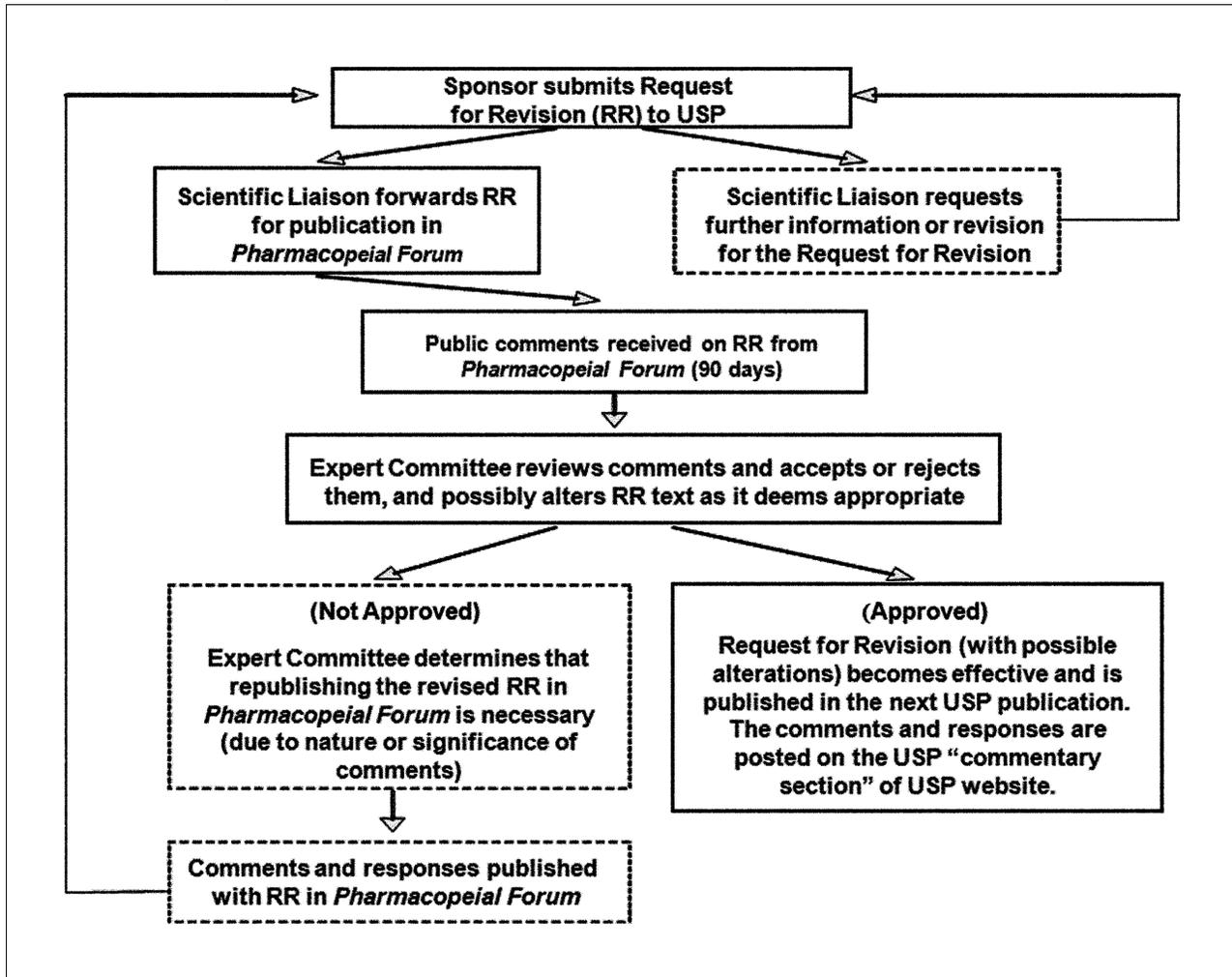
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USP welcomes comments and data on proposed revisions. A summary of comments received, along with USP’s responses, will be published in the *Revisions and Commentary* section of the USP website (<http://www.usp.org/USPNF/revisions/>).

The chart below shows the public review and comment process and its relationship to standards development.



Questions on the process should be addressed to the USP Executive Secretariat, United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852 (e-mail: execsec@usp.org).

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The various sections of *PF* are briefly described below. A more detailed description of each section is provided at the beginning of that section. A general description of the types and amount of information expected in a *Request for Revision* is available in the *Guideline for*

Submitting Requests for Revision to the USP–NF on the USP Web site (www.usp.org/USPNF/submitMonograph/subGuide.html). Note that the Expert Committee listing and the Scientific Staff Directory also are located on the USP Web site (see below for links).

Proposed and Adopted Revisions to the USP–NF

Section	Content	How Readers Can Respond
Proposed Interim Revision Announcements	Proposals for <i>Interim Revision Announcements (IRAs)</i> that will be published as official <i>USP</i> or <i>NF</i> standards BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Expert Committee and Scientific Liaisons who handled the monograph or general chapter.	Review material and send comments within 90 days of the <i>PF</i> publication in which the standard was proposed (or per the comment deadline listed in the Briefing section). Direct comments to the USP Scientific Liaison using the contact information provided at the end of each <i>Proposed IRA</i>
In-Process Revision	Proposals for standards that will be published as official in a future <i>USP–NF</i> book or <i>Supplement</i> . BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Expert Committee and USP Scientific Liaisons who handle the monograph or general chapter.	Review material and send comments within 90 days of the <i>PF</i> publication in which the revision was proposed (or per the comment deadline listed in the Briefing section). Direct comments to the USP Scientific Liaison using the contact information provided at the end of each proposed revision. For general inquiries or in cases where a Scientific Liaison is not identified, use the general USP telephone number 301-881-0666 or fax number 301-998-6839 or stdsmonographs@usp.org .

Section	Content	How Readers Can Respond
Stage 4 Harmonization	Revision proposals from the Pharmacopoeial Discussion Group (PDG), which comprises the European Pharmacopoeia, the Japanese Pharmacopoeia, and USP. The Stage 4 draft and the briefing are published in the forum of each pharmacopoeia for public comment. The draft is published in its entirety. BRIEFING: Scientific rationale for the potential inclusion or change or for the proposed change.	Review material and provide comments to the USP Scientific Liaison using the contact information provided at the end of each Stage 4 Harmonization. Individuals who wish to correspond with the European and Japanese Pharmacopoeias concerning monographs in the Stage 4 period of international harmonization should address their comments to the coordinating pharmacopoeia, with a copy to USP. PhEur Secretariat Ms. Lynn Kelso-Eleuterio Central Secretariat European Pharmacopoeia Department European Directorate for the Health Care Council of Europe 7, Allée Kastner CS 30026 67081 Strasbourg France Tel: +33 (3) 88 41 31 48 Fax: +33 (3) 88 41 27 71 lynn.kelso@edqm.eu JP Secretariat Mr. Issei Takayama Technical Officer Pharmaceuticals and Medical Devices Agency (PMDA) Shin-kasumigaseki Building 3-3-2, Kasumigaseki, Chiyoda-ku Tokyo, 100-0013 Japan Phone: +81-3-3506-9431 Fax: +81-3-3506-9440 takayama-issei@pmda.go.jp
Stimuli to the Revision Process	Articles on standards development topics authored by the USP Council of Experts, USP staff, or other interested parties on which USP desires public input prior to further development.	Review material and provide comments to the recipient indicated (usually footnoted in each <i>Stimuli</i> article).

Other Sections

Expert Committees

A listing of the 2010–2015 Expert Committees that work on the development of USP compendial standards

(<http://www.usp.org/aboutUSP/governance/councilOfExperts/expertCommittees.html>)

Staff Directory

Names and contact information of key USP scientific staff members who work on the development of USP compendial standards

(<http://www.usp.org/USPNF/devProcess/contactScientists.html>)

Proposed Interim Revision Announcements

This section includes proposals for *Interim Revision Announcements (IRAs)* that will be published as official *USP* or *NF* standards. There is a 90-day comment period for these proposals (see <http://www.usp.org/USPNF/pf/pfRedesign.html> for the *PF* comment schedule). Readers should review material in this section and provide comments to the Scientific Liaison using the contact information appearing at the end of each proposed *IRA*. Information on how to comment can be found under the "Participation" section of www.usp.org. It is important to send comments promptly so that the Expert Committee members can consider reader's input as they are deciding whether to advance standards to official status. The approved official text will be published under *IRAs* in the "New Official Text" section of USP's Web site.

Each proposal is preceded by a Briefing that indicates the proposed revisions.

Symbols—New text is enclosed in symbols and set off from the current official text as shown in the following example:

•new text•

Where the symbols appear together with no enclosed text, such as

••

, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the date the proposal, if approved, will become official as an *IRA*. For example, •(IRA 1-Apr-2011)

BRIEFING

《 87 》 **Biological Reactivity Tests, In Vitro**, *USP 38* page 156. On the basis of comments received, it is proposed to make the following revisions. USP Positive Bioreaction RS, which is used as a *Positive Control* in the current chapter, is being discontinued due to the inability to acquire the material needed for the replacement lot. In the effort to find a replacement, a polyurethane film containing zinc diethyldithiocarbamate (ZDEC) and zinc dibutyldithiocarbamate (ZDBC) from Hatano Research Institute was identified and determined to be suitable as a *Positive Control*. Thus, the chapter is being revised to remove the reference to USP Positive Bioreaction RS and will include a reference for an alternate *Positive Control* that is suitable.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

The comment period for this revision ends May 31, 2015. In the absence of any significant adverse comments, it is proposed to implement this revision via an *Interim Revision Announcement*, with an official date of September 1, 2015.

(GCPS: D. Hunt.)

Correspondence Number—C154278

Comment deadline: July 31, 2015

《 87 》 **BIOLOGICAL REACTIVITY TESTS, IN VITRO**

The following tests are designed to determine the biological reactivity of mammalian cell cultures following contact with the elastomeric plastics and other polymeric materials with

direct or indirect patient contact or of specific extracts prepared from the materials under test. It is essential that the tests be performed on the specified surface area. When the surface area of the specimen cannot be determined, use 0.1 g of elastomer or 0.2 g of plastic or other material for every mL of extraction fluid. Exercise care in the preparation of the materials to prevent contamination with microorganisms and other foreign matter.

Three tests are described (i.e., the *Agar Diffusion Test*, the *Direct Contact Test*, and the *Elution Test*).¹ The decision as to which type of test or the number of tests to be performed to assess the potential biological response of a specific sample or extract depends upon the material, the final product, and its intended use. Other factors that may also affect the suitability of sample for a specific use are the polymeric composition; processing and cleaning procedures; contacting media; inks; adhesives; absorption, adsorption, and permeability of preservatives; and conditions of storage. Evaluation of such factors should be made by appropriate additional specific tests before determining that a product made from a specific material is suitable for its intended use. Materials that fail the *in vitro* tests are candidates for the *in vivo* tests described in *Biological Reactivity Tests* (88), *In Vivo*.

Change to read:

•

TEST CONTROL

Positive Control

Polyurethane film containing zinc diethyldithiocarbamate (ZDEC)² or zinc dibutyldithiocarbamate (ZDBC) •(IRA 1-Sep-2015)

Cell Culture Preparation

Prepare multiple cultures of L-929 (ATCC cell line CCL 1, NCTC clone 929; alternative cell lines obtained from a standard repository may be used with suitable validation) mammalian fibroblast cells in serum-supplemented minimum essential medium having a seeding density of about 10^5 cells per mL. Incubate the cultures at $37 \pm 1^\circ$ in a humidified incubator for NLT 24 h in a $5 \pm 1\%$ carbon dioxide atmosphere until a monolayer, with greater than 80% confluence, is obtained. Examine the prepared cultures under a microscope to ensure uniform, near-confluent monolayers. [Note—The reproducibility of the *In Vitro Biological Reactivity Tests* depends upon obtaining uniform cell culture density.]

Extraction Solvents

Sodium Chloride Injection [see monograph—use Sodium Chloride Injection containing 0.9% of sodium chloride (NaCl)]. Alternatively, serum-free mammalian cell culture media or serum-supplemented mammalian cell culture media may be used. Serum supplementation is used when extraction is done at 37° for 24 h.

APPARATUS

Autoclave

Employ an autoclave capable of maintaining a temperature of $121 \pm 2^\circ$, equipped with a thermometer, a pressure gauge, a vent cock, a rack adequate to accommodate the test containers above the water level, and a water cooling system that will allow for cooling of the

test containers to about 20° , but not below 20° , immediately following the heating cycle.

Oven

Use an oven, preferably a mechanical convection model, that will maintain operating temperatures in the range of 50° – 70° within $\pm 2^{\circ}$.

Incubator

Use an incubator capable of maintaining a temperature of $37 \pm 1^{\circ}$ and a humidified atmosphere of $5 \pm 1\%$ carbon dioxide in air.

Extraction Containers

Use only containers, such as ampuls or screw-cap culture test tubes, or their equivalent, of Type I glass. If used, culture test tubes, or their equivalent, are closed with a screw cap having a suitable elastomeric liner. The exposed surface of the elastomeric liner is completely protected with an inert solid disk 50–75 μm in thickness. A suitable disk can be fabricated from polytef.

Preparation of Apparatus

Cleanse all glassware thoroughly with chromic acid cleansing mixture and, if necessary, with hot nitric acid followed by prolonged rinsing with Sterile Water for Injection. Sterilize and dry by a suitable process for containers and devices used for extraction, transfer, or administration of test material. If ethylene oxide is used as the sterilizing agent, allow NLT 48 h for complete degassing.

PROCEDURE

Preparation of Sample for Extracts

Prepare as directed in the *Procedure* under $\langle 88 \rangle$.

Preparation of Extracts

Prepare as directed for *Preparation of Extracts* in $\langle 88 \rangle$ using either Sodium Chloride Injection [0.9% sodium chloride (NaCl)] or serum-free mammalian cell culture media as *Extraction Solvents*. [Note—If extraction is done at 37° for 24 h in an incubator, use cell culture media supplemented by serum. The extraction conditions should not in any instance cause physical changes, such as fusion or melting of the material pieces, other than a slight adherence.]

AGAR DIFFUSION TEST

This test is designed for elastomeric closures in a variety of shapes. The agar layer acts as a cushion to protect the cells from mechanical damage while allowing the diffusion of leachable chemicals from the polymeric specimens. Extracts of materials that are to be tested are applied to a piece of filter paper.

Sample Preparation

Use extracts prepared as directed, or use portions of the test specimens having flat surfaces

NLT 100 mm² in surface area.

Positive Control Preparation

Proceed as directed for *Sample Preparation*.

Negative Control Preparation

Proceed as directed for *Sample Preparation*.

Procedure

Using 7 mL of cell suspension prepared as directed under *Cell Culture Preparation*, prepare the monolayers in plates having a 60-mm diameter. Following incubation, aspirate the culture medium from the monolayers, and replace it with serum-supplemented culture medium containing NMT 2% of agar. [Note—The quality of the agar must be adequate to support cell growth. The agar layer must be thin enough to permit diffusion of leached chemicals.] Place the flat surfaces of *Sample Preparation*, *Negative Control Preparation*, and *Positive Control Preparation* or their extracts in an appropriate extracting medium, in duplicate cultures in contact with the solidified agar surface. Use no more than three specimens per prepared plate. Incubate all cultures for NLT 24 h at $37 \pm 1^{\circ}$, preferably in a humidified incubator containing $5 \pm 1\%$ of carbon dioxide. Examine each culture around each *Sample*, *Negative Control*, and *Positive Control*, under a microscope, using a suitable stain, if desired.

Interpretation of Results

The biological reactivity (cellular degeneration and malformation) is described and rated on a scale of 0–4 (see *Table 1*). Measure the responses of the cell cultures to the *Sample Preparation*, the *Negative Control Preparation*, and the *Positive Control Preparation*. The cell culture test system is suitable if the observed responses to the *Negative Control Preparation* is grade 0 (no reactivity) and to the *Positive Control Preparation* is at least grade 3 (moderate). The *Sample* meets the requirements of the test if the response to the *Sample Preparation* is not greater than grade 2 (mildly reactive). Repeat the procedure if the suitability of the system is not confirmed.

Table 1. Reactivity Grades for Agar Diffusion Test and Direct Contact Test

Grade	Reactivity	Description of Reactivity Zone
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen and less than 0.45 cm beyond specimen
3	Moderate	Zone extends 0.45–1.0 cm beyond specimen
4	Severe	Zone extends greater than 1.0 cm beyond specimen

DIRECT CONTACT TEST

This test is designed for materials in a variety of shapes. The procedure allows for simultaneous extraction and testing of leachable chemicals from the specimen with a serum-supplemented medium. The procedure is not appropriate for very low- or high-density materials that could cause mechanical damage to the cells.

Sample Preparation

Use portions of the test specimen having flat surfaces NLT 100 mm² in surface area.

Positive Control Preparation

Proceed as directed for *Sample Preparation*.

Negative Control Preparation

Proceed as directed for *Sample Preparation*.

Procedure

Using 2 mL of cell suspension prepared as directed under *Cell Culture Preparation*, prepare the monolayers in plates having a 35-mm diameter. Following incubation, aspirate the culture medium from the cultures, and replace it with 0.8 mL of fresh culture medium. Place a single *Sample Preparation*, a *Negative Control Preparation*, and a *Positive Control Preparation* in each of duplicate cultures. Incubate all cultures for NLT 24 h at $37 \pm 1^{\circ}$ in a humidified incubator containing $5 \pm 1\%$ of carbon dioxide. Examine each culture around each *Sample*, *Negative Control*, and *Positive Control Preparation*, under a microscope, using a suitable stain, if desired.

Interpretation of Results

Proceed as directed for *Interpretation of Results* under *Agar Diffusion Test*. The *Sample* meets the requirements of the test if the response to the *Sample Preparation* is not greater than grade 2 (mildly reactive). Repeat the procedure if the suitability of the system is not confirmed.

ELUTION TEST

This test is designed for the evaluation of extracts of polymeric materials. The procedure allows for extraction of the specimens at physiological or nonphysiological temperatures for varying time intervals. It is appropriate for high-density materials and for dose-response evaluations.

Sample Preparation

Prepare as directed in *Preparation of Extracts*, using either Sodium Chloride Injection [0.9% sodium chloride (NaCl)] or serum-free mammalian cell culture media as *Extraction Solvents*. If the size of the *Sample* cannot be readily measured, a mass of NLT 0.1 g of elastomeric material or 0.2 g of plastic or polymeric material per mL of extraction medium may be used. Alternatively, use serum-supplemented mammalian cell culture media as the extracting medium to simulate more closely physiological conditions. Prepare the extracts by heating for 24 h in an incubator containing $5 \pm 1\%$ of carbon dioxide. Maintain the extraction temperature at $37 \pm 1^{\circ}$, because higher temperatures may cause denaturation of serum proteins.

Positive Control Preparation

Proceed as directed for *Sample Preparation*.

Negative Control Preparation

Proceed as directed for *Sample Preparation*.

Procedure

Using 2 mL of cell suspension prepared as directed under *Cell Culture Preparation*, prepare the

monolayers in plates having a 35-mm diameter. Following incubation, aspirate the culture medium from the monolayers, and replace it with extracts of the *Sample Preparation*, *Negative Control Preparation*, or *Positive Control Preparation*. The serum-supplemented and serum-free cell culture media extracts are tested in duplicate without dilution (100%). The Sodium Chloride Injection extract is diluted with serum-supplemented cell culture medium and tested in duplicate at 25% extract concentration. Incubate all cultures for 48 h at $37 \pm 1^\circ$ in a humidified incubator preferably containing $5 \pm 1\%$ of carbon dioxide. Examine each culture at 48 h, under a microscope, using a suitable stain, if desired.

Interpretation of Results

Proceed as directed for *Interpretation of Results* under *Agar Diffusion Test* but use *Table 2*. The *Sample* meets the requirements of the test if the response to the *Sample Preparation* is not greater than grade 2 (mildly reactive). Repeat the procedure if the suitability of the system is not confirmed. For dose-response evaluations, repeat the procedure, using quantitative dilutions of the sample extract.

Table 2. Reactivity Grades for Elution Test

Grade	Reactivity	Conditions of All Cultures
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Less than or equal to 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Greater than 20% to less than or equal to 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	Moderate	Greater than 50% to less than 70% of the cell layers contain rounded cells or are lysed
4	Severe	Nearly complete destruction of the cell layers

Change to read:

USP Reference Standards (11)—USP High-Density Polyethylene RS (Negative Control). ~~USP Positive Bioreaction RS.~~

••(IRA 1-Sep-2015)

¹ Further details are given in the following publications of the American Society for Testing and Materials, 1916 Race St., Philadelphia, PA 19103: "Standard Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity," ASTM Designation F 895-84; "Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices," ASTM Designation F 813-83.

•²

ZDECand ZDBC polyurethanes are available from the Food and Drug Safety Center, Hatano Research Institute, Ochiai 729-5, Hadanoshi, Kanagawa 257, Japan. •(IRA 1-Sep-2015)

BRIEFING

Powdered Digitalis, *USP 38* page 3111. The following revisions are proposed:

1. Because of the discontinuation of USP Gitoxin RS, it is proposed to replace *Identification* test *B* by thin-layer chromatography with an HPLC procedure.

2. *Identification* test A is revised to remove the subsections for *Unground Digitalis* and *Histology* and to update the *Botanic Characteristics* for *Ground Digitalis*.
3. The *USP Reference Standards* section is revised to remove USP Digitoxin RS and USP Gitoxin RS and to add USP Digoxin RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

The comment period for this revision ends on July 31, 2015. In the absence of significant comments, it is proposed to implement this revision through an *Interim Revision Announcement*, with an official date of November 1, 2015.

(DSB: N. Kim.)

Correspondence Number—C152051

Comment deadline: July 31, 2015

Powdered Digitalis

DEFINITION

Powdered Digitalis is *Digitalis* (*Digitalis purpurea*) dried at a temperature not exceeding 60°, reduced to a fine or a very fine powder, and adjusted, if necessary, to conform to the official potency by admixture with sufficient Lactose, Starch, or exhausted marc of digitalis, or with Powdered Digitalis having either a lower or a higher potency.

The potency of Powdered Digitalis is such that, when assayed as directed, 100 mg is equivalent to 1 USP Digitalis Unit.¹

[Note—When Digitalis is prescribed, Powdered Digitalis is to be dispensed.]

IDENTIFICATION

Change to read:

- **A. Botanic Characteristics**

Unground Digitalis: ~~This occurs as more or less crumpled or broken leaves. The leaf blades are ovate, oblong-ovate to ovate-lanceolate, mostly 10–35 cm in length and 4–11 cm in width and contracted into a winged petiole. The apex is obtuse; the margin irregularly crenate or serrate; the lower surface densely pubescent, the upper surface wrinkled and finely hairy. The venation is conspicuously reticulate, the mid-rib and principal veins broad and flat, and the lower veins are continued into the wings of the petiole. The color of the upper surface is dark green, of the lower surface grayish from the dense pubescence, the larger veins often purplish. The odor is slight when dry, peculiar and characteristic when moistened.~~

Histology: ~~Digitalis shows an upper epidermis whose cells possess slightly wavy anticlinal walls, numerous hairs, and no stomata; a lower epidermis with wavy anticlinal walls, numerous oval stomata, and many hairs, and frequently not attached over irregular areas to the cell layer within, especially near the veins; a broad chlorenchyma of a single layer of short palisade cells and several layers of spongy parenchyma; and numerous vascular bundles in the larger veins and petioles, separated by vascular rays one cell in width. On the apex of each marginal tooth, one or two water stomata occur.~~

●●(IRA 1-Nov-2015)

Ground Digitalis: This is dark green in color.

•The powder is dark green in color. •(IRA 1-Nov-2015)

Present are chiefly numerous irregular fragments of epidermis and chlorenchyma; nonglandular hairs that are frequently curved or crooked, up to 500 μm in length, uniseriate, two- to eight-celled, some of the cells collapsed so that the planes of adjoining cells may be at right angles, the terminal cell pointed or rounded; few, small glandular hairs, usually with a one- or two-celled stalk, and a one- or two-celled head; fragments of veins and petioles with annular, reticulate, spiral and simple pitted vessels and tracheids. Calcium oxalate is absent.

Delete the following:

•• **B. Procedure**

(See *Chromatography* (621).)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Sample solution: Transfer 100 mg to a 15-mL centrifuge tube containing 2.0 mL of diluted alcohol and 1.0 mL of lead acetate TS, shake, and boil for 2 min. Centrifuge, decant the supernatant into a second 15-mL centrifuge tube, and add 2.0 mL of chloroform. Centrifuge, then remove the lower layer, and filter it through a chloroform-washed small column of anhydrous sodium sulfate (100 to 300 mg) into a 5-mL centrifuge tube. Evaporate the chloroform solution under a stream of nitrogen to dryness, and dissolve the residue in 100 μL of a mixture of methanol and chloroform (1:1).

Standard solutions: Prepare a *Standard solution* in the same manner, using 100 mg of USP Digitalis RS (*Standard solution A*). Prepare a second *Standard solution* by dissolving USP Digitoxin RS and USP Gitoxin RS in a mixture of methanol and chloroform (1:1) such that the final concentration of each is approximately 0.2 mg/mL (*Standard solution B*).

Application volume: 10 μL

Developing solvent system: Ethyl acetate, methanol, and water (30:4:3)

Analysis

Samples: Apply the *Sample solution*, *Standard solution A*, and *Standard solution B*, each as a narrow band about 15 mm long, to a thin-layer chromatographic plate.

Allow the bands to dry. Develop the chromatogram in a saturated chamber, using a solvent system, until the solvent front has moved about 15 cm from the origin. Mix 10 mL of chloramine T solution (3 in 100) with 40 mL of a solution (1 in 4) of trichloroacetic acid in alcohol (store the mixture in a cool place, and use it within 1 week), and spray the air-dried chromatographic plate with this mixture. Heat the plate at 110° for 15–20 min, and examine it under long-wavelength UV light. Locate the 2 prominent bands from *Standard solution A* corresponding in R_f value to the 2 bands obtained from *Standard solution B*. The chromatogram obtained from the solution under test shows bands corresponding to them, and also shows bands corresponding to the 3 other bands most prominent in the chromatogram from *Standard solution A* but of lower R_f value.

Acceptance criteria: Relative R_f values for the 5 bands are: 1.0 (digitoxin); 0.8 to 0.9 (gitoxin); 0.6 to 0.7; 0.4 to 0.5; and 0.3 to 0.4. •(IRA 1-Nov-2015)

Add the following:

•• **B.**

Solution A: Water

Solution B: Acetonitrile

Mobile phase: (See *Table 1.*)

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
35	70	30
45	60	40
48	60	40
48.1	80	20
50	80	20

Diluent: Water and acetonitrile (80:20)

Standard solution A: 0.1 mg/mL of USP Digoxin RS in *Diluent*

Standard solution B: Extract 0.5 g of USP Digitalis RS, accurately weighed, with 10 mL of 70% aqueous methanol by sonication at room temp for 15 min. Centrifuge at 4000 rpm for 3 min, and pass through a membrane filter of 0.45- μ m pore size.

Sample solution: Prepare a sample as directed in *Standard solution B.*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability.*)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 7.5-cm; 3.5- μ m packing L1

Column temperature: 20 $^{\circ}$

Flow rate: 1.0 mL/min

Injection volume: 10 μ L

Analysis

Samples: *Standard solution A, Standard solution B, and Sample solution*

Using the chromatograms of *Standard solution A* and *Standard solution B* and *Table 2*, identify the retention times of the peaks corresponding to deacetyllanatoside C, digoxigenin-bis-digitoxoside, gitoxigenin, digitoxigenin, α -acetyldigoxin, β -acetyldigoxin, gitoxin, lanatoside A, and digitoxinin the *Sample solution*.

Table 2

Name	Relative Retention Time
Deacetyllanatoside C	0.57
Digoxigenin-bis-digitoxoside	0.59
Gitoxigenin	0.69
Digoxin (standard)	1.00
Digitoxigenin	1.48
α -Acetyldigoxin	1.70
β -Acetyldigoxin	1.95

Name	Relative Retention Time
Gitoxin	2.12
Lanatoside A	2.57
Digitoxin	2.68

*(IRA 1-Nov-2015)

ASSAY

Change to read:

● Procedure

Standard solution: Weigh the contents of 1 container of USP Digitalis RS to the nearest mg, either in the original container or in a weighing bottle, and transfer to a dry, hard-glass, glass-stoppered container or centrifuge tube of at least 50-mL capacity. Complete the weighing within 5 min after opening the ampul. Add a menstruum consisting of 4 volumes of alcohol and 1 volume of water so that the total volume of menstruum added corresponds to 10 mL for each g of powder. Insert the stopper, the upper third of which is greased lightly with petrolatum. Shake the mixture for 24 ± 2 h at $25 \pm 5^\circ$ by mechanical means, which continuously brings the solid material into fresh contact with the liquid phase. Immediately thereafter transfer, if necessary, to a centrifuge tube, centrifuge, and decant the supernatant tincture into a dry, hard-glass bottle having a tight closure. Preserve under refrigeration, and use within 30 days.

Sample solution: Transfer 5 g of Digitalis, reduced to a fine powder, to a hard-glass, glass-stoppered container or centrifuge tube of at least 50-mL capacity. Proceed as directed under *Standard solution*, beginning with "Add a menstruum". Preserve under refrigeration, and use within 30 days.

Pigeons: Use adult pigeons free from gross evidence of disease or emaciation, and of such weight that the heaviest weighs less than twice the weight of the lightest. Divide the pigeons into groups as nearly alike as practicable with respect to breed and weight so that the average weight of the group assigned by random choice to the *Standard solution* shall not differ by more than 30% from the average weight of the group assigned to the preparation to be assayed. Withhold food but not water for 16–28 h before use. Preparatory to injection, lightly anesthetize the pigeon with ether, and immobilize it; expose an alar vein, and cannulate with a suitable cannula. Maintain the anesthesia during cannulation and throughout the subsequent injection period at such a level that pain is absent, the pupillary and corneal reflexes are present, and the voluntary musculature is not relaxed beyond permitting the pigeon to make some voluntary movement occasionally.

Preparation of sample dilutions: On the day of the *Assay*, dilute portions of the *Standard solution* and of the preparation to be assayed (*Sample solution*) with isotonic sodium chloride solution in such a way that the estimated fatal dose of each dilution will be 15 mL/kg of body weight.

Injection of sample dilutions: Arrange to inject the appropriate sample dilution by suitable means such as a small-bore buret calibrated to 0.05 mL. Start the injection after ensuring the absence of air bubbles from the injection apparatus, by infusing, within a few seconds, a volume of the test dilution equivalent to 1 mL/kg of body weight. Repeat this dose at 5-min intervals thereafter until the pigeon dies of cardiac arrest. Use a total of

NLT 6 pigeons for the *Standard solution* and NLT 6 pigeons for the preparation to be assayed. If the average number of doses for any given dilution required to produce death is less than 13 or greater than 19, or if the larger exceeds the smaller in the same assay by more than 4 doses, regard these data as preliminary. Use them as a guide, and repeat with a fresh, higher or lower dilution. Complete the assay within 30 days for preservation of the *Standard solution* and *Sample solution*.

Calculation of potency: Tabulate and average the number of doses of the *Standard solution*, designating the average \bar{z}_S , and likewise obtain the corresponding average, \bar{z}_U , for the *Sample solution*. Compute the potency in USP Digitalis Units/mL (i.e., per 100 mg) of the *Sample solution* as:

$$\text{Potency} = \bar{z}_S R / \bar{z}_U$$

$$\text{Potency} = [\bar{z}_S \times (v_S/v_U)] / \bar{z}_U \text{ (IRA 1-Nov-2015)}$$

\bar{z}_S = average number of doses of the *Standard solution* (IRA 1-Nov-2015)

$R = v_S/v_U$

(IRA 1-Nov-2015)

v_S = number of USP Digitalis Units/mL of *Standard solution* dilution

v_U = volume of *Sample solution* per mL of dilution (mL)

\bar{z}_U = average number of doses of the *Sample solution* (IRA 1-Nov-2015)

Compute the confidence interval, L [see Equation (31) in *Design and Analysis of Biological Assays* (111), *The Confidence Interval and Limits of Potency, Confidence Intervals for Individual Assays*]. If L exceeds 0.30, repeat the Assay or inject more pigeons with one or both preparations until the confidence interval is 0.30 or less.

Acceptance criteria: The potency of Powdered Digitalis, calculated from that of the *Sample solution*, is satisfactory if the result is NLT 0.85 USP Digitalis Units and NMT 1.20 USP Digitalis Units/100 mg.

IMPURITIES

- **Articles of Botanical Origin** (561), *Acid-Insoluble Ash*: NMT 5.0%

SPECIFIC TESTS

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): It meets the requirements of the tests for absence of *Salmonella species*.
- **Water Determination** (921), *Method III, Procedure for Articles of Botanical Origin*: NMT 5.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. A package of a suitable desiccant may be enclosed in the container.

Change to read:

- **USP Reference Standards** (11)

USP Digitalis RS
~~USP Digitoxin RS~~
~~USP Gitoxin RS~~
 • USP Digoxin RS

• (IRA 1-Nov-2015)

¹ One USP Digitalis Unit represents the potency of 100 mg of USP Digitalis RS.

BRIEFING

Digoxin, USP 38 page 3115. The following revisions are proposed:

1. Because of the discontinuation of USP Gitoxin RS, a new quantitative HPLC procedure is being added in the test for *Related Glycosides*.
2. *Identification* test C is being deleted to accommodate this revision.
3. In the *Assay*, the *Chromatographic system* is being updated with new column dimensions, column temperature, and flow rate.
4. The *USP Reference Standards* section is revised to remove USP Gitoxin RS.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

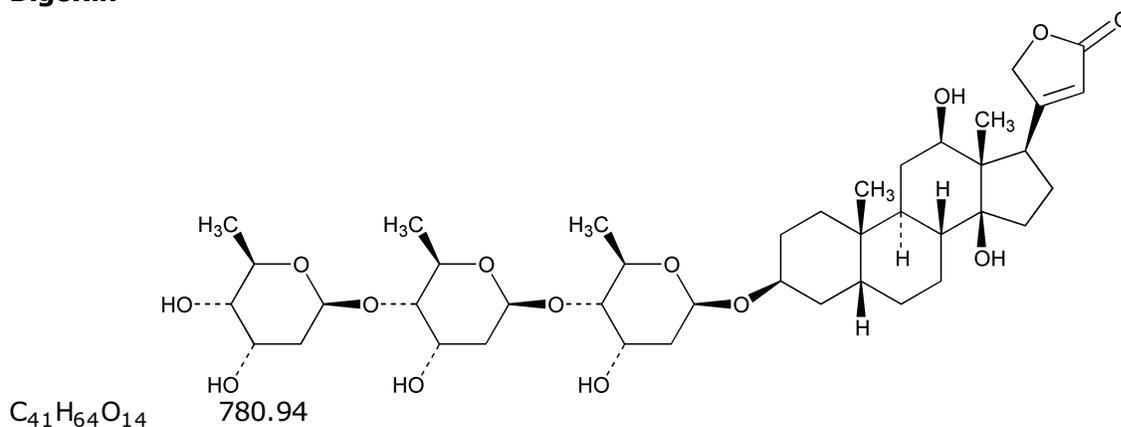
The comment period for this revision ends on July 31, 2015. In the absence of significant comments, it is proposed to implement this revision through an *Interim Revision Announcement*, with an official date of November 1, 2015.

(DSB: N. Kim.)

Correspondence Number—C152052

Comment deadline: July 31, 2015

Digoxin



Card-20(22)-enolide, 3-[(O-2,6-dideoxy- β -D-ribo-hexopyranosyl-(1 \rightarrow 4)-O-2,6-dideoxy- β -D-ribo-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy- β -D-ribo-hexopyranosyl)oxy]-12,14-dihydroxy-, (3 β ,5 β ,12 β)-;

Digoxin;

3 β -[(O-2,6-Dideoxy- β -D-ribo-hexopyranosyl-(1 \rightarrow 4)-O-2,6-dideoxy- β -D-ribo-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy- β -D-ribo-hexopyranosyl)oxy]-12 β ,14-dihydroxy-5 β -card-20(22)-

enolide [20830-75-5].

DEFINITION

Digoxin is a cardiotonic glycoside obtained from the leaves of *Digitalis lanata* Ehrhart (Fam. Scrophulariaceae). It contains NLT 95.0% and NMT 101.0% of digoxin ($C_{41}H_{64}O_{14}$), calculated on the dried basis. [**Caution**—Handle Digoxin with exceptional care, because it is extremely poisonous.]

IDENTIFICATION

- **A. Infrared Absorption** 〈 197K 〉
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Delete the following:

•• ~~C. Procedure~~

~~**Analysis:** Examine in visible light the thin-layer chromatograph prepared as directed in the test for *Related Glycosides*.~~

~~**Acceptance criteria:** The R_f value of the principal blue spot of the *Sample solution* corresponds to that of the *Standard solution*. •(IRA 1-Nov-2015)~~

ASSAY

Change to read:

• Procedure

Mobile phase: Acetonitrile and water (13:37)

System suitability solution: 40 µg/mL each of USP Digoxin RS and digoxigenin in diluted alcohol

Standard solution: 0.25 mg/mL of USP Digoxin RS in diluted alcohol. [Note—Use a sonic bath to aid dissolution.]

Sample solution: 0.25 mg/mL of Digoxin in diluted alcohol. [Note—Dissolve using sonication, before make-up to final volume.]

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 218 nm

Column: ~~4.2-mm × 25-cm; packing L1~~

•4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 25° •(IRA 1-Nov-2015)

~~**Guard column:** 3.2-mm × 15-mm; packing L1~~

• •(IRA 1-Nov-2015)

Flow rate: 3 mL/min

•2 mL/min •(IRA 1-Nov-2015)

Injection volume: 10 µL

System suitability**Sample:** *System suitability solution***Suitability requirements****Resolution:** NLT 4.0 between digoxin and digoxigenin**Column efficiency:** NLT 1200 theoretical plates for the digoxin peak**Tailing factor:** NMT 2.0 for the digoxin peak**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of digoxin ($C_{41}H_{64}O_{14}$) in the portion of Digoxin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak response of digoxin from the *Sample solution* r_S peak response of digoxin from the *Standard solution* C_S concentration of USP Digoxin RS in the *Standard solution* (mg/mL) C_U concentration of Digoxin in the *Sample solution* (mg/mL)**Acceptance criteria:** 95.0%–101.0% on the dried basis**IMPURITIES**

- **Residue on Ignition** (281): NMT 0.5%, a 100-mg specimen being used

Change to read:

- **Related Glycosides**

Diluent: Chloroform and methanol (2:1)**Standard solution:** 10 mg/mL of USP Digoxin RS in *Diluent***Gitoxin standard solution:** 0.30 mg/mL of USP Gitoxin RS in *Diluent***Sample solution:** 10 mg/mL of Digoxin in *Diluent***Chromatographic system***(See Chromatography (621), Thin-Layer Chromatography.)***Mode:** TLC**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture to which octadecylsilane (C18) is permanently bonded**Application volume:** 10 μ L**Developing solvent system:** Methanol and water (7:3)**Spray reagent:** 1.53 M trichloroacetic acid in dehydrated alcohol and 30 mg/mL chloramine T (freshly prepared) (4:1). [Note—Mix before using.]**Analysis****Samples:** *Standard solution*, *Gitoxin standard solution*, and *Sample solution*

Apply *Samples* on a line parallel to and about 2.5 cm from the bottom edge of a reversed-phase thin-layer chromatographic plate. Allow the spots to dry, and place the plates in a developing chamber. Develop the chromatogram until the solvent front has moved about 15 cm above the line of application. Remove the plate, and allow the solvent to evaporate. Spray the plate with *Spray reagent*, and heat in an oven at 110 °C.

for 10 min. Examine the plate under long-wavelength UV light.

Acceptance criteria: No spot from the *Sample solution*, except that due to digoxin, is more intense than the spot from the *Gitoxin standard solution* (NMT 3% of any related glycoside as gitoxin).

• **Solution A:** Acetonitrile and water (10:90)

Solution B: Water and acetonitrile (10:90)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	78	22
5	78	22
15	30	70
16	78	22
30	78	22

Standard solution: 0.5 mg/mL of USP Digoxin RS in methanol

System suitability solution: Dissolve 50 mg of lanatoside C in methanol, and dilute with methanol to 100 mL. To 1.0 mL of this solution, add 1.0 mL of the *Standard solution*, and dilute with methanol to 20 mL.

Sample solution: Accurately weigh 25 mg of digoxin, transfer into a 50-mL volumetric flask, and adjust with methanol to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 25°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between the digoxin and lanatoside C peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the peak areas of total impurities, gitoxin, and digitoxin against the principle peak in the *Standard solution*.

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Digoxin (standard)	1.00	—
Gitoxin	2.16	0.5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Digitoxin	2.62	0.5
Total impurities	—	3.5

*(IRA 1-Nov-2015)

- **Residual Solvents** 〈 467 〉: 2000 µg/g for methylene chloride and for chloroform

SPECIFIC TESTS

- **Loss on Drying** 〈 731 〉

Analysis: Dry under vacuum at 105° for 1 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Digoxin RS

~~USP Gitoxin RS~~

*(IRA 1-Nov-2015)

BRIEFING

Omega-3-Acid Ethyl Esters, *USP 38* page 4627; *PF 40(5)* [Sept.–Oct. 2014]. On the basis of comments received on the proposed revisions to the Omega-3-Acid Ethyl Esters monograph published in *PF 40(5)*, the following additional changes are proposed:

1. The *Definition* section is revised by removing some of the statements related to acceptance criteria and purification processes because ingredients obtained through more than one purification process are now permitted in the monograph.
2. A new *Identification* test to distinguish the approved APIs from other non-approved lower concentrations of omega-3-acid ethyl esters is added. The new test is also linked to a labeling requirement to identify the type of omega-3-acid ethyl esters contained in the drug products as type A.
3. The *Assay* acceptance criteria is revised by adding a new column titled *Acceptance criteria II* in *Table 1*, for articles labeled as containing Omega-3-Acid Ethyl Esters type A.
4. A labeling requirement is added to the *Labeling* section to indicate whether the article contains Omega-3-Acid Ethyl Esters type A.
5. The *Impurities* test for the limit of lead, arsenic, cadmium, and mercury in the revised monograph is retained, based on the implementation of *Elemental Impurities—Limits* 〈 232 〉.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

The comment period for this revision ends on July 31, 2015. In the absence of significant comments, it is proposed to implement this revision through an *Interim Revision Announcement*, with an official date of November 1, 2015.

(DS: H. Dinh.)

Correspondence Number—C155627

Comment deadline: July 31, 2015

Omega-3-Acid Ethyl Esters

DEFINITION

Change to read:

~~Omega-3-Acid Ethyl Esters are obtained by transesterification of the body oil obtained from fish of families such as *Engraulidae*, *Carangidae*, *Clupeidae*, *Osmeridae*, *Salmonidae*, and *Scombridae*~~

~~•or from animals of the class *Cephalopoda*, •(IRA 1-Nov-2015)~~

and subsequent purification processes including urea fractionation followed by molecular distillation

~~•or other processes. •(IRA 1-Nov-2015)~~

The content of eicosapentaenoic acid ethyl ester (EPAee) plus the content of docosahexaenoic acid ethyl ester (DHAee) is NLT ~~800~~

~~•700 •(IRA 1-Nov-2015)~~

mg/g and NMT 880 mg/g, with NLT ~~430~~

~~•365 •(IRA 1-Nov-2015)~~

mg/g and NMT 495 mg/g of EPAee and NLT ~~347~~

~~•290 •(IRA 1-Nov-2015)~~

mg/g and NMT 403 mg/g of DHAee. It contains NLT ~~90~~

~~•78 •(IRA 1-Nov-2015)~~

% (w/w) of the sum of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), moroctic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), eicosapentaenoic acid ethyl ester (EPAee) (C20:5 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), docosapentaenoic acid ethyl ester (C22:5 n-3, EE), and docosahexaenoic acid ethyl ester (DHAee) (C22:6 n-3, EE). Tocopherol may be added as an antioxidant.

~~•Omega-3-Acid Ethyl Esters is a mixture of ethyl esters principally of eicosapentaenoic acid (EPAee) (C20:5 n-3, EE) and docosahexaenoic acid (DHAee) (C22:6 n-3, EE). It may also contain ethyl esters of alpha-linolenic acid (C18:3 n-3, EE), moroctic acid (C18:4 n-3, EE), eicosatetraenoic acid (C20:4 n-3, EE), heneicosapentaenoic acid (C21:5 n-3, EE), and docosapentaenoic acid (C22:5 n-3, EE). Tocopherol may be added as an antioxidant.~~

~~•(IRA 1-Nov-2015)~~

IDENTIFICATION

Change to read:

- **A.** The retention times of the peaks for eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester

• principal peaks • (IRA 1-Nov-2015)

in *Test solution*

• 4 • (IRA 1-Nov-2015)

correspond to those respective compounds

• of eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester • (IRA 1-Nov-2015)

in *Standard solution*

• 1b and *Standard solution 1a*, • (IRA 1-Nov-2015)

as obtained in the Assay.

Add the following:

- **B.** It meets the acceptance criteria in *Table 1* of the Assay. • (IRA 1-Nov-2015)

ASSAY

Change to read:

- **Content of EPAee, DHAee, and Total Omega-3-Acids Ethyl Esters**

(See *Fats and Fixed Oils* 〈 401 〉, *Omega-3 Fatty Acids Determination and Profile*.)

- **Standard solution 1a, Standard solution 1b, Test solution 3, Test solution 4, System suitability solution 1, Chromatographic system, and System suitability:**

Proceed as directed in *Fats and Fixed Oils* 〈 401 〉, *Omega-3 Fatty Acids Determination and Profile*. • (IRA 1-Nov-2015)

Analysis

Samples: ~~Standard solution and Test solution~~

• *Standard solution 1a, Standard solution 1b, Test solution 3, and Test solution 4*

• (IRA 1-Nov-2015)

Calculate the content of EPAee and DHAee in the portion of Omega-3-Acid Ethyl Esters taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak area ratio of the EPAee or DHAee peak to the internal standard peak from *Test solution 3*

R_S = peak area ratio of the EPAee

• to the internal standard peak from *Standard solution 1b* • (IRA 1-Nov-2015)
or DHAee peak to the internal standard peak from ~~*Standard Solution 1*~~

• *Standard solution 1a* • (IRA 1-Nov-2015)

C_S = concentration of USP Eicosapentaenoic Acid Ethyl Ester RS

• in *Standard solution 1b* • (IRA 1-Nov-2015)
or USP Docosahexaenoic Acid Ethyl Ester RS in ~~*Standard Solution 1*~~

• *Standard solution 1a* • (IRA 1-Nov-2015)

(mg/mL)

C_U = concentration of Omega-3 Ethyl Esters in *Test solution 3* (g/mL)

Calculate the content of total omega-3-acids ethyl esters in the portion of Omega-3-Acid Ethyl

Esters taken:

$$\text{Result} = r_{\text{FAn-3ee}} [(EPAee + DHAee)/(r_{\text{EPAee}} + r_{\text{DHAee}})] + EPAee + DHAee$$

$r_{\text{FAn-3ee}}$ = sum of the peak areas of alpha-linolenic acid ethyl ester (C18:3 n₋₃, EE), moroctic acid ethyl ester (C18:4 n₋₃, EE), eicosatetraenoic acid ethyl ester (C20:4 n₋₃, EE), heneicosapentaenoic acid ethyl ester (C21:5 n₋₃, EE), and docosapentaenoic acid ethyl ester (C22:5 n₋₃, EE) in *Test solution 4*

$EPAee$ = content of EPAee (mg/g)

$DHAee$ = content of DHAee (mg/g)

r_{EPAee} = peak area of EPAee in *Test solution 4*

r_{DHAee} = peak area of DHAee in *Test solution 4*

Acceptance criteria: It conforms to the acceptance criteria in *Table 1*.

★Articles labeled as Omega-3-Acid Ethyl Esters type A meet *Acceptance criteria II*.

Table 1

Name	Relative Retention Time	Acceptance Criteria I		Acceptance Criteria II (For articles labeled as Omega-3-Acid Ethyl Esters type A)	
		NLT	NMT	NLT	NMT
C18:3 n ₋₃ , EE ^a	0.585	—	—	—	—
C18:4 n ₋₃ , EE ^b	0.608	—	—	—	—
C20:4 n ₋₃ , EE ^c	0.777	—	—	—	—
C20:5 n ₋₃ , EE (EPAee) ^d	0.796	430 mg/g	495 mg/g	365 mg/g	435 mg/g
C21:5 n ₋₃ , EE ^e	0.889	—	—	—	—
C22:5 n ₋₃ , EE ^f	0.977	—	—	—	—
C22:6 n ₋₃ , EE (DHAee) ^g	1.000	347 mg/g	403 mg/g	290 mg/g	360 mg/g
EPAee + DHAee	—	800 mg/g	880 mg/g	700 mg/g	749 mg/g
Total omega-3-acid ethyl esters	—	90% (w/w)	—	78% (w/w)	—

^a Alpha-linolenic acid ethyl ester.

^b Moroctic acid ethyl ester.

^c Eicosatetraenoic acid ethyl ester.

^d Eicosapentaenoic acid ethyl ester.

e Heneicosapentaenoic acid ethyl ester.

f Docosapentaenoic acid ethyl ester (clupanodonic acid ethyl ester).

g Docosahexaenoic acid ethyl ester.

*(IRA 1-Nov-2015)

IMPURITIES

- **Fats and Fixed Oils** 〈 401 〉: NMT 0.1 ppm each of lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg)

Change to read:

- **Cholesterol**

Internal standard stock solution: 3 mg/mL of 5 α -cholestane in *n*-heptane. [Note—Prepare fresh before use.]

Internal standard solution: 0.3 mg/mL of 5 α -cholestane in *n*-heptane. [Note—Prepare fresh before use.]

Standard stock solution: 3.0 mg/mL of cholesterol in *n*-heptane. [Note—This solution is stable for 6 months stored in a freezer.] Transfer 1.0 mL of this solution to a 10.0-mL volumetric flask. Dilute with *n*-heptane to volume. [Note—Prepare this solution fresh daily.]

Standard solution: Transfer 1.0 mL of *Standard stock solution* and 1.0 mL of *Internal standard solution* to a 15-mL centrifuge tube. Proceed as directed in the *Sample solution* beginning with "Evaporate to dryness".

Alpha tocopherol stock solution: 1.5–2.0 mg/mL of USP Alpha Tocopherol RS in *n*-heptane. [Note—This solution is stable for 12 months stored in a freezer.]

System suitability solution: Mix 1.0 mL of *Standard stock solution*, 1.0 mL of *Internal standard stock solution*, and 2.0 mL of *Alpha tocopherol stock solution* in a 50-mL volumetric flask. Evaporate to dryness with the aid of heat, and dilute with ethyl acetate to volume. Dilute 1.0 mL of this solution with ethyl acetate to 10.0 mL. [Note—This solution is stable for 6 months stored in a freezer.]

Sample solution: Transfer 100 mg of Omega-3-Acid Ethyl Esters to a 15-mL centrifuge tube. Add 1.0 mL of *Internal standard solution*. Evaporate to dryness at about 50° with a gentle stream of nitrogen. Add 0.5 mL of 50% potassium hydroxide and 3 mL of alcohol, fill the tube with nitrogen, and cap. Heat the sample at 100° for 60 min, using a heating block. Cool for about 10 min. Add 6 mL of water to the tube, and shake for 1 min. Extract the solution four times with 2.5-mL portions of ethyl ether, using a vortex mixer or suitable shaker for 1 min for each extraction. Transfer and combine the extracts into a large centrifuge tube, and wash with 5 mL of water, mixing completely with gentle inversion. Remove the water phase, and add 5 mL of 0.5 M potassium hydroxide to the ether phase, mixing carefully to avoid an emulsion. Remove the potassium hydroxide, and add another 5 mL of water, mixing carefully. Transfer the ether phase to a small centrifuge tube. [Note—If an emulsion has occurred, a small amount of sodium chloride may be added to obtain a separation of the phases.] Evaporate the ether phase to dryness under a stream of nitrogen with careful heating. Dissolve the sample in 600 μ L of ethyl acetate, and mix well.

Transfer 200 µL of this solution to a sample vial, and dilute with ethyl acetate to about 2 mL.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m capillary; coated with a G27 phase of 0.25-µm thickness

Temperatures

Injection port: 320°

Detector: 300°

Column: See the temperature program table below

•Table 2.

Table 2 • (IRA 1-Nov-2015)

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	0	170	1
170	4	320	1.5

Carrier gas: Helium

Flow rate: 1.3 mL/min

Injection volume: 1 µL

Injection type: Splitless injection system

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.2 between alpha tocopherol and cholesterol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the content of total cholesterol in the portion of Omega-3-Acid Ethyl Esters taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U)$$

R_U = peak area ratio of the cholesterol peak to the internal standard from the *Sample solution*

R_S = peak area ratio of the cholesterol peak to the internal standard from the *Standard solution*

W_S = weight of cholesterol in the *Standard solution* (mg)

W_U = weight of Omega-3-Acid Ethyl Esters in the *Sample solution* (g)

Acceptance criteria: NMT 3.0 mg/g

• Oligomers

Mobile phase: Tetrahydrofuran

System suitability solution: Monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin in *Mobile phase*, with concentrations of about 0.5, 0.3, and 0.2 mg/mL, respectively. [Note—Suitable grades of monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin may be obtained from Nu-Chek Prep.]

Sample solution 1: 5.0 mg/mL of Omega-3-Acid Ethyl Esters in tetrahydrofuran

Sample solution 2: [Note—Use *Sample solution 2* where the results of this test using *Sample solution 1* exceed the *Acceptance criteria* due to the presence of monoglycerides.] Weigh 50 mg of Omega-3-Acid Ethyl Esters into a quartz tube, add 1.5 mL of a 20-g/L solution of sodium hydroxide in methanol, cover with nitrogen, cap tightly with a polytef-lined cap, mix, and heat on a water bath for 7 min. Allow to cool. Add 2.0 mL of boron trichloride–methanol solution, cover with nitrogen, cap tightly, mix, and heat on a water bath for 30 min. Cool to 40°–50°, add 1 mL of isooctane, cap, and shake vigorously for NLT 30 s. Immediately add 5 mL of saturated sodium chloride solution, cover with nitrogen, cap, and shake thoroughly for NLT 15 s. Transfer the upper layer to a separate tube. Shake the methanol layer with 1 mL of isooctane. Wash the combined isooctane extracts with 2 quantities, each of 1 mL of water. Carefully evaporate the solvent under a stream of nitrogen, then add 10.0 mL of tetrahydrofuran to the residue. Add a small amount of anhydrous sodium sulfate, and filter.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Differential refractometer

Columns: Three concatenated 7.8-mm × 30-cm columns; 7-μm packing L21, with pore sizes in the range 5–50 nm, arranged with decreasing pore size from the injector to the detector to fulfill the system suitability requirements.

Flow rate: 0.8 mL/min

Injection volume: 40 μL

System suitability

Sample: *System suitability solution*

Suitability requirements

Elution order: Tridocosahexaenoin, didocosahexaenoin, and monodocosahexaenoin

Resolution: NLT 2.0 between monodocosahexaenoin and didocosahexaenoin; NLT 1.0 between didocosahexaenoin and tridocosahexaenoin

Analysis

Samples: *Sample solution 1* and *Sample solution 2*

Measure the areas of the major peaks.

Calculate the percentage of oligomers in the portion of Omega-3-Acid Ethyl Esters taken to prepare *Sample solution 1*:

$$\text{Result} = (r_I/r_T) \times 100$$

r_I sum of the areas of the peaks with a retention time less than that of the ethyl esters peaks

r_T sum of the areas of all peaks

Calculate the percentage of oligomers in the portion of Omega-3-Acid Ethyl Esters taken to prepare *Sample solution 2*:

$$\text{Result} = (r_I/r_T) \times 100$$

r_I sum of the areas of all peaks with a retention time less than that of the methyl esters peaks

r_T sum of the areas of all peaks

Acceptance criteria: NMT 1.0% of oligomers

- **Limit of Dioxins, Furans, and Polychlorinated Biphenyls (PCBs):** Determine the content of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by method No. 1613 revision B of the Environmental Protection Agency. Determine the content of polychlorinated biphenyls (PCBs) by method No. 1668 revision A of the Environmental Protection Agency.

Acceptance criteria: The sum of PCDDs and PCDFs is NMT 1 pg/g of WHO toxic equivalents. The sum of PCBs (polychlorinated biphenyls, IUPAC congeners PCB-28, PCB-52, PCB-101, and PCB-118, PCB-138, PCB-153, PCB-180) is NMT 0.5 ppm.

Change to read:

- **Limit of Total Unidentified Fatty Acids Ethyl Esters**

● [Note—This test is not required for the articles labeled as Omega-3-Acid Ethyl Esters type A.]

● (IRA 1-Nov-2015)

From the chromatogram obtained with *Test solution 4* in the *Assay for Content of EPAee, DHAee, and Total Omega-3-Acids Ethyl Esters*, determine the peak area of the largest single unidentified peak with a relative retention time different from those in the following table

● *Table 3.*

Table 3 ● (IRA 1-Nov-2015)

Identified Ethyl Ester	Relative Retention Time
Phytanic acid	0.416
C16:3 n-4	0.431
C16:4 n-1	0.468
C18:3 n-6	0.557
C18:3 n-4	0.574
C18:3 n-3	0.585
C18:4 n-3	0.608
C18:4 n-1	0.618
Furan acid 5	0.691
C19:5	0.710
C20:3 n-6	0.720
C20:4 n-6	0.736
Furan acid 7	0.744
C20:4 n-3	0.777
Furan acid 8	0.783
EPA	0.796
Furan acid 9	0.867
C21:5 n-3	0.889

Identified Ethyl Ester	Relative Retention Time
C22:4	0.917
Furan acid 10	0.922
C22:5 n-6	0.939
Furan acid 11	0.963
C22:5 n-3	0.977
DHA	1.000

Calculate the content of unidentified fatty acids ethyl esters in area percentage:

$$\text{Result} = 100 - (100 \times \Sigma A_{iee}/r_T)$$

A_{iee} = peak area of each identified ethyl ester in the table above

★*Table 3*★(IRA 1-Nov-2015)

r_T = sum of the areas of all peaks except solvents and BHT

Acceptance criteria: The area of the largest single unidentified peak is NMT 0.5% of the total area. The total area of unidentified peaks as calculated above is NMT 2%.

Add the following:

●● **Limit of Non-Omega-3-Acid Ethyl Esters**

[Note—This test is only required for the articles labeled as Omega-3-Acid Ethyl Esters type A.]

From the chromatogram obtained with *Test solution 4* in the *Assay for Content of EPA_{ee}, DHA_{ee}, and Total Omega-3-Acids Ethyl Esters*, calculate the amounts of C18:1 n-9 ethyl ester and C20:4 n-6 ethyl ester in the portion of Omega-3-Acid Ethyl Esters taken:

$$\text{Result} = (A_{iep}/r_T) \times 100$$

A_{iep} = peak area of C18:1 n-9 ethyl ester or C20:4 n-6 ethyl ester

r_T = sum of the areas of all peaks except solvents and BHT

Acceptance criteria

C18:1 n-9 ethyl ester: NMT 6.0%

C20:4 n-6 ethyl ester: NMT 4.0%

★(IRA 1-Nov-2015)

SPECIFIC TESTS

- **Fats and Fixed Oils** (401), *Acid Value:* NMT 2.0 mg of KOH/g
- **Fats and Fixed Oils** (401), *Anisidine Value:* NMT 15
- **Fats and Fixed Oils** (401), *Peroxide Value :* NMT 10.0
- **Absorbance**

Sample solution: Transfer 300 mg, accurately weighed, to a 50-mL volumetric flask.

Dissolve in and dilute immediately with isooctane to volume. Pipet 2.0 mL into a 50-mL volumetric flask, and dilute with isooctane to volume.

Acceptance criteria: NMT 0.55, determined at 233 nm, with isooctane being used as the blank

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers under a nitrogen atmosphere. Store at controlled room temperature.

Change to read:

- **Labeling:** The label states the content of DHA ethyl ester and EPA ethyl ester in mg/g, the sum of the EPA and DHA ethyl esters contents in mg/g, and the content of the total omega-3-acid ethyl esters in percentage (w/w). It also states the name of any added antioxidant.
- Articles intended to meet *Acceptance criteria II* of the *Assay* and the *Limit of Non-Omega-3-Acid Ethyl Esters* are labeled as Omega-3-Acid Ethyl Esters type A. (IRA 1-Nov-2015)

● USP Reference Standards (11)

USP Alpha Tocopherol RS

USP Docosahexaenoic Acid Ethyl Ester RS

All *cis*-4,7,10,13,16,19-docosahexaenoic ethyl ester.

C₂₄H₃₆O₂ 356.55

USP Eicosapentaenoic Acid Ethyl Ester RS

All *cis*-5,8,11,14,17-eicosapentaenoic ethyl ester.

C₂₂H₃₄O₂ 330.51

USP Methyl Tricosanoate RS

Tricosanoic acid methyl ester.

C₂₄H₄₈O₂ 368.64

BRIEFING

Omega-3-Acid Ethyl Esters Capsules, *USP 38* page 4630; *PF 40(5)* [Sept.–Oct. 2014]. On the basis of comments received on the proposed revision published in *PF 40(5)*, the following additional changes are proposed:

1. Add a new *Identification* test to distinguish the Capsule contents with APIs that contain different concentrations of Omega-3-Acid Ethyl Esters with reference to the *Acceptance criteria in Specific Tests, Concentration of Omega-3-Acid Ethyl Esters*.
2. Add the *Concentration of Omega-3-Acid Ethyl Esters* test in *Specific Tests*. The *Acceptance criteria* for this test will include *Acceptance criteria I*, for APIs having NLT 90% (w/w) of total omega-3-acid ethyl esters, and *Acceptance criteria II*, for APIs having NLT 78% (w/w) of total omega-3-acid ethyl esters, which is intended for Capsules labeled as containing Omega-3-Acid Ethyl Esters type A.
3. Add a requirement to the *Labeling* section to indicate whether the Capsules contain Omega-3-Acid Ethyl Esters type A.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

The comment period for this revision ends on July 31, 2015. In the absence of significant comments, it is proposed to implement this revision through an *Interim Revision Announcement*, with an official date of November 1, 2015.

(DS: N. Davydova.)

Correspondence Number—C157916

Comment deadline: July 31, 2015

Omega-3-Acid Ethyl Esters Capsules

DEFINITION

Change to read:

~~Omega-3-Acid Ethyl Esters, which are obtained by transesterification of the body oil obtained from fish of families such as *Engraulidae*, *Carangidae*, *Clupeidae*, *Osmeridae*, *Salmonidae*, and *Scombridae* and subsequent purification processes including urea fractionation followed by molecular distillation with NLT 95.0% and NMT 105.0% of the labeled sum of eicosapentaenoic acid ethyl ester (EPAee) and docosahexaenoic acid ethyl ester (DHAee). The content of EPAee plus the content of DHAee is NLT 800 mg/g and NMT 880 mg/g, with NLT 430 mg/g and NMT 495 mg/g of EPAee and NLT 347 mg/g and NMT 403 mg/g of DHAee.~~

• Omega-3-Acid Ethyl Esters Capsules contain Omega-3-Acid Ethyl Esters, with NLT 95.0% and NMT 105.0% of the labeled sum of eicosapentaenoic acid ethyl ester (EPAee) and docosahexaenoic acid ethyl ester (DHAee) and NLT 95% of the labeled amount of total omega-3-acid ethyl esters, as the sum of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), morotic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), eicosapentaenoic acid ethyl ester (EPAee) (C20:5 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), docosapentaenoic acid ethyl ester (C22:5 n-3, EE), and docosahexaenoic acid ethyl ester (DHAee) (C22:6 n-3, EE). •(IRA 1-Nov-2015)

Tocopherol may be added as an antioxidant.

IDENTIFICATION

Change to read:

- **A.** The retention times of the peaks for eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay for Content of EPAee and DHAee* and *Total Omega-3-Acids Ethyl Esters*. •(IRA 1-Nov-2015)

Add the following:

- **B.** It complies with the *Acceptance criteria* of the test for *Concentration of Omega-3-Acid Ethyl Esters*, in *Specific Tests*. •(IRA 1-Nov-2015)

ASSAY

Change to read:

- **Content of EPAee and DHAee**

•and Total Omega-3-Acids Ethyl Esters•(IRA 1-Nov-2015)

[Note—Carry out the procedure as rapidly as possible, avoiding exposure to actinic light, oxidizing agents, oxidation catalysts (i.e., copper and iron), and air.]

Antioxidant solution: 50 mg/L of butylated hydroxytoluene in isooctane

Internal standard solution: 7.0 mg/mL of USP Methyl Tricosanoate RS in *Antioxidant solution*

System suitability solution: 5.5 mg/mL of docosahexaenoic acid methyl ester and 0.5 mg/mL of tetracos-15-enoic acid methyl ester in *Antioxidant solution*

Standard solution: Dissolve 60.0 mg of USP Docosahexaenoic Acid Ethyl Ester RS and 90.0 mg of USP Eicosapentaenoic Acid Ethyl Ester RS in 10.0 mL of *Internal standard solution*.

• **Retention time identification solution:** Prepare a mixture containing suitable concentrations of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), moroctic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), and docosapentaenoic acid ethyl ester (C22:5 n-3, EE) in *Antioxidant solution*.¹•(IRA 1-Nov-2015)

Sample solution: Weigh NLT 10 Capsules in a tared weighing bottle. With a sharp blade, carefully open the Capsules, without loss of shell material, and transfer the combined Capsule contents to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of diethyl ether. Discard the washings, and allow the empty Capsules to air-dry over a period of NMT 30 min, taking precautions to avoid uptake or loss of moisture. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average fill weight per Capsule (W_{AF}). Transfer ~~250 mg of the combined Capsule contents~~

•an amount of the combined Capsule contents equivalent to 225 mg of the labeled amount of total omega-3-acid ethyl esters•(IRA 1-Nov-2015)

to a suitable flask, and dissolve with 10.0 mL of *Internal standard solution*.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 25–50-m fused silica capillary; coated with a 0.25-µm film of G16

Temperatures

Injection port: 250°

Detector: 270°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	0	170	2
170	3.5	255	9

Carrier gas: Hydrogen or helium

Linear velocity: Adjust to obtain a retention time for docosahexaenoic acid ethyl ester of 26 ± 3 min.

Injection volume: 1 µL

Injection type: Split; split ratio,1:220

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between docosahexaenoic acid methyl ester and tetracos-15-enoic acid methyl ester peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% for the ratios of the peak responses of DHAee and EPAee relative to the internal standard, *Standard solution*

Analysis

Samples: *Standard solution*,

• *Retention time identification solution*, • (IRA 1-Nov-2015)

and *Sample solution*

• Identify the retention times of the relevant fatty acid ethyl esters by comparing the peaks in the chromatogram of the *Sample solution* with those in the chromatogram of the *Retention time identification solution*. • (IRA 1-Nov-2015)

Calculate the content, in mg/g, of EPAee and DHAee in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the *Sample solution*

R_S = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the *Standard solution*

C_S = concentration of USP Eicosapentaenoic Acid Ethyl Ester RS or USP Docosahexaenoic Acid Ethyl Ester RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (g/mL)

Acceptance criteria: *Table 2*

Table 2

Name	Acceptance Criteria, NLT (mg/g)	Acceptance Criteria, NMT (mg/g)
EPAee	430	495
DHAee	347	403
EPAee + DHAee	800	880

• • (IRA 1-Nov-2015)

Calculate the percentage of the labeled sum of EPAee and DHAee in the portion of Capsules taken:

$$\text{Result} = (EPAee + DHAee) \times W_{AF} \times (100/L)$$

$EPAee$ = content of EPAee in the portion of Capsules taken (mg/g)

$DHAee$ = content of DHAee in the portion of Capsules taken (mg/g)

W_{AF} = average fill weight of the Capsules taken (g)

L = sum of the labeled content of EPAee and DHAee (mg/Capsule)

• Calculate the percentage of the labeled amount of total omega-3-acids ethyl esters in the

portion of Capsules taken:

$$\text{Result} = \{r_{FA\Omega-3ee} \times [(EPAee + DHAee)/(r_{EPAee} + r_{DHAee})] + EPAee + DHAee\} \times W_{AF} \times (100/L)$$

$r_{FA\Omega-3ee}$ = sum of the peak areas of alpha-linolenic acid ethyl ester (C18:3 n₃, EE), morotic acid ethyl ester (C18:4 n₃, EE), eicosatetraenoic acid ethyl ester (C20:4 n₃, EE), heneicosapentaenoic acid ethyl ester (C21:5 n₃, EE), and docosapentaenoic acid ethyl ester (C22:5 n₃, EE) from the *Sample solution*

$EPAee$ = content of EPAee (mg/g)

$DHAee$ = content of DHAee (mg/g)

r_{EPAee} = peak area of EPAee from the *Sample solution*

r_{DHAee} = peak area of DHAee from the *Sample solution*

W_{AF} = average fill weight of the Capsules taken (g)

L = label claim of total omega-3-acids ethyl esters (g/Capsule)

Acceptance criteria: 95.0%–105.0% of the labeled sum of EPAee and DHAee and NLT 95% of the labeled amount of total omega-3-acid ethyl esters per Capsule (IRA 1-Nov-2015)

PERFORMANCE TESTS

- **Uniformity of Dosage Units** { 905 }, *Weight Variation*: Meet the requirements
- **Disintegration** { 701 }
 - Medium, tier 1:** Water
 - Medium, tier 2:** Simulated gastric fluid TS
 - Time:** 30 min
 - Analysis:** Perform the test with water as *Medium, tier 1*. Repeat the test with simulated gastric fluid TS as *Medium, tier 2*, if the disintegration time is more than 30 min in *Medium, tier 1*.
 - Acceptance criteria:** Meet the requirements

IMPURITIES

- **Oligomers**
 - Mobile phase:** Tetrahydrofuran
 - System suitability solution:** Monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin in *Mobile phase*, with concentrations of about 0.5, 0.3, and 0.2 mg/mL, respectively. [Note—Suitable grades of monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin may be obtained from Nu-Chek Prep.]
 - Sample solution 1:** 5.0 mg/mL of the Capsule contents in tetrahydrofuran
 - Sample solution 2:** [Note—Use *Sample solution 2* where the results of this test using *Sample solution 1* exceed the *Acceptance criteria* due to the presence of monoglycerides.] Weigh 50 mg of the Capsule contents into a quartz tube, add 1.5 mL of a 20-g/L solution of sodium hydroxide in methanol, cover with nitrogen, cap tightly with a polytef-lined cap, mix, and heat on a water bath for 7 min. Allow to cool. Add 2.0 mL of boron trichloride–methanol solution, cover with nitrogen, cap tightly, mix, and heat on a water bath for 30 min. Cool to 40°–50°, add 1 mL of isooctane, cap, and shake vigorously for NLT 30 s. Immediately add 5 mL of saturated sodium chloride solution, cover with

nitrogen, cap, and shake thoroughly for NLT 15 s. Transfer the upper layer to a separate tube. Shake the methanol layer with 1 mL of isoctane. Wash the combined isoctane extracts with 2 quantities, each of 1 mL of water. Carefully evaporate the solvent under a stream of nitrogen, then add 10.0 mL of tetrahydrofuran to the residue. Add a small amount of anhydrous sodium sulfate, and filter.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Differential refractometer

Columns: Three concatenated 7.8-mm × 30-cm columns; 7-μm packing L21, with pore sizes in the range 5–50 nm, arranged with decreasing pore size from the injector to the detector to fulfill the system suitability requirements

Flow rate: 0.8 mL/min

Injection volume: 40 μL

System suitability

Sample: *System suitability solution*

Suitability requirements

Elution order: Tridocosahexaenoin, didocosahexaenoin, and monodocosahexaenoin

Resolution: NLT 2.0 between monodocosahexaenoin and didocosahexaenoin; NLT 1.0 between didocosahexaenoin and tridocosahexaenoin

Analysis

Samples: *Sample solution 1* and *Sample solution 2*

Measure the areas of the major peaks.

Calculate the percentage of oligomers in the portion of omega-3-acid ethyl esters taken to prepare *Sample solution 1*:

$$\text{Result} = (r_I/r_T) \times 100$$

r_I sum of the peak areas with retention times less than that of the ethyl esters peak

r_T sum of the areas of all peaks

Calculate the percentage of oligomers in the portion of the Capsules contents taken to prepare *Sample solution 2*:

$$\text{Result} = (r_I/r_T) \times 100$$

r_I sum of the peak areas with retention times less than that of the methyl esters peak

r_T sum of the areas of all peaks

Acceptance criteria: NMT 2% of oligomers

SPECIFIC TESTS

Add the following:

•• Concentration of Omega-3-Acid Ethyl Esters

Antioxidant solution, Internal standard solution, System suitability solution, Standard solution, Retention time identification solution, Sample solution, Chromatographic system, System suitability, and Analysis: Proceed as directed in the *Assay for Content of EPAee and DHAee and Total Omega-3-Acids Ethyl Esters*.

Calculate the concentration, in mg/g, of EPAee and DHAee in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the *Sample solution*

R_S = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the *Standard solution*

C_S = concentration of USP Eicosapentaenoic Acid Ethyl Ester RS or USP Docosahexaenoic Acid Ethyl Ester RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (g/mL)

Calculate the concentration, in mg/g, of total omega-3-acids ethyl esters in the portion of Capsules taken:

$$\text{Result} = r_{FA\omega-3ee} \times [(EPAee + DHAee)/(r_{EPAee} + r_{DHAee})] + EPAee + DHAee$$

$r_{FA\omega-3ee}$ = sum of the peak areas of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), moroctic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), and docosapentaenoic acid ethyl ester (C22:5 n-3, EE) from the *Sample solution*

$EPAee$ = content of EPAee (mg/g)

$DHAee$ = content of DHAee (mg/g)

r_{EPAee} = peak area of EPAee from the *Sample solution*

r_{DHAee} = peak area of DHAee from the *Sample solution*

Acceptance criteria: It meets the requirements in *Table 2*. Capsules labeled as containing Omega-3-Acid Ethyl Esters type A meet *Acceptance criteria II*.

Table 2

Name	Acceptance criteria I		Acceptance criteria II (for Capsules labeled as containing Omega-3-Acid Ethyl Esters type A)	
	NLT	NMT	NLT	NMT
EPAee	430 mg/g	495 mg/g	365 mg/g	435 mg/g
DHAee	347 mg/g	403 mg/g	290 mg/g	360 mg/g
EPAee + DHAee	800 mg/g	880 mg/g	700 mg/g	749 mg/g
Total omega-3-acid ethyl esters ^a	90% (w/w)	—	78% (w/w)	—

^a Sum of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), moroctic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), eicosapentaenoic acid ethyl ester (EPAee) (C20:5 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), docosapentaenoic acid ethyl ester (C22:5 n-3, EE), and docosahexaenoic acid ethyl ester (DHAee) (C22:6 n-3, EE).

- **Fats and Fixed Oils** 〈 401 〉, *Acid Value*

Sample solution: Dissolve about 5.0 g of the oil, accurately weighed, in 100 mL of a mixture of equal volumes of alcohol and ether (which has been neutralized to phenolphthalein with 0.1 M potassium hydroxide) contained in a flask.

Acceptance criteria: NMT 2.0 mg KOH/g

- **Fats and Fixed Oils** 〈 401 〉, *Anisidine Value:* NMT 25

- **Fats and Fixed Oils** 〈 401 〉, *Peroxide Value:* NMT 10 mEq/kg

- **Absorbance**

Sample solution: Transfer 300 mg, accurately weighed, to a 50-mL volumetric flask.

Dissolve in and dilute immediately with isooctane to volume. Pipet 2.0 mL into a 50-mL volumetric flask, and dilute with isooctane to volume.

Acceptance criteria: NMT 0.60, determined at 233 nm in a 1-cm cell, with isooctane being used as the blank

- **Microbial Enumeration Tests** 〈 61 〉: NMT 10^3 cfu/g for the total aerobic microbial count, and NMT 10^2 cfu/g for the total combined yeasts and molds count.

- **Tests for Specified Microorganisms** 〈 62 〉: Meet the requirements for absence of *Escherichia coli* in 1 g and for absence of *Salmonella* species in 10 g

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature. Do not freeze. Protect from light.

Change to read:

- **Labeling:** The label states the amount of docosahexaenoic acid (DHA) ethyl ester and eicosapentaenoic acid (EPA) ethyl ester, and the minimum amount of total content of omega-3-acid ethyl esters in mg/Capsule.

● Capsules intended to meet *Acceptance criteria II* of the test for *Concentration of Omega-3-Acid Ethyl Esters* are labeled as containing Omega-3-Acid Ethyl Esters type A.

● (IRA 1-Nov-2015)

It also states the name and content of any added antioxidant.

- **USP Reference Standards** 〈 11 〉

USP Docosahexaenoic Acid Ethyl Ester RS

All *cis*-4,7,10,13,16,19-docosahexaenoic ethyl ester.

$C_{24}H_{36}O_2$ 356.55

USP Eicosapentaenoic Acid Ethyl Ester RS

All *cis*-5,8,11,14,17-eicosapentaenoic ethyl ester.

$C_{22}H_{34}O_2$ 330.51

USP Methyl Tricosanoate RS

Tricosanoic acid methyl ester.

$C_{24}H_{48}O_2$ 368.64

●¹

The relevant fatty acid ethyl esters are available from Nu-Chek-Prep (www.nu-chekprep.com); Cayman Chemical (www.caymanchem.com); and Carbosynth (www.carbosynth.com). ● (IRA 1-Nov-2015)

BRIEFING

Oxycodone Hydrochloride, *USP 38* page 4705. On the basis of comments received, it is proposed to make the following changes:

1. Add *Identification* test C based on the retention time agreement using the existing *Assay* procedure.
2. Update the *Run time* for the *Assay* to be consistent with the current *USP* style.
3. Move the *Limit of Alcohol* test from the *Organic Impurities* section to another section under *Impurities*. The designations for the existing procedures under *Organic Impurities* are revised accordingly.
4. Add *Procedure 3* to the *Organic Impurities* section to accommodate products approved with noroxycodone as a potential impurity. The proposed HPLC procedure is based on analyses performed using the Inertsil ODS-3 brand of L1 column. The typical retention time for oxycodone is about 38 min.
5. Add chemical names of impurities to *Table 1* and update the chemical information in the *USP Reference Standards* section.
6. Add one Reference Standard to the *USP Reference Standards* section to support the proposed test for *Organic Impurities, Procedure 3*.
7. Add a *Labeling* section to indicate with which organic impurity procedure the article complies.

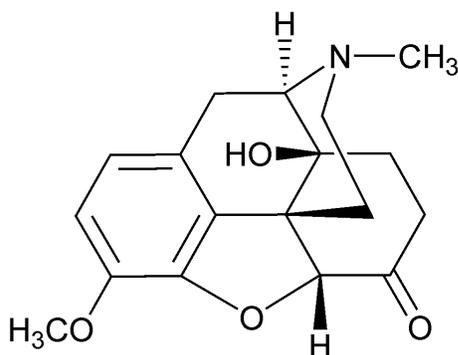
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

The comment period for this revision ends on July 31, 2015. In the absence of significant adverse comments, it is proposed to implement this revision through an *Interim Revision Announcement*, with an official date of November 1, 2015.

(SM2: H. Cai.)

Correspondence Number—C148391

Comment deadline: July 31, 2015

Oxycodone Hydrochloride

• HCl

$C_{18}H_{21}NO_4 \cdot HCl$ 351.82

Morphinan-6-one, 4,5-epoxy-14-hydroxy-3-methoxy-17-methyl-, hydrochloride, (5 α)-;
4,5 α -Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one hydrochloride [124-90-3].

DEFINITION

Oxycodone Hydrochloride contains NLT 97.0% and NMT 103.0% of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$), calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

• A. Procedure

Sample solution: Dissolve 250 mg in 25 mL of water.

Analysis: Render the 25 mL of *Sample solution* alkaline with 6 N ammonium hydroxide. Allow the mixture to stand until a precipitate is formed. Filter, wash the precipitate with 50 mL of cold water, and dry at 105° for 2 h.

Acceptance criteria: The precipitate melts between 218° and 223° , but the range between the beginning and the end of melting does not exceed 2° (see *Melting Range or Temperature* 〈 741 〉).

- **B. Infrared Absorption** 〈 197K 〉: Use a portion of the dried precipitate obtained in *Identification test A*.

Add the following:

- **C.** The retention time of the oxycodone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. •(IRA 1-Nov-2015)

ASSAY

Change to read:

• Procedure

Mobile phase: 0.005 M sodium 1-hexanesulfonate, methanol, triethylamine, and phosphoric acid (900:100:2:5). Adjust with 50% sodium hydroxide solution to a pH of 2.5 ± 0.1 , and filter.

System suitability solution: 13 $\mu\text{g/mL}$ of codeine phosphate and 9 $\mu\text{g/mL}$ of oxycodone in *Mobile phase*

Standard solution: 0.9 mg/mL of USP Oxycodone RS in *Mobile phase*

Sample solution: 1 mg/mL of Oxycodone Hydrochloride in *Mobile phase*. [Note—Pass a portion of this solution through a filter of 0.5- μm or finer pore size, and use the filtrate as the *Sample solution*.]

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 206 nm

Column: 3.9-mm \times 15-cm; 4- μm packing L7

Column temperature: 50°

Flow rate: 1.5 mL/min

Injection volume: 10 μL

•**Run time:** NLT 2 times the retention time of oxycodone •(IRA 1-Nov-2015)

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for codeine and oxycodone are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between codeine and oxycodone, *System suitability solution*

Tailing factor: 0.75–1.25, *Standard solution*

Relative standard deviation: NMT 2.0% from replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[~~Note—Record the chromatograms for a period of time that is twice the retention time of the main oxycodone peak.~~]

••(IRA 1-Nov-2015)

Calculate the percentage of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) in the portion of Oxycodone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxycodone Hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of oxycodone hydrochloride, 351.82

M_{r2} = molecular weight of oxycodone base, 315.37

Acceptance criteria: 97.0%–103.0% on the anhydrous, solvent-free basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.05%. [Note—Use of sulfuric acid is omitted.]

Add the following:

•• Limit of Alcohol

Internal standard stock solution: Transfer 6.0 mL of isopropyl alcohol to a 500-mL volumetric flask, and dilute with water to volume. [Note—The isopropyl alcohol must be free of alcohol impurities.]

Internal standard solution: Transfer 5.0 mL of the *Internal standard stock solution* to a 100-mL volumetric flask, and dilute with water to volume.

Standard stock solution: 16 mg/mL of alcohol (C_2H_5OH) in water

Standard solution: Pipet 3.0 mL of the *Standard stock solution* and 5.0 mL of the *Internal standard stock solution* into a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer about 240 mg of Oxycodone Hydrochloride to a 15-mL centrifuge tube, add 5.0 mL of the *Internal standard solution*, and mix to dissolve.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 4-mm × 1.8-m glass; packed with 80- to 100-mesh support S3

Carrier gas: Helium

Temperatures**Injection port:** 170°**Detector:** 170°**Column:** 150°. [Note—Condition the column overnight at 235° with a slow flow of carrier gas.]**Injection volume:** 5 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 2 between isopropyl alcohol and alcohol**Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of alcohol (C₂H₅OH) in the portion of Oxycodone Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U peak response ratio of the alcohol peak to the isopropyl alcohol from the *Sample solution*

R_S peak response ratio of the alcohol peak to the isopropyl alcohol from the *Standard solution*

C_S concentration of alcohol in the *Standard solution* (mg/mL)

C_U concentration of Oxycodone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0% (IRA 1-Nov-2015)**Change to read:**

- Organic Impurities**

- ~~Procedure 1: Limit of Alcohol~~

~~**Internal standard stock solution:** Transfer 6.0 mL of isopropyl alcohol to a 500-mL volumetric flask, and dilute with water to volume. [Note—The isopropyl alcohol must be free of alcohol impurities.]~~

~~**Internal standard solution:** Transfer 5.0 mL of the *Internal standard stock solution* to a 100-mL volumetric flask, and dilute with water to volume.~~

~~**Standard stock solution:** 16 mg/mL of alcohol (C₂H₅OH) in water~~

~~**Standard solution:** Pipet 3.0 mL of the *Standard stock solution* and 5.0 mL of the *Internal standard stock solution* into a 100-mL volumetric flask, and dilute with water to volume.~~

~~**Sample solution:** Transfer about 240 mg of Oxycodone Hydrochloride to a 15-mL centrifuge tube, add 5.0 mL of the *Internal standard solution*, and mix to dissolve.~~

~~**Chromatographic system**~~

~~(See *Chromatography* (621), *System Suitability*.)~~

~~**Mode:** GC~~~~**Detector:** Flame ionization~~~~**Column:** 4 mm × 1.8 m glass; packed with 80 to 100 mesh support S3~~~~**Carrier gas:** Helium~~~~**Temperature**~~~~**Column:** 150 °. [Note—Condition the column overnight at 235 ° with a slow flow of carrier gas.]~~~~**Injector:** 170 °~~~~**Detector:** 170 °~~~~**Injection volume:** 5 µL~~~~**System suitability**~~~~**Sample:** Standard solution~~~~**Suitability requirements**~~~~**Resolution:** NLT 2 between isopropyl alcohol and alcohol~~~~**Tailing factor:** NMT 1.5~~~~**Relative standard deviation:** NMT 2.0%~~~~**Analysis**~~~~**Samples:** Standard solution and Sample solution~~~~Calculate the percentage of alcohol (C₂H₅OH) in the portion of Oxycodone Hydrochloride taken:~~

~~$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$~~

~~*R_U* peak response ratio of the alcohol peak to the isopropyl alcohol from the *Sample solution*~~~~*R_S* peak response ratio of the alcohol peak to the isopropyl alcohol from the *Standard solution*~~~~*C_S* concentration of alcohol in the *Standard solution* (mg/mL)~~~~*C_U* concentration of Oxycodone Hydrochloride in the *Sample solution* (mg/mL)~~~~**Acceptance criteria:** NMT 1.0%~~

* [Note—On the basis of the synthetic route, perform either (a) *Procedure 1* and *Procedure 2*, or (b) *Procedure 3*. *Procedure 1* and *Procedure 2* are recommended if noroxymorphone or 8^β-hydroxyoxycodone (7,8-dihydro-8^β-14-dihydroxycodone) is a potential impurity. *Procedure 3* is recommended if noroxycodone is a potential impurity.]

*(IRA 1-Nov-2015)

Procedure 2

1(IRA 1-Nov-2015)

Analysis: Use the chromatogram of the *Sample solution* in the *Assay* to calculate the percentage of each impurity in the portion of Oxycodone Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_T peak response for each impurity

$r_{\bar{r}}$ sum of the responses of all the peaks

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxymorphone	0.31	0.15
Noroxymorphone		
^a (IRA 1-Nov-2015)	0.33	0.15
10-Hydroxyoxycodone		
^b (IRA 1-Nov-2015)	0.53	0.15
6- α Oxycodol		
^c (IRA 1-Nov-2015)	0.67	0.25
7,8-Dihydro-8 β -14-dihydroxycodeinone		
^d 8 β -Hydroxyoxycodone (7,8-dihydro-8 β -14-dihydroxycodeinone) ^d (IRA 1-Nov-2015)	0.71	0.15
Hydrocodone	1.19	0.15
Individual unspecified impurity	—	0.10
Total impurities	—	2.0
^a 4,5 α -Epoxy-3,14-dihydroxymorphinan-6-one.		
^b 4,5 α -Epoxy-10 α ,14-dihydroxy-3-methoxy-17-methylmorphinan-6-one.		
^c 4,5 α -Epoxy-3-methoxy-17-methylmorphinan-6 α ,14-diol.		
^d 4,5 α -Epoxy-10 α ,14-dihydroxy-3-methoxy-17-methylmorphinan-6-one.		
(IRA 1-Nov-2015)		

Procedure 3

***2:** (IRA 1-Nov-2015)

Limit of Oxycodone Related Compound A (14-Hydroxycodeinone) and Oxycodone Related Compound C (Codeinone)

Solution A: Dissolve 3.45 g of monobasic sodium phosphate in 1000 mL of water. Add 5.41 g of sodium dodecyl sulfate, and mix. Filter, and adjust with 50% (w/v) sodium hydroxide solution to a pH of 7.50 ± 0.05 .

Solution B: Water and phosphoric acid (9:1)

Mobile phase: Prepare a mixture of acetonitrile, methanol, and *Solution A* (15.8: 12.0: 72.2), and adjust with *Solution B* to a pH of 7.80 ± 0.01 .

Diluent: Prepare a mixture of water and *Solution B* (9:1).

Unspiked oxycodone hydrochloride solution: 50 mg/mL of USP Oxycodone Hydrochloride RS in *Diluent*

System suitability solution: 100 μ g/mL of USP Oxycodone Hydrochloride RS and 5 μ g/mL

each of USP Oxycodone Related Compound A RS and USP Oxycodone Related Compound C RS in *Diluent*

Standard solution: 50 mg/mL of USP Oxycodone Hydrochloride RS and 0.5 µg/mL each of USP Oxycodone Related Compound A RS and USP Oxycodone Related Compound C RS in *Diluent*

Sample solution: 50 mg/mL of Oxycodone Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.0-mm × 15-cm; 3.5-µm packing L1

Column temperature: 40°

Flow rate: 0.7 mL/min

Injection volume: 5 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for oxycodone related compound C, oxycodone related compound A, and oxycodone are about 0.44, about 0.85, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4 between oxycodone related compound A and oxycodone related compound C, *System suitability solution*

Tailing factor: NMT 2.0, *System suitability solution*

Relative standard deviation: NMT 20% for oxycodone related compound A and oxycodone related compound C, *Standard solution*

Analysis

Samples: *Diluent*, *Standard solution*, *Unspiked oxycodone hydrochloride solution*, and *Sample solution*

Calculate the percentage of oxycodone related compound A and oxycodone related compound C in the portion of Oxycodone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of oxycodone related compound A or oxycodone related compound C from the *Sample solution*

r_S peak response of oxycodone related compound A or oxycodone related compound C minus the response of the *Unspiked oxycodone hydrochloride solution* from the *Standard solution*

C_S concentration of USP Oxycodone Related Compound A RS or USP Oxycodone Related Compound C RS in the *Standard solution* (mg/mL)

C_U concentration of Oxycodone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxycodone related compound C ^a	0.44	0.001
Oxycodone related compound A ^b	0.85	0.001
Oxycodone	1.0	—
^a Codeinone (C ₁₈ H ₁₉ NO ₃).		
^b 14-Hydroxycodone (C ₁₈ H ₂₁ NO ₄).		

(Procedure 3

2 (IRA 1-Nov-2015)

postponed indefinitely)

Procedure 3

Buffer: Mix 4.0 mL of heptafluorobutyric acid with 2000 mL of water and adjust with ammonium hydroxide to a pH of 2.3 ± 0.1.

Solution A: Methanol and *Buffer* (23:77)

Solution B: Methanol, tetrahydrofuran, and *Buffer* (20:3:77)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
30	0	100
55	0	100
55.1	100	0
65	100	0

Diluent: Mix 3.0 mL of trifluoroacetic acid with 1000 mL of water.

System suitability solution: 0.0067 mg/mL of USP Hydrocodone RS, 0.0067 mg/mL of USP Oxycodone Related Compound A RS, and 3.0 mg/mL of USP Oxycodone Hydrochloride RS in *Diluent*

Standard solution: 0.0067 mg/mL of USP Hydrocodone RS in *Diluent*

Sample solution: 3.0 mg/mL of Oxycodone Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 3-µm packing L1

Column temperature: 38°

Flow rate: 0.8 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between oxycodone and hydrocodone; NLT 1.0 between hydrocodone and oxycodone related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oxycodone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U

= peak response of each impurity from the *Sample solution*

r_S

= peak response of hydrocodone from the *Standard solution*

C_S

= concentration of USP Hydrocodone RS in the *Standard solution* (mg/mL)

C_U

= concentration of Oxycodone Hydrochloride in the *Sample solution* (mg/mL)

M_{r1}

= molecular weight of hydrocodone hydrochloride, 335.83

M_{r2}

= molecular weight of hydrocodone, 299.36

F

= relative response factor (see *Table 4*)

Acceptance criteria: See *Table 4*. Disregard any peaks below 0.03%.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxymorphone hydrochloride	0.54	0.93	0.15
1-Hydroxyoxycodone hydrochloride ^a	0.69	1.00	0.15
6-Oxycodol hydrochloride ^b	0.79	1.16	0.25
Oxycodone hydrochloride	1.00	—	—
Hydrocodone hydrochloride	1.14	1.00	0.50
14-Hydroxycodeinone hydrochloride (oxycodone related compound A hydrochloride) ^c	1.18	0.99	0.25
Noroxycodone hydrochloride ^d	1.26	0.94	0.50
Individual unspecified impurity	—	—	0.10
Total impurities	—	—	1.5

^a 4,5 α -Epoxy-1,14-dihydroxy-3-methoxy-17-methylmorphinan-6-one hydrochloride.

^b 4,5 α -Epoxy-3-methoxy-17-methylmorphinan-6,14-diol hydrochloride.

c 4,5 α -Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-7-ene-6-one (oxycodone related compound A hydrochloride salt).

d 4,5 α -Epoxy-3-methoxy-17-methylmorphinan-6-one hydrochloride.

*(IRA 1-Nov-2015)

SPECIFIC TESTS

• Content of Chloride

Sample solution: 6 mg/mL in methanol

Analysis: To 50 mL of *Sample solution* add 5 mL of glacial acetic acid, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride.

Acceptance criteria: 9.8%–10.4% on the anhydrous, solvent-free basis

• Optical Rotation $\langle 781S \rangle$, *Specific Rotation*

Sample solution: 25 mg/mL of Oxycodone Hydrochloride in water on the anhydrous, solvent-free basis

Acceptance criteria: -137° to -149°

• Water Determination $\langle 921 \rangle$, *Method I*: NMT 7.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

Add the following:

•• **Labeling:** The label states with which *Organic Impurities* procedure the article complies if *Organic Impurities, Procedure 1* is not used. *(IRA 1-Nov-2015)

Change to read:

• **USP Reference Standards $\langle 11 \rangle$**

• USP Hydrocodone RS

*(IRA 1-Nov-2015)

USP Oxycodone RS

USP Oxycodone Hydrochloride RS

USP Oxycodone Related Compound A RS

~~14-Hydroxycodone-~~

• Also known as 14-Hydroxycodone

4,5 α -Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-7-ene-6-one.

$C_{18}H_{19}NO_4$ 313.35

*(IRA 1-Nov-2015)

USP Oxycodone Related Compound C RS

~~Codeinone-~~

• Also known as Codeinone

4,5~~α~~-Epoxy-3-methoxy-17-methylmorphinan-7-ene-6-one.

C₁₈H₁₉NO₃ 297.35

•(IRA 1-Nov-2015)

In-Process Revision

This section contains proposals for adoption as official *USP* or *NF* standards (either proposed *new* standards or proposed *revisions* of current *USP* or *NF* standards). These may be any of the following: (1) proposed revisions placed directly under *In-Process Revision*, or (2) modifications of revisions previously proposed under *In-Process Revision*. Readers should review material in this section and provide comments to the Scientific Liaison using the contact information appearing at the end of each proposal. Information on how to comment can be found under the "Participation" section of www.usp.org. It is important to send comments promptly, using the comment deadline listed after each title.

Briefings Each Proposal is preceded by a Briefing in the following format:

Briefing
Name of Item , citations of the most recent USP publications in which this item appeared. Rationale for the revision. Other relevant information. (For example, if a chromatographic method is being proposed, column specifications and retention times for compounds of interest.) Finally, the Expert Committee acronym (the name of the Scientific Liaison who handled the particular monograph or general chapter, and the USP tracking correspondence number, as shown in the example below:
(Expert Committee Acronym: Liaison Name.)
Correspondence Number—CXXXXX

Symbols Proposed revisions are shown with language proposed for deletion or replacement crossed out. All *USP–NF* revisions use the following symbols that indicate the final destination of the official text:

•new text•

if slated for an *IRA*;

▲new text▲

if slated for *USP–NF*;

■new text■

if slated for a *Supplement to USP–NF*. The same symbols indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as

• •
, or

■ ■
, or

▲▲

, it means that text has been deleted and no new text was proposed to replace it.

In all revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA*, *Supplement*, or the *USP* or *NF* as the publication where the revision will appear if approved. For example, ■

2S (*USP 34*) indicates that the proposed revision is slated for the *Second Supplement to USP 34*, and ▲

USP35 and  *NF30* indicates that the revisions are proposed for *USP 35* and *NF 30*, respectively.

Official Title Changes Where the specification "**Monograph title change**" is found, it indicates that the official title stated after that specification will be substituted for the former title in the appropriate places throughout that monograph once this revision becomes official.

BRIEFING

《 17 》 **Prescription Container Labeling**, *USP 38* page 96. The Nomenclature, Safety, and Labeling Expert Committee proposes revisions to this general chapter to accomplish two objectives:

1. To point to methodologies that simplify administration instructions for oral dosage forms.
2. To provide information on alternative-access methods that can meet the needs of visually impaired individuals.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(NSL: D. Bohannon.)

Correspondence Number—C151044

Comment deadline: July 31, 2015

《 17 》 PRESCRIPTION CONTAINER LABELING

Change to read:

INTRODUCTION

Medication misuse has resulted in more than 1 million adverse drug events per year in the United States. Patients' best source (and often only source) of information regarding the medications they have been prescribed is on the prescription container label. Although other written information and oral counseling ~~sometimes~~

■ ■ 1S (*USP39*)

may be available, the prescription container label must fulfill the professional obligations of the prescriber and pharmacist. These obligations include giving patients the ~~most~~

■ ■ 1S (*USP39*)

essential information ~~needed~~

■ they will need ■ 1S (*USP39*)

to understand how to safely and appropriately use the medication and

■ how ■ 1S (*USP39*)

to adhere to the prescribed medication regimen.

Inadequate understanding of prescription directions for use and auxiliary information

■ provided ■ 1S (*USP39*)

on dispensed containers is widespread. Studies have found that 46% of patients misunderstood one or more dosage instructions, and 56% misunderstood one or more auxiliary warnings. The problem of misunderstanding is particularly

■ common and ■ 1S (USP39)

troublesome in patients with low or marginal literacy and in patients receiving multiple medications that are scheduled for administration using unnecessarily complex, nonstandardized time periods. In one study, patients with low literacy were 34 times more likely to misinterpret prescription medication warning labels. However, even patients with adequate literacy often misunderstand common prescription directions and warnings. In addition, there is great variability in the actual auxiliary warning and supplemental instructional information that has been applied by individual practitioners to the same prescription. The specific evidence to support a given auxiliary statement often is unclear, and patients often ignore such information. The essential

■ 1S (USP39)

need for, and benefit of, auxiliary label information (both text and icons) in

■ for ■ 1S (USP39)

improving patient understanding about safe and appropriate use of their medications vs-

■ requires further study in comparison to ■ 1S (USP39)

explicit, simplified language alone. ~~require further study.~~

■ 1S (USP39)

Lack of universal standards for labeling on dispensed prescription containers is a root cause for

■ of ■ 1S (USP39)

patient misunderstanding, nonadherence, and medication errors. ~~On May 18, 2007, the USP Safe Medication Use Expert Committee established an Advisory Panel to: 1) determine optimal prescription label content and format to promote safe medication use by critically reviewing factors that promote or distract from patient understanding of prescription medication instructions and 2) create universal prescription label standards for format/appearance and content/language.~~

~~In November 2009, the Health Literacy and Prescription Container Labeling Advisory Panel presented its recommendations to the Safe Medication Use Expert Committee, which then requested that USP develop~~

■ USP developed ■ 1S (USP39)

patient-centered label standards for the format, appearance, content, and language of prescription medication instructions to promote patient understanding. These recommendations form the basis of this general chapter.

Note—These standards do not apply when a prescription drug will be administered to a patient by licensed personnel who are acting within their scope of practice.

Change to read:

PRESCRIPTION CONTAINER LABEL STANDARDS TO PROMOTE PATIENT UNDERSTANDING

Organize the Prescription Label in a Patient-Centered Manner

Information shall be organized in a way that best reflects how most patients seek out and understand medication instructions. Prescription container labeling should feature only the most important patient information needed for safe and effective understanding and use.

Emphasize Instructions and Other Information Important to Patients

Prominently display information that is critical for the patient's safe and effective use of the medicine. At the top of the label, specify the patient's name, the drug name (spell out full generic and brand name) and

■ drug ■ 1S (USP39)

strength, and explicit clear directions for use in simple language.

The prescription directions should follow a standard format so

■ that ■ 1S (USP39)

the patient can expect that each element will be

■ presented ■ 1S (USP39)

in a regimented

■ specific, predictable ■ 1S (USP39)

order each time a prescription is received.

■ Use of a methodology that simplifies the administration instructions for the patient's medication should improve the individual's ability to understand prescription instructions, to take the correct dose, and to organize multi-drug regimens. Employing best practices of patient-centered instructions—which utilize the principles of health literacy, medication therapy management, and education, to explicitly describe how to take daily-use, solid dose-form medications—has resulted in improved patient understanding. One such patient-centered method is the universal medication schedule (UMS). The UMS shifts medication-taking into four standardized time periods (morning, noon, evening, bedtime) and uses simplified language and formatting to promote understanding (e.g., “take 1 pill in the morning and 1 pill at bedtime” rather than “take one tablet twice daily”). Such methods may be particularly useful for simplifying daily medication regimens that include multiple oral therapies. [Note—The word “pill” is used in the UMS to enhance health literacy and may not reflect a USP definition for an oral dosage form (see *Compendial Nomenclature, Nomenclature Guidelines* on the USP website at www.usp.org/usp-nf/development-process).]

When oral liquid dosage forms are prescribed, the appropriate dosing component (e.g., oral syringe, dosing cup) shall be provided to the patient or caregiver to accurately measure and administer the oral medication. The graduations on the component shall be legible and indelible, and the associated volume markings shall be in metric units and limited to a single measurement scale that corresponds with the dose instructions on the prescription container label (see

Packaging and Storage Requirements (659)). ■ 1S (USP39)

Other, less-critical but important content (e.g., pharmacy name and phone number, prescriber name, fill date, refill information, expiration date, prescription number, drug quantity, physical description, and evidence-based auxiliary information) should not supersede critical patient information. Such less-critical information should be placed away from dosing instructions (e.g., at the bottom of the label or in another less prominent location) because it ~~distracts~~

■ can distract ■ 1S (USP39)

patients, which can impair their recognition and understanding.

Simplify Language

The language on the label should be clear, simplified, concise, and familiar, and should be used in a standardized manner. Only common terms and sentences should be used. Do not use unfamiliar words (including Latin terms) or medical jargon.

Use of readability formulas and software is not recommended to simplify

■ for simplifying ■ 1S (USP39)

short excerpts of text like

■ such as ■ 1S (USP39)

those on prescription labels. Instead, use simplified, standardized sentences that have been

■ were ■ 1S (USP39)

developed to ensure ease of understanding the instructions correctly

■ ■ 1S (USP39)

by seeking feedback from samples of diverse consumers.

■ Such language will promote correct understanding of the instructions. ■ 1S (USP39)

Give Explicit Instructions

Instructions for use (i.e., the SIG or signatur) should clearly separate the dose itself from the timing of each dose in order to explicitly convey the number of dosage units to be taken and when (e.g., specific time periods each day such as morning, noon, evening, and bedtime). Instructions shall include specifics on time periods. Do not use alphabetic characters for numbers. For example, write "Take 2 tablets in the morning and 2 tablets in the evening" rather than "Take two tablets twice daily").

Whenever available, use standardized directions (e.g., write "Take 1 tablet in the morning and 1 tablet in the evening" if the prescription reads b.i.d.). Vague instructions based on dosing intervals such as twice daily or 3 times daily, or hourly intervals such as every 12 hours, generally should be avoided because such instructions are implicit rather than explicit, they may involve numeracy skills, and patient interpretation may vary

■ differ ■ 1S (USP39)

from prescriber intent. Although instructions that use specific hourly times (e.g., 8 a.m. and 10 p.m.) may seem to be more easily understood than implicit vague instructions, recommending dosing by

■ at ■ 1S (USP39)

precise hours of the day is less readily understood and may present greater adherence issues due to individual lifestyle patterns, e.g., shift work,

■ (due to individual lifestyle patterns such as shift work) ■ 1S (USP39)

than more general time frames such as in the morning, in the evening, after breakfast, with lunch, or at bedtime. Consistent use of the same terms should help avoid patient confusion.

■ A set of standardized, explicit instructions (the universal medication schedule, UMS) were developed and tested in English and other languages to improve patient understanding.¹

■ 1S (USP39)

Ambiguous directions such as "take as directed" should be avoided unless clear and unambiguous supplemental instructions and counseling are provided (e.g., directions for use that will not fit on the prescription container label). A clear statement referring the patient to

such supplemental materials should be included on the container label.

Include Purpose for Use

If the purpose of the medication is included on the prescription, it should be included on the prescription container label unless the patient prefers that it not appear.

■ Practitioners should ~~■ 1S (USP39)~~

always ask patients their preference when ~~prescriptions are submitted for filling~~

■ writing the prescription. ■ 1S (USP39)

Confidentiality and FDA approval for intended use (e.g., labeled ~~vs.~~

■ versus ■ 1S (USP39)

off-label use) may limit inclusion of the purpose on labels. Current evidence supports inclusion of purpose-for-use language in clear, simple terms (e.g., "for high blood pressure" rather than "for hypertension").

Limit Auxiliary Information

Auxiliary information on the prescription container label should be evidence-based

■ and presented ■ 1S (USP39)

in simple explicit language that is minimized to avoid distracting patients with nonessential information. Most patients, particularly those with ~~low~~

■ limited ■ 1S (USP39)

literacy, pay little attention to auxiliary information. The information should be presented in a standardized manner and should be critical for patient understanding and safe medication use (e.g., warnings and critical administration alerts). Icons are frequently misunderstood by patients. In addition, icons that provide abstract imagery for messages that are difficult to ~~visually depict~~

■ depict visually ■ 1S (USP39)

may be ineffective at improving understanding compared with simplified text alone. Use only icons for which there is adequate evidence, through consumer testing, that they improve patient understanding about correct use. Evidence-based auxiliary information, both text and icons, should be standardized so that it is applied consistently and does not depend on individual practitioner choice.

Address Limited English Proficiency

Whenever possible, the directions for use on a prescription container label should be provided in the patient's preferred language. Otherwise there is a risk of misinterpretation of instructions by patients with limited English proficiency, which could lead to medication errors and adverse health outcomes. Additionally, whenever possible, directions for use should appear in English as well, to facilitate counseling; the drug ~~name~~

■ name(s) ■ 1S (USP39)

shall be in English so that emergency personnel and other intermediaries can have quick access to the information.

■ Standardized translations of universal medication schedule instructions are available.²

■ 1S (USP39)

Translations of prescription medication labels should be produced using a high-quality translation process. An example of a high-quality translation process is:

- Translation by a trained translator who is a native speaker of the target language
- Review of the translation by a second trained translator and reconciliation of any differences
- Review of the translation by a pharmacist who is a native speaker of the target language and reconciliation of any differences
- Testing of comprehension with target audience.

If a high-quality translation process cannot be provided, labels should be printed in English, ~~and~~ **with the use of** **1S (USP39)**

trained interpreter services ~~used~~

1S (USP39)

whenever possible to ensure patient comprehension. The use of computer-generated translations should be limited to programs with demonstrated quality because dosage instructions can be ~~inconsistent and~~

translated inconsistently or incorrectly, which is **1S (USP39)**

potentially hazardous. Standardized translated instructions and technology advances are needed to ensure the accuracy and safety of prescription container labeling for patients with ~~low~~

limited **1S (USP39)**

English proficiency.

Improve Readability

Labels should be designed and formatted so they are easy to read. Currently, no strong evidence supports the superiority, in

terms of **1S (USP39)**

legibility, of serif ~~vs.~~

versus **1S (USP39)**

sans serif typefaces, ~~so~~

therefore **1S (USP39)**

simple uncondensed fonts of either type can be used.

Optimize typography by using the following techniques:

- High-contrast print (e.g., black print on white background).
- Simple, uncondensed familiar fonts with sufficient space within letters and between letters (e.g., Times **New** **1S (USP39)**

Roman or Arial).

- Sentence case (i.e., punctuated like a sentence in English: Initial capital

letter **1S (USP39)**

followed by lower-case words except proper nouns).

- Large font size (e.g., minimum 12-point Times

New **1S (USP39)**

Roman or 11-point Arial) for critical information. Note that point size is not the actual size of the letter, so two fonts with the same nominal point size can have different actual letter sizes.

~~x-height, the height of the lower-case x in typeface,~~

■ The height of the typeface, x-height, ■ 1S (USP39)

has been used as a more accurate indicator of apparent size than point size. For example, for a given point size, the x-height and apparent size of Arial are actually bigger than those for Times

■ New ■ 1S (USP39)

Roman. Do not use type smaller than 10-point Times

■ New ■ 1S (USP39)

Roman or the equivalent size øf

■ in ■ 1S (USP39)

another font. Older adults, in particular, have difficulty reading small print.

- Adequate white space between lines of text (25%–30% of the point size).

- White space to distinguish

■ to separate ■ 1S (USP39)

sections on the label such as directions for use vs-

■ versus ■ 1S (USP39)

pharmacy information.

- Horizontal text only.

Other measures that can also improve readability:

- If possible,

- ■ 1S (USP39)

Minimize the need to turn the container in order to read lines of text.

- Never truncate or abbreviate critical information.

- ■ Use ■ 1S (USP39)

highlighting, bolding, and other typographical cues should

■ cues to ■ 1S (USP39)

preserve readability (e.g., high-contrast print and light color for highlighting) and should

■ ■ 1S (USP39)

emphasize patient-centric

■ patient-centered ■ 1S (USP39)

information or information that facilitates adherence (e.g., refill ordering).

- Limit the number of colors used for highlighting (e.g.,

■ i.e., ■ 1S (USP39)

no more than one or

■ ■ 1S (USP39)

two).

- Use of separate lines to distinguish when each dose should be taken.

Alternative-Access Methods to Address Visual Impairment

Patients with visual impairment who are unable to read printed prescription container labels often report inadvertently taking the wrong medication or amount, or taking it at the wrong time or under the wrong instructions, compromising their own safety. Similarly, if a caregiver is visually impaired, the patient they care for is at risk for medication errors. The magnitude of this problem increases with aging, as the risk of visual impairment and the number of prescribed medications both increase with age.

- Follow standards for patient-centered prescription labels. ■ 1S (USP39)

- Provide alternative access for visually impaired patients (e.g.,

■ alternative-access methods include ■ 1S (USP39)

tactile, auditory, or enhanced visual systems that may employ advanced mechanics of assistive technology).

■ 3

- Enhance communication between the pharmacist and visually impaired patients (and their designated representatives) such that the pharmacist can explain alternative-access options and together they can identify those best suited to the patient's needs.

- Once an alternative-access method is identified for the individual patient, the pharmacist shall provide the service or direct the patient to a pharmacy that offers that type of alternative access.

- Ensure that duplicate accessible labels preserve the integrity of the print prescription drug container label and provide the same sequence of information as the printed label.

- Follow specific best practices for each respective alternative-access format employed.

■ 1S (USP39)

■ 1

Explicit and Standardized Prescription Medicine Instructions. December 2014. Agency for Healthcare Research and Quality, Rockville, MD. <http://www.ahrq.gov/professionals/quality-patient-safety/pharmhealthlit/prescriptionmed-instr.html>. ■ 1S (USP39)

■ 2

Available at <http://www.ahrq.gov/professionals/quality-patient-safety/pharmhealthlit/prescriptionmed-instr.html>. ■ 1S (USP39)

■ 3

See Working Group Recommendations from Access Board Working Group on Accessible Prescription Drug Container Labels: <http://www.access-board.gov/guidelines-and-standards/health-care/about-prescription-drug-container-labels/working-group-recommendations>. ■ 1S (USP39)

BRIEFING

《 476 》 **Organic Impurities in Drug Substances and Drug Products**, PF 40(3) [May–June 2014]. This revision is proposed on the basis of public comments received on its previous publication in PF. As part of an ongoing monograph modernization initiative, USP is updating the general chapter *Impurities in Drug Substances and Drug Products* 《 1086 》 and proposing this new chapter that addresses organic impurities testing for articles subject to applicable monographs in compendia of the USP, including USP–NF. This new chapter has been created to align with current scientific and regulatory approaches and to help ensure the appropriate control of organic impurities and degradation products in drug substances and drug products. The goal is to provide a science-based approach for the control of impurities in relevant monographs, and thereby ensure the quality of the product as it relates to safety and efficacy. Over time, *Ordinary Impurities* 《 466 》 may be used less frequently and may be withdrawn.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCPA: A. Hernandez-Cardoso.)
Correspondence Number—C157665

Comment deadline: July 31, 2015

Change to read:

{ 476 }

■ CONTROL OF ■_{1S} (USP39)

ORGANIC IMPURITIES IN DRUG SUBSTANCES AND DRUG PRODUCTS

Change to read:

INTRODUCTION

~~This chapter covers formal requirements for controlling organic process impurities and degradation products in drug substances, and degradation products in drug products. Impurities are critical quality attributes of drug substances and drug products because they have the potential to affect safety and efficacy. Therefore, all drug substances and drug products are subject to control of impurities. When a detected impurity is not described in the individual monograph, the manufacturer is responsible for developing appropriate specifications (analytical procedures and acceptance criteria).~~

~~For medicines marketed in the United States, drug substances and drug products covered by this chapter can, in general, be placed in one of two categories: (1) those that are covered by ICH and/or Food and Drug Administration (FDA) guidances, for example, most New Drug Application (NDA) and Abbreviated New Drug Application (ANDA) products; and (2) those that are not covered by drug applications, for example, over the counter (OTC) products.~~

~~Impurities that arise from excipients present in the drug product or that are leached from the container-closure system are not covered in this chapter. Also excluded from this chapter are veterinary products, biological/biotechnological products, peptides, oligonucleotides, fermentation products and semisynthetic products derived from them, radiopharmaceuticals, herbal products, and crude products of animal or plant origin. These products are excluded from this chapter because impurities of these types of drug substances and drug products may require special considerations. Current regulatory guidance documents, such as those from ICH, and sound scientific principles may be used to control the level of impurities in products that are excluded from this chapter.~~

~~Also excluded from this chapter are the following:~~

- ~~• Extraneous contaminants that should not occur in drug substances or drug products and are more appropriately addressed as good manufacturing practice issues~~
- ~~• Polymorphic forms~~
- ~~• Impurities arising from residual solvents (see *Residual Solvents* ~~{467}~~)~~
- ~~• Elemental impurities (see *Elemental Impurities—Limits* ~~{232}~~ and *Elemental Impurities—Procedures* ~~{233}~~).~~

IDENTIFICATION OF IMPURITIES IN DRUG SUBSTANCES AND DRUG PRODUCTS

Drug substance and drug product organic impurities shall include process-related impurities that result from the manufacturing process, and degradation products observed during manufacture and stability studies. Identification of impurities shall be based on sound scientific appraisal of potential degradation pathways in the drug substance and drug product, including those impurities that arise from interactions of the drug with the environment, excipients, or the primary container closure system.

Impurities observed in stability studies conducted at the recommended storage conditions shall be identified when above the identification threshold, which can be established using currently applicable regulations or other scientific means. Impurities present at a level below the identification threshold generally do not require identification.

ANALYTICAL PROCEDURES FOR IMPURITIES AND DEGRADATION PRODUCTS

Manufacturers shall validate analytical procedures and demonstrate their suitability for the detection and quantitation of impurities. Manufacturers of drug substances and drug products shall refer to current applicable regulatory guidances and shall develop control strategies for establishing acceptance criteria for impurities. These acceptance criteria shall be justified with appropriate safety considerations.

Analytical procedures for OTC drug products may require a case-by-case approach, depending on the diversity and complexity of dosage forms. These procedures shall be verified or validated and shall be suitable for their intended purpose.

For impurities that are known or suspected to be highly toxic (e.g., genotoxic) or that produce undesired pharmacological effects, the quantitation/detection limit of the analytical procedures shall be commensurate with the acceptance criteria.

REPORTING IMPURITIES AND DEGRADATION PRODUCTS

Impurities present at a level above the reporting threshold (i.e., the disregard limit) shall be reported according to the relevant analytical method(s). The reporting threshold can be established using currently applicable regulations/guidances or other acceptable scientific means.

Quantitative test results shall be reported as numerical values and rounded according to conventional rules (see *General Notices 7.20*). Impurities at a level greater than the reporting threshold shall also be summed and reported as total impurities.

SETTING ACCEPTANCE CRITERIA FOR IMPURITIES AND DEGRADATION PRODUCTS

Acceptance criteria shall be set for all impurities expected to be present at levels above the reporting thresholds, and these criteria shall be based on an applicable guidance or on scientific rationale. Acceptance criteria should not be based solely on process capability. Acceptance criteria shall cover the drug substance through its intended period of use and the drug product through its shelf life. Generally, drug substance impurities need not be monitored or specified in drug products unless they are also degradation products.

~~The acceptance criteria shall include the following, where applicable:~~

- ~~• Each specified identified impurity and degradation product~~
- ~~• Each specified unidentified impurity and degradation product~~
- ~~• Any unspecified impurity or degradation product with an acceptance criteria NMT the identification threshold~~
- ~~• Total impurities and degradation products.~~

~~For a given impurity, manufacturers shall establish the acceptance criteria based on its qualified level, its increase during stability studies and proposed shelf life, and its labeled storage conditions.~~

~~For OTC products, manufacturers may need to determine acceptance criteria on a case-by-case basis. They also can consider the historical safety record of drug substances; the quantitative structure-activity relationship (QSAR); the route of administration; and the likely consumption patterns, such as duration of treatment for the patient population.~~

~~QUALIFICATION OF IMPURITIES AND DEGRADATION PRODUCTS~~

~~Establishment of acceptance criteria for impurities should focus on safety considerations. The level of any impurity or degradation product present in a drug substance or drug product that has been adequately tested in safety or clinical studies is generally considered qualified. However, highly toxic (e.g., genotoxic) impurities or degradation products shall be addressed using applicable guidances. Impurities or degradation products that are also significant metabolites are generally considered qualified.~~

~~Qualification of impurities shall be based on applicable guidances, scientific rationale, or history of product use. Higher or lower thresholds for qualification of impurities may be appropriate for some products based on scientific rationale and level of concern, including drug class effects and clinical experience.~~

~~For additional guidance and definition of terms, see *Impurities in Drug Substances and Drug Products* (1086).~~

INTRODUCTION

This chapter covers requirements for controlling organic impurities in drug substances and drug products described in *USP* monographs. All drug substances and drug products are subject to control of organic impurities. A threshold-based approach described in the ICH Q3A and Q3B guidances may be used for the control of organic impurities in drug substances or drug products generated during the manufacturing process or storage. The organic impurities to be controlled in the drug substance are the process impurities and degradation products. The organic impurities to be controlled in the drug product are only the degradation products of the drug substance or those resulting from the interaction of the drug substance with excipients and/or the primary container closure. Drug substance process impurities need not be controlled in the drug product unless they are also degradation products.

This chapter covers drug substances and drug products marketed in the United States based on approval by the Food and Drug Administration (FDA) either via New Drug Applications (NDAs) or Abbreviated New Drug Applications (ANDAs) or through the FDA over-the-counter (OTC)

monograph system.

This chapter does not cover veterinary products, biological/biotechnological products, peptides, oligonucleotides, fermentation products and semisynthetic products derived from them, polymorphic forms, radiopharmaceuticals, herbal products, or crude products of animal or plant origin. In addition, impurities present in the drug product originating from excipients or leached from the container–closure system, inorganic/elemental impurities, and residual solvents are out of the scope of this chapter.

If a new impurity is uncovered or when the level of a known impurity increases as compared to an existing monograph, the manufacturer is responsible for evaluating the impact on the safety and efficacy of the drug. If an individual monograph is inadequate to control an impurity, the manufacturer is responsible for developing and validating appropriate analytical procedures, establishing acceptance criteria, and communicating with USP.

IDENTIFICATION OF IMPURITIES AND DEGRADATION PRODUCTS

Impurities present at or above the identification threshold for drug substances and drug products at release and on storage shall be identified. The identification threshold can be established using current applicable regulatory guidances or other acceptable scientific means. Lower thresholds may be required for impurities known or suspected to be highly toxic (e.g., genotoxic) or that produce undesired pharmacological effects.

ANALYTICAL PROCEDURES FOR IMPURITIES AND DEGRADATION PRODUCTS

Manufacturers shall validate analytical procedures and must demonstrate their suitability for the detection and quantitation of impurities in drug substances and drug products. Manufacturers shall develop acceptance criteria for impurities justified by appropriate safety considerations and consistent with current applicable regulatory guidances.

Analytical procedures for FDA OTC monograph drug products may require a case-by-case approach, depending on the complexity of dosage forms. These procedures shall be verified or validated to be suitable for their intended purpose.

A lower limit of detection and limit of quantitation may be required for impurities known or suspected to be highly toxic (e.g., genotoxic) or to produce undesired pharmacological effects.

REPORTING IMPURITIES AND DEGRADATION PRODUCTS

Impurities present at or above the reporting threshold shall be reported according to the relevant analytical procedure. The reporting threshold can be established using current applicable regulatory guidances or other acceptable scientific means.

Impurity results shall be reported as numerical values and rounded according to conventional rules (see *General Notices and Requirements*, 7.20). Individual impurity values shall be summed and reported as total impurities.

SETTING ACCEPTANCE CRITERIA FOR IMPURITIES AND DEGRADATION PRODUCTS

Acceptance criteria shall be set for all impurities present at or above the qualification

thresholds for drug substances and drug products at release and through the shelf life. The acceptance criteria shall be based on applicable guidances or other acceptable scientific means, with safety as the primary consideration and not solely based on process capability. Drug substance process impurities need not be monitored or specified in drug products unless they are also degradation products.

The acceptance criteria for drug substances shall include the following, where applicable:

- Each specified identified impurity
- Each specified unidentified impurity
- Any unspecified impurity with an acceptance criterion of NMT the identification threshold
- Total impurities

The acceptance criteria for drug products shall include the following, where applicable:

- Each specified identified degradation product
- Each specified unidentified degradation product
- Any unspecified degradation product with an acceptance criterion of NMT the identification threshold
- Total degradation products

For FDA OTC monograph products, manufacturers may need to determine acceptance criteria on a case-by-case basis. Factors such as quantitative structure-activity relationship (QSAR) of the drug substance, route of administration, the likely consumption patterns such as duration of treatment and the patient population, and historical safety of the drug may be considered in justifying the acceptance criteria.

Acceptance criteria for highly toxic (e.g., genotoxic) impurities or degradation products shall be addressed using current applicable guidances.

QUALIFICATION OF IMPURITIES AND DEGRADATION PRODUCTS

Qualification of impurities shall be based on safety, applicable guidances, scientific rationale, or history of product use. Higher or lower qualification thresholds may be appropriate for some impurities based on scientific rationale.

The level of any impurity or degradation product present in a drug substance or drug product that has been adequately tested in safety and/or clinical studies would be considered qualified. Impurities or degradation products that are also significant metabolites present in animal and/or human studies are also generally considered qualified.

(For additional guidance and definition of terms, see *Impurities in Drug Substances and Drug Products* { 1086 }.)

■ 1S (USP39)

BRIEFING

{ 659 } **Packaging and Storage Requirements**, *USP 38* page 443. The General Chapters—Packaging, Storage, and Distribution Expert Committee proposes this revision to update and clarify definitions related to proper packaging and storage.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

The following Briefing list includes monographs and/or chapters that both reference the General Chapter under revision and require revision to keep references to the General Chapter accurate. Other monographs and/or chapters may also be listed, even where the reference to the General Chapter remains unchanged, as additional notice to stakeholders where there is believed to be potential for the change in the general chapter itself to affect pass-fail determinations for particular monograph articles. <http://www.usp.org/usp-nf/notices/general-chapter-packaging-and-storage-requirements>

(GCPS: D. Hunt.)

Correspondence Number—C141037

Comment deadline: July 31, 2015

〈 659 〉 PACKAGING AND STORAGE REQUIREMENTS

Delete the following:

■ **(Chapter to become official May 1, 2016)**

■ 1S (*USP39*)

Delete the following:



Every monograph in the *USP* and *NF* shall have packaging and storage requirements. For the packaging portion of the statement, the choice of containers is given in this chapter. For drug product packaging requirements, definitions are provided to guide selection and adaptation. For active pharmaceutical ingredients (APIs), the choice would be tight, well-closed or, where needed, a light-resistant container. For excipients, given their typical presentation as large-volume commodity items (containers ranging from drums to tank cars), a well-closed container is an appropriate default.

Where no specific directions or limitations are provided in the article's labeling, articles shall be protected from moisture, freezing, and excessive heat and, where necessary, from light during shipping and distribution. Drug substances are exempt from this standard. ▲*USP38*

Change to read:

PACKAGING

~~Packaging must not interact physically or chemically with official articles in any way that causes their safety, identity, strength, quality, or purity to fail to conform to requirements.~~

▲Packaging container choices are given in this chapter. For drug products and active pharmaceutical ingredients (APIs), the container choices are tight, well-closed, or, where needed, light-resistant. For excipients, given their typical presentation as large-volume commodity items (containers ranging from drums to tank cars), a well-closed container is an appropriate default. For articles other than drug substances and drug products, where no specific directions or limitations are provided, articles shall be protected from moisture,

freezing, and excessive heat, and, where necessary, from light during shipping and distribution.

The compendial requirements for the use of specified containers apply also to articles as packaged by the pharmacist or other dispenser, unless otherwise indicated in the individual monograph. **▲USP38**

GENERAL DEFINITIONS

Packaging system (also referred to as a container closure system): The sum of packaging components that together contain and protect the article. This includes primary packaging components and secondary packaging components, if the latter is intended to provide additional protection.

Container: A receptacle that holds an intermediate compound, active pharmaceutical ingredient, excipient, or dosage form and is

▲or may be ▲USP38

in direct contact with the articles.

▲The immediate container is that which is in direct contact with the article at all times. The closure is a part of the container. Before being filled, the container should be clean. Special precautions and cleaning procedures may be necessary to ensure that each container is clean and that extraneous matter is not introduced into or onto the article. **▲USP38**

Packaging component: Any single part of the package or container closure system including the container (e.g., ampuls, prefilled syringes, vials, bottles); container liners (e.g., tube cartridge liners); closures (e.g., screw caps, stoppers); ferrules and overseals; closure liners; inner seals; administration ports; overwraps; administration accessories; and labels.

Primary packaging component: Packaging components that are in direct contact or may become in direct contact with the article.

Secondary packaging component: Packaging components that are not and will not be in direct contact with the article.

▲▲USP38

Tertiary packaging: Packaging components that are not in direct contact with the article but facilitate the handling and transport in order to prevent damage from physical handling and storage conditions to which the article is subjected.

Materials of construction: Refers to the materials (e.g., glass, plastic, elastomers, metal) used to manufacture a packaging component.

Multiple-dose

▲container ▲USP38

(also referred to as multi-dose): A packaging system that permits withdrawal of successive portions of an article for parenteral administration without changing the safety, strength, quality, or purity of the remaining portion. See

▲

~~Multi-Dose Containers in Container Content for Injections (697):~~ ~~▲USP38~~

Multiple-unit

~~▲container:~~ ~~▲USP38~~

~~A packaging system that permits withdrawal of successive portions of an article without changing the safety, strength, quality, or purity of the remaining portion.~~

Single-unit

~~▲container:~~ ~~▲USP38~~

~~A packaging system that holds a quantity of an article intended for administration as a single dose or a single finished device intended for~~

~~▲use promptly after the container is opened. Preferably, the immediate container and/or the outer container or protective packaging shall be so designed as to show evidence of any tampering with the contents.~~ ~~▲USP38~~

Single-dose

~~▲container:~~

~~A single-dose container is a container of sterile medication for parenteral administration (injection or infusion) that is not required to meet the antimicrobial effectiveness testing criteria. [Note—For this definition only, container is synonymous with packaging system and container closure system.] A single-dose container is designed for use with a single patient as a single injection/infusion.³ A single-dose container is labeled as such and, when space permits, should include appropriate discard instructions on the label. Examples of single-dose containers are vials, ampuls, and prefilled syringes.~~ ~~▲USP38~~

Unit-dose

~~▲container:~~ ~~▲USP38~~

~~A single-unit packaging system for an article intended for administration by other than the parenteral route as a single dose.~~

Unit-of-use

~~▲container:~~ ~~▲USP38~~

~~A packaging system that contains a specific quantity of an article that is intended to be dispensed as such without further modification except for the addition of appropriate labeling. Unit-of-use packaging may not be repackaged for sale.~~

Pharmacy bulk package:A

~~▲packaging system~~ ~~▲USP38~~

~~of a sterile preparation for parenteral use that contains many single doses. The contents are intended for use in a pharmacy admixture program and are restricted to the preparation of admixtures for infusion or, through a sterile transfer device, for the filling of empty sterile syringes.~~

~~▲The closure shall be penetrated only one time after constitution, if necessary,~~ ~~▲USP38~~

~~with a suitable sterile transfer device or dispensing set that allows measured dispensing of the contents. The *Pharmacy bulk package* is to be used only in a suitable work area such as a~~

~~laminar flow hood (or an equivalent clean air compounding area).~~

Designation as a *Pharmacy bulk package* is limited to

~~▲Injection, for Injection, or Injectable Emulsion dosage forms as defined in~~

~~Nomenclature (1121), General Nomenclature Forms. ▲USP38~~

~~*Pharmacy bulk package*, although containing more than one single dose, is exempt from the multiple-dose container volume limit of 30 mL and the requirement that it contains a substance or suitable mixture of substances to prevent the growth of microorganisms.~~

~~▲See~~

~~Labeling (7) for labeling requirements. ▲USP38~~

Small-volume injections:

~~▲An injection that ▲USP38~~

~~is packaged in containers labeled as containing 100 mL or less.~~

Large-volume injections:

~~▲An ▲USP38~~

~~injection that is intended for intravenous use, and is packaged in containers labeled as containing more than 100 mL.~~

Child-resistant

~~▲packaging: ▲USP38~~

~~A packaging system designed or constructed to meet Consumer Product Safety Commission standards pertaining to opening by children (16 CFR §1700.20).~~

Senior-friendly

~~▲packaging: ▲USP38~~

~~A packaging system designed or constructed to meet Consumer Product Safety Commission standards pertaining to opening by senior adults (16 CFR §1700.20).~~

Tamper-evident

~~▲packaging: ▲USP38~~

~~A packaging system that may not be accessed without obvious destruction of the seal or some portion of the packaging system.~~

~~▲Tamper-evident packaging shall be used for a sterile article intended for ophthalmic or otic use, except where extemporaneously compounded for immediate dispensing on prescription.~~

~~Articles intended for sale without prescription are also required to comply with the tamper-evident packaging and labeling requirements of the FDA where applicable. Preferably, the immediate container and/or the outer container or protective packaging used by a manufacturer or distributor for all dosage forms that are not specifically exempt is designed so as to show evidence of any tampering with the contents.~~

~~**Hermetic container:** A packaging system that is impervious to air or any other gas under the ordinary or customary conditions of handling, shipment, storage, and distribution.~~

~~▲USP38~~

Tight**▲container:**▲USP38

A packaging system that protects the

▲contents▲USP38

—from contamination by extraneous liquids, solids, or vapors; from loss of the article; and from efflorescence, deliquescence, or evaporation under the ordinary or customary conditions of handling, shipment, storage, and distribution

▲and is capable of tight reclosure. Where a tight container is specified, it may be replaced by a hermetic container for a single dose of an article. [Note—

Where packaging and storage in a tight container or well-closed container is specified in the individual monograph, the container used for an article when dispensed on prescription meets the requirements in *Containers—Performance Testing* ~~(671)~~.]▲USP38

Well-closed**▲container:**▲USP38

A packaging system that protects the

▲contents▲USP38

—from contamination by extraneous solids

▲▲USP38

—and from loss of the article under the ordinary or customary conditions of handling, shipment, storage, and distribution. See *Containers—Performance Testing* ~~(671)~~.

Light-resistant**▲container:**▲USP38

A packaging system that protects

▲the contents▲USP38

—from the effects of light by virtue of the specific properties of the material of which it is composed, including any coating applied to it. A clear and colorless or a translucent container may be made light-resistant by means of an opaque covering or by use of secondary packaging, in which case the label of the container bears a statement that the opaque covering or secondary packaging is needed until the articles are to be used or administered.

▲Where it is directed to “protect from light” in an individual monograph, preservation in a light-resistant container is intended. See

Containers—Performance Testing ~~(671)~~, *Light Transmission Test*.

Black closure system or black bands:The use of a black closure system on a vial (e.g., a black cap-overseal and a black ferrule to hold the elastomeric closure) or the use of a black band or series of bands above the constriction on an ampul is prohibited, except for *Potassium Chloride for Injection Concentrate*. See *Labeling* ~~(7)~~.

▲USP38**INJECTION PACKAGING**

Validation of container-closure integrity must demonstrate no penetration of microbial contamination or chemical or physical impurities. In addition, the solutes and the vehicle must maintain their specified total and relative quantities or concentrations when exposed to

anticipated extreme conditions of manufacturing and processing, storage, shipment, and distribution. Closures for multiple-dose packaging systems permit the withdrawal of the contents without removal or destruction of the closure. The closure permits penetration by a needle and, upon withdrawal of the needle, closes at once, protecting the contents against contamination. Validation of the multiple-dose container closure integrity must include verification that such a package prevents microbial contamination or loss of product contents under anticipated conditions of multiple entry and use.

Piggyback packaging systems are usually intravenous infusion container closure systems used to administer a second infusion through a connector of some type or an injection port on the administration set of the first fluid, thereby avoiding the need for another injection site on the patient's body. Piggyback packaging systems also are known as secondary infusion containers.

The volume of injection in a single-dose packaging system provides the amount specified for one-time parenteral administration and in no case is more than sufficient to permit the withdrawal and administration of 1 L.

Preparations intended for intraspinal, intracisternal, or peridural administration are packaged only in single-dose packaging systems.

Unless otherwise specified in the individual monograph, a multiple-dose packaging system contains a volume of injection sufficient to permit the withdrawal of NMT 30 mL.

The following injections are exempt from the 1-L restriction of the foregoing requirements relating to packaging:

1. Injections packaged for extravascular use as irrigation solutions or peritoneal dialysis solutions.
2. Injections packaged for intravascular use as parenteral nutrition or as replacement or substitution fluid to be administered continuously during hemofiltration.

Injections packaged for intravascular use that may be used for intermittent, continuous, or bolus replacement fluid administration during hemodialysis or other procedures, unless excepted above, must conform to the 1-L restriction. Injections labeled for veterinary use are exempt from packaging and storage requirements concerning the limitation to single-dose packaging systems and the limitation on the volume of multiple-dose containers.

Sterile solids packaging: Containers, including the closures, for dry solids intended for injection do not interact physically or chemically with the preparation in any manner to alter the strength, quality, or purity beyond the official requirements under the ordinary or customary conditions of handling, shipment, storage, sale, and use. A packaging system for a sterile solid permits the addition of a suitable solvent and withdrawal of portions of the resulting solution or suspension in such manner that the sterility of the product is maintained. Where the Assay in a monograph provides a procedure for the *Sample solution*, in which the total withdrawable contents are to be withdrawn from a single-dose packaging system with a hypodermic needle and syringe, the contents are to be withdrawn as completely as possible into a dry hypodermic syringe of a rated capacity not exceeding three times the volume to be withdrawn and fitted with a 21-gauge needle NLT 2.5 cm (1 inch) in length, with care being taken to expel any air bubbles, and discharged into a container for dilution and assay.

MEDICAL GAS PACKAGING

Gas cylinder: A gas cylinder is a metallic packaging system constructed of steel or aluminum designed to hold medical gases under pressure. Medical gases include Carbon Dioxide USP, Helium USP, Medical Air USP, nitric oxide, Nitrous Oxide USP, Nitrogen NF, and Oxygen USP. As a safety measure, for carbon dioxide, cyclopropane, helium, medical air, nitrous oxide, and oxygen, the Pin Index Safety System of matched fittings is recommended for cylinders of Size E or smaller.

ASSOCIATED COMPONENTS

Many associated components are graduated for dose administration. It is the responsibility of the manufacturer to ensure that the appropriate dosing component is provided or that a general purpose component, such as those described in this section, is specified for delivering the appropriate dose with the intended accuracy. The graduations should be legible and indelible.

Graduated associated components described in this section are for general use. Graduated markings should be legible, indelible, and on an extraoral nonproduct contact surface. Under ideal conditions of use, the volume error incurred in measuring liquids for individual dose administration by means of such graduated components should be NMT 10% of the indicated amount of the liquid preparation with which the graduated component will be used. Few liquid preparations have the same surface and flow characteristics. Therefore, the volume delivered varies materially from one preparation to another.

Polymers and ingredients added to polymers that are used in the fabrication of associated components must conform to the requirements in the applicable sections of the Code of Federal Regulations, Title 21, *Indirect Food Additives*.

Dosing cup: A measuring device consisting of a small cup that is packaged with oral liquid articles or that may be sold and purchased separately.

Dosing spoon: A measuring device consisting of a bowl and a handle that is packaged with oral liquid articles or that may be sold and purchased separately. The handle may be a graduated tube.

Medicine dropper: A measuring device consisting of a transparent or translucent barrel or tube that is generally fitted with a collapsible bulb. It is packaged with oral liquid articles or may be sold and purchased separately.

Droppers typically vary in capacity; however, the delivery end should be a round opening having an external diameter of about 3 mm. The barrel may be graduated. [Note—Few medicinal liquids have the same surface and flow characteristics as water, and therefore the size of drops varies materially from one preparation to another.]

Oral syringe: A measuring device consisting of a plunger and barrel made of suitable rigid, transparent or translucent plastic material and a seal on the end. It is packaged with oral liquid articles or may be sold and purchased separately. The syringe should expel a measured amount of a liquid article directly into the patient's mouth. Finger grips located at the open end of the barrel should be the appropriate size, shape, and strength, and should allow the syringe to be held securely during use. The barrel may be graduated.

Teaspoon: A measuring device consisting of a shallow bowl, oval or round, at the end of a

handle. A teaspoon has been established as containing 4.93 ± 0.24 mL. For the practice of administering articles, the teaspoon may be regarded as representing a volume of 5 mL.

Articles intended for administration by teaspoon should be formulated on the basis of dosage in 5 mL units, such that any component used to administer liquid articles should deliver 5 mL wherever a teaspoon calibration is indicated. A household spoon is not an acceptable alternative to the graduated teaspoon described herein.

POISON PREVENTION PACKAGING ACT (PPPA)

This act requires special packaging of most human oral prescription drugs, oral controlled drugs, certain non-oral prescription drugs, certain dietary supplements, and many over-the-counter (OTC) drug preparations in order to protect the public from personal injury or illness from misuse of these preparations (16 CFR §1700.14).

The immediate packaging of substances regulated under the PPPA must comply with the special packaging standards (16 CFR §1700.15 and 16 CFR §1700.16) and applies to all packaging types including reclosable, nonclosable, and unit-dose types.

Special packaging is not required either for drugs dispensed within a hospital setting for inpatient administration or by manufacturers and packagers of bulk-packaged prescription drugs repackaged by the pharmacist. PPPA-regulated prescription drugs may be dispensed in nonchild-resistant packaging upon the request of the purchaser or when directed in a legitimate prescription (15 U.S.C. §1473).

Manufacturers or packagers of PPPA-regulated OTC preparations are allowed to package one size in nonchild-resistant packaging as long as popular size, special packages are also supplied. The nonchild-resistant packaging requires special labeling (16 CFR §1700.5).

STORAGE CONDITIONS

Specific directions are stated in some monographs with respect to storage conditions, e.g., the temperature or humidity at which an article must be stored and shipped. Such directions apply, except where the label on the article has different storage conditions that are based on stability studies. Where no specific storage conditions are provided in the individual monograph, but the label of an article states storage conditions based on stability studies, such labeled storage directions apply.

▲▲USP38

Freezer: A place in which the temperature is maintained between -25° and -10° (-13° and 14° F).

Refrigerator: A

▲cold ▲USP38

place in which the temperature is maintained between 2° and 8° (36° and 46° F).

Cold: Any temperature not exceeding 8° (46° F).

Cool: Any temperature between 8° and 15° (46° and 59° F). [Note—An article for which storage in a cool place is directed may, alternatively, be stored and shipped as refrigerated, unless otherwise specified by the individual monograph.]

Room temperature: The temperature prevailing in a work area.

Controlled room temperature: The temperature maintained

▲thermostatically that encompasses ▲USP38

at the usual and customary working environment of 20°–25° (68°–77° F). The following conditions also apply.

▲Mean kinetic temperature not to exceed 25° . ▲USP38

Excursions between 15° and 30° (59° and 86° F) that are experienced in pharmacies, hospitals, and warehouses, and during shipping are allowed.

▲Provided ▲USP38

the mean kinetic temperature does not exceed 25° ,

▲transient ▲USP38

spikes up to 40° are permitted as long as they do not

▲exceed ▲USP38

24 h. Spikes above 40° may be permitted only if the manufacturer so instructs.

Articles may be labeled for storage at “controlled room temperature” or at “up to 25° ,” or other wording based on the same mean kinetic temperature.

An article for which storage at *Controlled room temperature* is directed may, alternatively, be stored and shipped in a cool place or refrigerated, unless otherwise specified in the individual monograph or on the label.

Warm: Any temperature between 30° and 40° (86° and 104° F).

Excessive heat: Any temperature above 40° (104° F).

Dry place: The term “dry place” denotes a place that does not exceed 40% average relative humidity at 20° (68° F) or the equivalent water vapor pressure at other temperatures. The determination may be made by direct measurement at the place or may be based on reported climatic conditions. Determination is based on NLT 12 equally spaced measurements that encompass either a season, a year, or, where recorded data demonstrate, the storage period of the article. There may be values of up to 45% relative humidity provided that the average value does not exceed 40% relative humidity. Storage in a container validated to protect the article from moisture vapor, including storage in bulk, is considered a dry place.

Protection from freezing: Where, in addition to the risk of breakage of the container, freezing subjects an article to loss of strength or potency, or to destructive alteration of its characteristics, the container label bears an appropriate instruction to protect the article from freezing.

Protection from light: Where light subjects an article to loss of strength or potency, or to destructive alteration of its characteristics, the container label bears an appropriate instruction

~~to protect the article from light.~~

INTRODUCTION

The purpose of this chapter is to provide packaging definitions, auxiliary packaging information, and storage condition definitions relevant to the storage and distribution of active ingredients, excipients, and medical products, such as pharmaceuticals, devices, combination products (e.g., drug-eluting stents), and dietary supplements.

PACKAGING

Packaging materials must not interact physically or chemically with a packaged article in a manner that causes its safety, identity, strength, quality, or purity to fail to conform to established requirements. Any plastic material used to construct a packaging system must meet the applicable requirements of *Plastic Materials of Construction* 〈 661.1 〉. All Packaging systems must meet the applicable requirements specified in *Elastomeric Closures for Injections* 〈 381 〉, *Containers—Glass* 〈 660 〉, *Plastic Packaging Systems for Pharmaceutical Use* 〈 661.2 〉, and *Auxiliary Packaging Components* 〈 670 〉.

Packaging component/system choices are described in this chapter. For excipients, given their typical presentation as large-volume commodity items (*Packaging systems* ranging from drums to tank cars), a well-closed container is an appropriate default requirement. Articles must be protected from moisture, freezing, and excessive heat (see *General Definitions*) when no specific directions or limitations are provided.

The compendial requirements for the use of specified containers apply also to articles packaged by *Dispensers*, *Repackagers*, or other individuals, unless otherwise indicated in the individual drug product monograph.

POISON PREVENTION PACKAGING ACT (PPPA)

This act, which is administered by the United States Consumer Product Safety Commission (CPSC), requires special packaging for most human oral prescription drugs, oral controlled drugs, certain non-oral prescription drugs, certain dietary supplements, and many over-the-counter (OTC) drug preparations, to protect the public from personal injury or illness from misuse of these preparations (16 CFR§1700.14).

The primary packaging of substances regulated under the PPPA must comply with the special packaging standards (16 CFR§1700.15 and 16 CFR§1700.16) and which apply to all packaging types, including reclosable, non-closable, and unit-dose types.

Special packaging is not required for drugs dispensed within a hospital setting for inpatient administration. Also, special packaging does not need to be used by manufacturers and packagers of bulk-packaged prescription drugs that will be repackaged by the pharmacist. PPPA-regulated prescription drugs may be dispensed in non-*Child-resistant packaging* upon the request of the purchaser or when directed in a legitimate prescription (15 U.S.C.§1473).

Manufacturers or packagers of PPPA-regulated OTC preparations are allowed to package one

size in non-*Child-resistant packaging* as long as popular-size, special packages are also supplied. The non-*Child-resistant packaging* requires special labeling (16 CFR§1700.5).

TEMPERATURE AND STORAGE

Specific directions are stated in some monographs with respect to storage conditions, e.g., the temperature or humidity, at which an article must be stored and shipped. Such directions apply except where the label on the article has different storage conditions that are based on stability studies. Where no specific storage conditions are provided in the individual monograph, the labeled article storage conditions, based on stability studies, will apply.

GENERAL DEFINITIONS

Packaging Definitions

Packaging system (also referred to as a Container–closure system): The sum of packaging components and materials that together contain and protect the article. This includes *Primary packaging components* as well as *Secondary* and *Tertiary packaging components* when such components are required to provide additional protection.

Container: A receptacle that holds an intermediate compound, active pharmaceutical ingredient, excipient, or dosage form and is in direct contact with the article.

Closure: A material that seals an otherwise open space of a *Container* and provides protection for the contents. It also provides access to the contents of the *Container*.

Packaging component: Any single part of the *Package* or *Container–closure system* including: the *Container* (e.g., ampules, prefilled syringes, vials, and bottles); *Closures* (e.g., screw caps and stoppers); ferrules and overseals; closure liners (e.g., tube cartridge liners); inner seals; administration ports; overwraps; administration accessories; labels; cardboard box; and shrink wrap.

Primary packaging component: A *Packaging component* that is in direct contact with or may come into direct contact with the article.

Secondary packaging component: A *Packaging component* that is in direct contact with a *Primary packaging component* and may provide additional protection for the article.

Tertiary packaging component: A *Packaging component* that is in direct contact with a *Secondary packaging component* and may provide additional protection for the article during transportation and/or storage.

Ancillary component: A component or entity that may come into contact with a *Tertiary packaging component* during the distribution, storage, and/or transportation of the packaged article (e.g., pallets, skids, shrink wrap, and active containers).

Associated component: A *Packaging component* that is typically intended to deliver the drug article to the patient but is not stored in contact with the article for its entire shelf life (e.g., spoons, dosing cups, and dosing syringes).

Materials of construction: The materials (e.g., glass, plastic, elastomers, and metal) of which a packaging component consists.

Small-volume injection (Small-volume parenteral): An injectable dosage form that is packaged in *Containers* labeled as containing 100 mL or less.

Large-volume injection (Large-volume parenteral): An injectable dosage form that is packaged in *Containers* labeled as containing more than 100 mL.

Child-resistant packaging: A *Packaging system* designed or constructed to meet CPSC standards pertaining to opening by children (16 CFR § 1700.20 et seq).

Senior-friendly packaging: A *Packaging system* designed or constructed to meet CPSC standards pertaining to opening by senior adults (16 CFR § 1700.15 and 16 CFR § 1700.20).

Restricted delivery system: A *Packaging system* designed or constructed to restrict the amount of the drug product that may be delivered in order to limit access by children. *Restricted delivery systems* should meet and may exceed CPSC standards for special packaging [*Child-resistant* and *Senior-friendly packaging* (16 CFR § 1700.15 et seq)]. For oral medicinal liquids, surface and flow characteristics vary. It is the responsibility of the manufacturer to ensure that all components of the *Restricted delivery system* provide the intended safety protection. One component of the *Restricted delivery system* is the flow restrictor, which is a packaging component that restricts the flow of liquid. The flow restrictor may be used as part of a *Restricted delivery system* or as an adaptor to facilitate use of a measuring device for oral medicinal liquids. A flow restrictor should not compromise CPSC standards for special packaging [*Child-resistant* and *Senior-friendly packaging* (16 CFR § 1700 et seq)].

Tamper-evident packaging: A *Packaging system* that may not be accessed without obvious destruction of the seal or some portion of the *Packaging system*. *Tamper-evident packaging* must be used for sterile drug products intended for ophthalmic or otic use, except where extemporaneously compounded for immediate dispensing on prescription. Drug products intended for sale without prescription are also required to comply with the *Tamper-evident packaging* and labeling requirements of the Food and Drug Administration (FDA) where applicable. Preferably, the immediate *Container* and/or the outer *Container* or protective packaging used by a manufacturer or distributor for all dosage forms that are not specifically exempt is designed to show evidence of any tampering with the contents.

Reclosables packaging: A package that after it has been initially opened is capable of being reclosed with a similar degree of security, and is capable of being used a sufficient number of times to dispense the total contents without loss of security. *Reclosable packaging* may incorporate child-resistance capabilities.

Non-reclosables packaging: A package or part of a package that cannot be closed again after all or part of the contents have been removed. Examples of *Non-reclosables packaging* are blisters, sachets, strips, and other single-unit containers. *Non-reclosables packaging* may include cold-formed foil blisters, foil strip packs, and PVC/Aclar® combining multilayer materials that are thermo-formed or cold-formed foil blisters. *Non-reclosables packaging* may be child resistant depending on the intended use and place of use. Household non-reclosables are subject to the PPPA as defined in 16 CFR § 1700.14.

Tight container: Well-closed container; Light-resistant container: See *Containers—Performance Testing* (671).

Equivalent container–closure system: A *Packaging system* that is as protective as or more protective than the original manufacturer's packaging system in terms of moisture-vapor

transmission rate, oxygen transmission, light transmission, and compatibility. System equivalency extends to any special protective materials, such as those for seals or desiccants associated with the original packaging system.

Table 1. Packaging Systems Definitions: Injection versus Non-Injection

Injection	Non-Injection
Multiple-dose	Multiple-unit
Single-dose	Single-unit
—	Unit-dose
—	Unit-of-use
Single-patient-use	—
Pharmacy bulk package	—
Imaging bulk package	—

Injection Packaging Systems

Multiple-dose container (also referred to as Multi-dose): A *Packaging system* that holds a sterile medication for parenteral administration (injection or infusion); that has met antimicrobial effectiveness testing requirements, or is excluded from such testing requirements by FDA regulation. A *Multiple-dose container* is intended to contain more than one dose of an article. When space permits, a *Multiple-dose container* is labeled as such. *Multiple-dose containers* are generally expected to contain 30 mL or less of medications. The beyond-use date for an opened or entered (e.g., needle-punctured) *Multiple-dose container* is 28 days unless otherwise specified by the manufacturer on the label. An example of a *Multiple-dose container* is a vial.

Single-dose container: A *Packaging system* that holds a sterile medication for parenteral administration (injection or infusion) that is not required to meet the antimicrobial effectiveness testing requirements. A *Single-dose container* is designed for use with a single patient as a single injection/infusion¹. When space permits a *Single-dose container* is labeled as such and should include on the label appropriate discard statements. Examples of *Single-dose containers* are vials, ampules, and prefilled syringes.

Single-patient-use container: A *Packaging system* that holds a sterile medication for parenteral administration (injection or infusion) that is intended to be used multiple times with a single patient. When space permits, a *Single-patient-use container* is labeled as such and should include on the label appropriate discard statements. Examples of *Single-patient-use containers* are patient controlled analgesia cartridges and certain pens for injection.

Pharmacy bulk package: A *Packaging system* of a sterile preparation for parenteral use that contains multiple single doses. The contents are intended for use in a pharmacy admixture program and are restricted to the preparation of admixtures for infusion or, through a sterile transfer device, for the filling of empty sterile syringes. The *Closure* must be penetrated only one time after constitution, if necessary, with a suitable sterile transfer device or dispensing set that allows measured dispensing of the contents. The *Pharmacy bulk package* is to be used only in a suitable work area such as a laminar flow hood (or an equivalent clean-air compounding area). Designation as a *Pharmacy bulk package* is limited to *Injection*, for *Injection*, or *Injectable Emulsion* dosage forms as defined in *Nomenclature* { 1121 }, *General Nomenclature Forms*.

Pharmacy bulk packages, although containing more than one single dose, are exempt from the *Multiple-dose container* volume limit of 30 mL and the requirement that they contain a substance or suitable mixture of substances to prevent the growth of microorganisms. See *Labeling* 〈 7 〉 for labeling requirements.

Imaging bulk package: A *Packaging system* that holds a sterile preparation for parenteral use that contains multiple single doses of a contrast agent (medical imaging drug product) for use with a medical imaging device. The contents are restricted to use in direct conjunction with either an automated imaging injection system or contrast management system which has features to mitigate the risk of cross-contamination and which is approved or cleared for use with an *Imaging bulk package*. The sterility assurance of the *Imaging bulk package* contents in part is dependent upon the automated contrast imaging injection system or the contrast management system.

The *Imaging bulk package closure* shall be penetrated only one time with a suitable sterile dispensing set that allows measured dispensing of the *Imaging bulk package* contents. The *Imaging bulk package* is to be used only in a room designated for at least minimally invasive radiological procedures that involve intravascular administration of a contrast agent. Using aseptic technique, the *Imaging bulk package closure* shall be penetrated only one time with a suitable sterile component of the automated contrast injection system or contrast management system. This procedure room should have a door, a separate ventilation system and be restricted to designated personnel only while the *Imaging bulk package* is in use. If the integrity of the bulk package and the delivery system cannot be assured through direct continuous supervision, the *Imaging bulk package* and all associated disposables for the automated contrast the imaging injection system or contrast management system should be discarded.

Designation as an *Imaging bulk package* is limited to *Injections, for Injections, or Injectable Emulsions* dosage forms as defined in *Injections and Implanted Drug Products* 〈 1 〉, and in *General Nomenclature Forms* 〈 1121 〉.

Imaging bulk packages, although containing more than one single dose, are exempt from the *Multiple-dose container* volume limit of 30 mL. The contents of the *Imaging bulk package* must have demonstrated the ability to limit the growth of microorganisms over the labeled period of use.

Where a *Container* is offered as an *Imaging bulk package*, the label shall 1) state prominently "Imaging bulk package" and in juxtaposition with this statement include the following use statement: "For use only with an automated imaging contrast injection system or contrast management system approved or cleared for use with this contrast agent in this Imaging Bulk Package"; 2) bear a statement limiting the time frame in which the container may be used once it has been entered, provided it is held under the labeled storage conditions; 3) bear the statement "See drug and device labeling for information on devices indicated for use with this Imaging bulk package and techniques to help assure safe use."

Non-Injection Packaging Systems

Multiple-unit container: A *Packaging system* that permits withdrawal of successive portions of a non-injection article without changing the safety, strength, quality, or purity of the remaining portion (e.g., bottle of capsules, tablets, and oral or topical liquids).

Single-unit container: A *Packaging system* that holds a quantity of a non-injection article intended for administration as a single dose and intended for use promptly after the *Packaging system* is opened.

Unit-dose container: A single-unit *Packaging system* for an article intended for administration by other than the parenteral route as a single dose.

Unit-of-use container: A *Packaging system* that contains a specific quantity of an article that is intended to be dispensed as such without further modification except for the addition of appropriate labeling. It is not permitted to repackage *Unit-of-Use containers* for sale.

Miscellaneous

Repackaging: The act of removing a drug product from the original manufacturer's *Packaging system* (bulk or marketed container) and placing it into another *Packaging system*, usually one of smaller size.

Repackager: A firm that repackages drug products for distribution (e.g., for resale to distributors, hospitals, or pharmacies), a function that is beyond the regular practice of a pharmacy. The distribution is not patient specific in that there are no prescriptions. Unlike dispensers, repackaging organizations are required to register with the FDA and to comply with the Current Good Manufacturing Practice regulations in 21 CFR § 210 and § 211.

Contract packager/contract repackager: A firm that is contracted by another organization, such as a manufacturer, to package bulk into a marketed container of a drug product. A *Contract packager* does not take ownership from the manufacturer and generally receives the assigned expiration date from the manufacturer.

Dispenser: A licensed or registered practitioner who is legally responsible for providing the patient with a preparation that is in compliance with a prescription or a medication order and contains a specific patient label. In addition, dispensers may prepare limited quantities in anticipation of a prescription or medication order from a physician. *Dispensers* are governed by the board of pharmacy of the individual state. The terms "dispenser" and "pharmacy" are used interchangeably.

Beyond use date: See ⟨ 7 ⟩.

Expiration date: See ⟨ 7 ⟩.

Black closure system or black bands: The use of a *Black closure system* on a vial (e.g., a black cap overseal and a black ferrule to hold the elastomeric closure) or the use of a *Black band* or series of bands above the constriction on an ampule is prohibited, except for *Potassium Chloride for Injection Concentrate*. See ⟨ 7 ⟩.

INJECTION PACKAGING

Packaging for sterile products intended for injection must be validated as meeting the containment and protection requirements that are essential for maintaining the article's quality.

Refer to *Sterile Product Packaging—Integrity Evaluation* ⟨ 1207 ⟩ for further information regarding sterile product container closure integrity testing and validation. *Closures* for *Multiple-dose containers* permit the withdrawal of the contents without removal or destruction of the

Closure. The *Closure* permits penetration by a needle and, upon withdrawal of the needle, closes at once, protecting the contents against contamination. Refer to *Elastomeric Closures for Injections* (381) for *Closure* reseal tests that are useful for screening multiple-dose closures for their reseal properties. Additional testing may be needed to ensure that the specific closure selected for a product package is able to prevent loss of product contents and microbial contamination under anticipated conditions of multiple entry and use. Piggyback packaging systems are usually intravenous infusion *Container-closure systems* that are used to administer a second infusion through a connector of some type or an injection port on the administration set of the first fluid, thereby avoiding the need for another injection site on the patient's body. Piggyback packaging systems also are known as secondary infusion containers.

The volume of injection in a *Single-dose container* provides the amount specified for one-time parenteral administration, and in no case is more than sufficient to permit the withdrawal and administration of 1 L. Preparations intended for intraspinal, intracisternal, or peridural administration are packaged in *Single-dose containers* only. Unless otherwise specified in the individual monograph, a *Multiple-dose container* contains a volume of injection sufficient to permit the withdrawal of NMT 30 mL.

The following injections are exempt from the 1-L restriction of the foregoing requirements relating to packaging:

- Injections packaged for extravascular use as irrigation solutions or peritoneal dialysis solutions.
- Injections packaged for intravascular use as parenteral nutrition or as replacement or substitution fluid to be administered continuously during hemofiltration.

Injections packaged for intravascular use that may be used for intermittent, continuous, or bolus replacement fluid administration during hemodialysis or other procedures, unless excepted above, must conform to the 1-L restriction. Injections labeled for veterinary use are exempt from the packaging and storage requirements concerning the limitation to single-dose packaging systems and the limitation on the volume of *Multiple-dose containers*.

Packaging for constitution: *Containers*, including the *Closures*, for dry solids intended for injection must not interact physically or chemically with the preparation in any manner that alters the strength, quality, or purity beyond the official requirements under the ordinary or customary conditions of handling, shipment, storage, sale, and use. A *Packaging system* for a sterile solid permits the addition of a suitable solvent and withdrawal of portions of the resulting solution or suspension in such manner that the sterility of the product is maintained. Where the Assay in a monograph provides a procedure for the Sample solution, in which the total withdrawable contents are to be withdrawn from a *Single-dose container* with a hypodermic needle and syringe, the contents are to be withdrawn as completely as possible into a dry hypodermic syringe of a rated capacity not exceeding three times the volume to be withdrawn and fitted with a 21-gauge needle NLT 2.5 cm (1 in) in length. Care must be taken to expel any air bubbles, and the contents are then discharged into a container for dilution and assay.

MEDICAL GAS PACKAGING

Gas cylinder: A metallic *Packaging system* constructed of steel or aluminum and designed to hold medical gases under pressure; these gases may include: Carbon Dioxide USP, Helium USP, Medical Air USP, Nitric Oxide, Nitrous Oxide USP, Nitrogen NF, and Oxygen USP. As a safety

measure, for carbon dioxide, cyclopropane, helium, medical air, nitrous oxide, and oxygen, the Pin-Index Safety System of matched fittings is recommended for cylinders of Size E or smaller.

ASSOCIATED COMPONENTS

Many associated components are graduated for dose administration. It is the responsibility of the manufacturer to ensure that the appropriate dosing component is provided or that a general purpose component, such as those described in this section, is specified for delivering the appropriate dose with the intended accuracy.

Graduated associated components described in this section are for general use. Graduated markings should be legible, indelible, and on an extraoral surface that does not contact the product. The associated volume markings shall be in metric units and limited to a single measurement scale that corresponds with the dose instructions on the prescription container label (see *Prescription Container Labeling* 〈 17 〉). Under expected conditions of use, the volume error incurred in measuring liquids for individual dose administration by means of such graduated components should be NMT 10% of the indicated amount of the liquid preparation with which the graduated component will be used.

Dosing cup: A measuring device consisting of a small cup that may be packaged with oral liquid articles.

Dosing spoon: A measuring device consisting of a bowl and handle that may be packaged with oral liquid articles. The handle may be a graduated tube.

Medicine dropper: A measuring device consisting of a transparent or translucent barrel or tube that is generally fitted with a collapsible bulb. It may be packaged with oral liquid articles.

Oral syringe: A measuring device consisting of a plunger and barrel made of transparent or translucent plastic material and a seal on the end. It may be packaged with oral liquid articles. The syringe should deliver a measured amount of a liquid drug product.

TEMPERATURE AND STORAGE DEFINITIONS

Freezer: A place in which the temperature is controlled between -25° and -10° (-13° and 14° F).

Refrigerator: A cold place in which the temperature is controlled between 2° and 8° (36° and 46° F).

Cold: Any temperature not exceeding 8° (46° F).

Cool: Any temperature between 8° and 15° (46° and 59° F). [Note—An article for which storage in a cool place is directed may, alternatively, be stored and shipped as refrigerated, unless otherwise specified by the individual monograph.]

Room temperature (also referred to as Ambient temperature): The temperature prevailing in a working environment.

Controlled room temperature: The temperature maintained thermostatically that

encompasses the usual and customary working environment of 20°–25° (68°–77° F). The following conditions also apply.

Mean kinetic temperature not to exceed 25°. Excursions between 15° and 30° (59° and 86° F) that are experienced in pharmacies, hospitals, and warehouses, and during shipping are allowed. Provided the mean kinetic temperature does not exceed 25°, transient spikes up to 40° are permitted as long as they do not exceed 24 h. Spikes above 40° may be permitted only if the manufacturer so instructs.

Articles may be labeled for storage at “controlled room temperature” or at “20°–25°,” or other wording based on the same mean kinetic temperature [see also *Good Storage and Distribution Practices for Drug Products* (1079), *Quality Management System, Mean Kinetic Temperature (MKT) Calculation*].

An article for which storage at *Controlled room temperature* is directed may, alternatively, be stored and shipped in a cool place or refrigerated, unless otherwise specified in the individual monograph or on the label.

Warm: Any temperature between 30° and 40° (86° and 104° F).

Excessive heat: Any temperature above 40° (104° F).

Dry place: A place that does not exceed 40% average relative humidity at 20° (68° F) or the equivalent water vapor pressure at other temperatures. The determination may be made by direct measurement at the place. Determination is based on NLT 12 equally spaced measurements that encompass either a season, a year, or, where recorded data demonstrate, the storage period of the article. There may be values of up to 45% relative humidity provided that the average value does not exceed 40% relative humidity. Storage in a *Container* validated to protect the article from moisture vapor, including storage in bulk, is considered a *Dry place*.

Protect from freezing: The *Container* label will bear an appropriate instruction to protect the article from freezing in cases where freezing exposes an article to loss of strength or potency or to destructive alteration of its characteristics. These risks are present in addition to the risk that the container may break if exposed to freezing temperatures.

Protect from light: Where light subjects an article to loss of strength or potency or to destructive alteration of its characteristics, the *Container* label bears an appropriate instruction to protect the article from light. The article must be packaged in a light resistant *Container*.

■ 1S (USP39)

¹ Exceptions may be considered only under conditions described in *Pharmaceutical Compounding—Sterile Preparations* (797).

BRIEFING

(791) pH, USP 38 page 556. The revision proposal harmonizes some terminology for

consistency with other pharmacopoeia and explicitly permits more calibration options. There are no revisions to the instrument performance requirements or calibration procedures.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCCA: A. Hernandez-Cardoso.)
Correspondence Number—C153850

Comment deadline: July 31, 2015

〈 791 〉 pH

Change to read:

INTRODUCTION

For compendial purposes, pH is defined as the value given by a suitable, properly ~~standardized~~ **calibrated**, **1S (USP39)**

potentiometric sensor and measuring system. [Note—The measuring system has traditionally been referred to as the “pH meter.” While the pH meter is still in common use, the measuring system can also be embedded inside the pH sensor, and the pH signal can be transmitted digitally to an external device such as a computer, Programmable Logic Controller (PLC), Distributed Control System (DCS), data acquisition system, terminal, or other microprocessor-controlled device.] By definition, pH is equal to $-\log_{10}[a_{\text{H}^+}]$ where a_{H^+} is the activity of the hydrogen (H^+) or hydronium ion (H_3O^+), and the hydrogen ion activity very closely approximates the hydrogen ion concentration.

The practical pH scale is defined:

$$\text{pH} = \text{pH}_s + [(E - E_s)/k]$$

E = measured potential where the galvanic cell contains the solution under test (pH)

E_s = measured potential where the galvanic cell contains the appropriate buffer solution for ~~standardization~~

calibration, **1S (USP39)**

(pH_s)

k = change in potential/unit change in pH and is derived from the Nernst equation (as follows)

$$k = \log_e(10) \times (RT/nF)$$

R = 8.314 J/mole/ $^\circ\text{K}$

T = temperature ($^\circ\text{K}$)

n = moles/half-reaction

F = Faraday constant, 96485 C/mole

The resulting equation is $[0.05916 + 0.0001984(T - 25^\circ)]$ volts at temperature T . Values of k

from 15°–35° are provided in *Table 1*.

Table 1. Values of k for Various Temperatures

Temperature (°C)	k (V)
15.00	0.05718
20.00	0.05817
25.00	0.05916
30.00	0.06016
35.00	0.06115

Values of k at other temperatures can be determined from the equation above. For practical purposes, values of k are determined from pH sensor calibration.

PH MEASUREMENT SYSTEM

The measurement system consists of: (1) a measuring electrode sensitive to hydrogen-ion activity, typically a glass electrode, though other electrode types are possible, (2) a suitable reference electrode, for example, a silver–silver chloride electrode, and (3) a voltage measurement system with an input resistance capable of measuring at a high input impedance of the pH sensor. The measuring and reference electrode may be separated or combined. The voltage measurement system may be separated from the pH sensor or integrated into the sensor. For most applications, a temperature measurement will be necessary for compensation of the Nernst temperature influence described above. A temperature device may be embedded into the pH sensor, or an external temperature device may be used.

Change to read:

INSTRUMENT REQUIREMENTS

The measurement system shall be capable of performing a 2-point

■ (or more) ■ 1S (USP39)

pH calibration (see below). The accuracy of the pH measurement system is described in the *Calibration* section. The resolution of the pH measurement system shall be at least 0.01 pH. The instrument shall be capable of temperature-compensating the pH sensor measurement to convert the millivolt signal to pH units at any temperature, either automatically using a temperature device built into the sensor system or by manual entry of the sample temperature into the measurement system. The accuracy of the temperature measurement system shall be $\pm 1^\circ$. The resolution of the temperature measurement system shall be at least 0.1° . Lab-based pH measurements are typically performed at $25 \pm 2^\circ$ unless otherwise specified in the individual monograph or herein. However, temperatures outside this range are acceptable if samples are more conveniently prepared at alternative temperatures. Examples of non-lab-based measurements include test samples inside process pipes, vessels, tanks, and other non-standard processing conditions. [Note—The definitions of pH, the pH scale, and the values assigned to the buffer solutions for standardization

■ calibration ■ 1S (USP39)

are for the purpose of establishing a practical, operational system so that results may be compared between laboratories. The measured pH values do not necessarily correspond exactly to those obtained by the definition, $\text{pH} = -\log a_{\text{H}^+}$, rather the values obtained are closely related to the activity of the hydrogen-ion in aqueous solutions.] [Note—Where a pH measurement system is **standardized**

■ calibrated ■ 1S (USP39)

by use of an aqueous buffer and then used to measure the “pH” of nonaqueous systems, the ionization constant of the acid or base, the dielectric constant of the medium, the liquid-junction potential (which may give rise to errors of approximately 1 pH unit), and the hydrogen-ion response of the glass electrode are all changed. For these reasons, the values so obtained with solutions that are only partially aqueous in character can be regarded only as apparent pH values.]

Change to read:

BUFFER SOLUTIONS FOR STANDARDIZATION

■ CALIBRATION ■ 1S (USP39)

OF THE pH MEASUREMENT SYSTEM

Buffer solutions for **standardization**

■ calibration ■ 1S (USP39)

are prepared as directed in *Table 2*.¹ Buffer salts of requisite purity can be obtained from the National Institute of Standards and Technology, other national authorities, or other suppliers. Buffer solutions should be stored in appropriate containers that ensure stability of the pH through the expiry date, and fitted with a tight closure. For buffer solutions greater than 11, the storage should be in containers that are resistant to or reduce carbon dioxide intrusion which would lower the pH of the buffer. For buffer solutions lower than 11, they should typically be prepared at intervals not to exceed 3 months. For buffer solutions greater than 11, they should typically be prepared and used fresh unless carbon dioxide ingress is restricted. All buffer solutions should be prepared using *Purified Water*. *Table 2* indicates the pH of the buffer solutions as a function of temperature. The instructions presented here are intended for the preparation of solutions having the designated molal (m) concentrations. However, in order to facilitate their preparation, the instructions are given in molarity. The difference in concentration between molality and molarity preparations for these buffer solutions is less than 1%, and the pH difference is negligible. Calibration using buffer solutions shall be done in the temperature range of the buffers listed in *Table 2*. [Note—The Nernst temperature compensation corrects only for the electrode millivolt output change with temperature, not the actual pH change of the buffer solution with temperature which is unique for each buffer.] Features such as automatic buffer recognition or buffer pH-temperature correction are available for convenience in accommodating the temperature influence on buffer solutions. The pH-temperature response can be determined from the values in *Table 2*.

Table 2. pH Values of Buffer Solutions for Standardization

■ Calibration ■ 1S (USP39)

Temperature (°C)	Potassium Tetraoxalate, 0.05 m	Potassium Hydrogen Tartrate Saturated at 25°	Potassium Dihydrogen Citrate, 0.05 M	Potassium Biphthalate, 0.05 m	Equimolal Phosphate, 0.05 m	Potassium Dihydrogen Phosphate, 0.0087 M, and Disodium Hydrogen Phosphate, 0.0303 M	Sodium Tetraborate, 0.01 m	Sodium Carbonate, 0.025 M, and Sodium Bicarbonate, 0.025 M	Calcium Hydroxide, Saturated at 25°
10	1.67	—	—	4.00	6.92	—	9.33	—	13.00
15	1.67	—	3.80	4.00	6.90	7.45	9.28	10.12	12.81
20	1.68	—	3.79	4.00	6.88	7.43	9.23	10.06	12.63
25	1.68	3.56	3.78	4.01	6.86	7.41	9.18	10.01	12.45
30	1.68	3.55	3.77	4.02	6.85	7.40	9.14	9.97	12.29
35	1.69	3.55	3.76	4.02	6.84	7.39	9.10	9.93	12.13
40	1.69	—	—	4.04	6.84	—	9.07	—	11.98
45	1.70	—	—	4.05	6.83	—	9.04	—	11.84
50	1.71	—	—	4.06	6.83	—	9.01	—	11.71
55	1.72	—	—	4.08	6.83	—	8.99	—	11.57
60	1.72	—	—	4.09	6.84	—	8.96	—	11.45
$\Delta pH/\Delta ^\circ C$	0.0010	—0.0014	—0.0022	0.0018	—0.0016	—0.0028	—0.0074	—0.0096	—0.0310

Preparation of alternative volumes at the same concentrations to those indicated below is acceptable.

Potassium tetraoxalate, 0.05 m: Dissolve 12.61 g of $\text{KH}_3(\text{C}_2\text{O}_4)_2 \cdot 2\text{H}_2\text{O}$, and dilute with water to make 1000.0 mL.

Potassium hydrogen tartrate, saturated at 25°: Add $\text{C}_4\text{H}_5\text{KO}_6$ to water until saturation is exceeded at 25°. Then filter or decant.

Potassium dihydrogen citrate, 0.05 M: Dissolve 11.41 g of $\text{C}_6\text{H}_7\text{KO}_4$, and dilute with water to make 1000.0 mL.

Potassium biphthalate, 0.05 m: Dissolve 10.12 g of $\text{KHC}_8\text{H}_4\text{O}_4$, previously dried at 110° for 1 h, and dilute with water to make 1000.0 mL.

Equimolal phosphate, 0.05 m: Dissolve 3.53 g of disodium hydrogen phosphate (Na_2HPO_4) and 3.39 g of potassium dihydrogen phosphate (KH_2PO_4), each previously dried at 120° for 2 h, and dilute with water to make 1000.0 mL.

Potassium dihydrogen phosphate, 0.0087 M, and disodium hydrogen phosphate, 0.0303 M: Dissolve 1.18 g of KH_2PO_4 and 4.30 g Na_2HPO_4 , both dried for 2 h at $120 \pm 2^\circ$, and dilute with water to make 1000.0 mL.

Sodium tetraborate, 0.01 m: Dissolve 3.80 g of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, and dilute with water to make 1000.0 mL. Protect from absorption of carbon dioxide.

Sodium carbonate, 0.025 M, and sodium bicarbonate, 0.025 M: Dissolve 2.64 g of sodium carbonate (Na_2CO_3) and 2.09 g of sodium bicarbonate (NaHCO_3), and dilute with water to make 1000.0 mL.

Calcium hydroxide, saturated at 25°: Add $\text{Ca}(\text{OH})_2$ to water until saturation is exceeded at 25°. Use water that has been recently boiled and protected from the atmosphere to limit carbon dioxide absorption. Then filter or decant.

Change to read:

CALIBRATION

Because of variations in the nature and operation of the available pH measurement systems, it is not practical to provide universal directions for the ~~standardization~~

■ calibration ■ 1S (USP39)

of the measurement system. However, the general principles to be followed are set forth in the following paragraphs. Examine the electrodes, especially the reference electrode and electrolyte level, if a liquid electrolyte is used. If necessary, replenish electrolyte supply, and observe other precautions indicated by the instrument and electrode manufacturers.

The calibration or verification of the pH measurement system should be periodically executed. The historical performance of the measurement system, the criticality of the pH measurement, the maintenance of the pH sensor, and the frequency of measurement operation is used to determine the frequency of the calibration/verification.

■ In addition to the 2-point calibration process described below, other multipoint methods may be available for use. These are acceptable if the pH sensor slope and the offset criteria (see step 10 below) as well as the calibration accuracy (see step 14 below) are met. ■ 1S (USP39)

If the pH of the buffer is sensitive to ambient carbon dioxide, then use *Purified Water* that has been recently boiled, and subsequently stored in a container designed to minimize ingress of carbon dioxide.

1. To ~~standardize~~

■ calibrate ■ 1S (USP39)

the pH measurement system, select three buffer solutions for ~~standardization~~

■ calibration, ■ 1S (USP39)

preferably from those given in *Table 2*, such that the expected pH of the material under test falls within their range. Two of the buffers are used for the calibration process, and the third buffer is used for verification. The value of the verification buffer ~~should be between~~

■ shall be between two of ■ 1S (USP39)

the calibration buffers. If the operational range of the pH sensor is beyond the pH range of the buffer solutions in *Table 2*, then either 1) select two nearby pH buffers from *Table 2* or 2) select one from *Table 2* and another documented prepared buffer that is outside the range.

2. Rinse the pH sensor several times with water, then with the first buffer solution.
3. Immerse the pH sensor in the first buffer solution at a temperature within the range of *Table 2*.
4. If automatic temperature measurement and compensation is not included in the measuring system, manually enter the temperature of the buffer and pH value of the buffer solution at that temperature into the instrument. For temperatures not listed in *Table 2*, use linear interpolation to determine the pH value as a function of temperature.
5. Initiate the 2-point calibration sequence with the first buffer according to the manufacturer's instructions.
6. Remove the pH sensor from the first buffer and rinse the electrode(s) with water, and then with the second buffer solution.
7. Immerse the pH sensor in the second buffer at a temperature within the range of *Table 2*.
8. If automatic temperature measurement and compensation is not included in the measuring system, manually enter the temperature of the buffer and pH value of the buffer solution at that temperature into the instrument.

9. Continue the 2-point calibration sequence with the second buffer according to the manufacturer's instructions.
10. After completion of the 2-point calibration process, verify that the pH slope and offset are within acceptable parameters. Typical acceptable parameters ~~are slopes ranging from 90%–105% and an offset of ± 30~~
 - are a slope of 90.0%–105.0% and an offset of 0.0 ± 30.0 ■ 1S (USP39)
 - mV (0.5 pH units at 25° C). Depending on the pH instrumentation, the pH slope and offset may be determined in software or by manual methods.
 - If using manual methods, follow supplier instructions to calculate the pH sensor slope/offset. ■ 1S (USP39)
 - If these parameters are not within acceptable parameters, the sensor should be properly cleaned, replenished, serviced, or replaced, and the 2-point calibration process shall be repeated.
11. Remove the pH sensor from the second buffer, and rinse thoroughly with water, and then the verification buffer.
12. Immerse the pH sensor in the verification buffer at a temperature within the range of *Table 2*.
13. If automatic temperature measurement and compensation are not included in the measuring system, manually enter the temperature of the buffer and pH value of the buffer solution at that temperature into the instrument.
14. The pH reading shall be within ± 0.05 pH of the value in *Table 2* at the buffer solution temperature.

Change to read:

OPERATION

All test samples should be prepared using *Purified Water*, unless otherwise specified in the monograph. All test measurements should use manual or automated Nernst temperature compensation.

1. Prepare the test material according to requirements in the monograph or according to specific procedures. If the pH of the test sample is sensitive to ambient carbon dioxide, then use *Purified Water* that has been recently boiled, and subsequently stored in a container designed to minimize ingress of carbon dioxide.
2. Rinse the pH sensor with water, then with a few portions of the test material.
3. Immerse the pH sensor into the test material and read
 - record ■ 1S (USP39)

the pH value and temperature.

In all pH measurements, allow sufficient time for stabilization of the temperature and pH measurement.

Diagnostic functions such as glass or reference electrode resistance measurement may be available to determine equipment deficiencies. Refer to the electrode supplier for diagnostic tools to assure proper electrode function.

Where approximate pH values suffice, indicators and test papers (see *Indicators* and *Indicator and Test Papers*) may be suitable.

For a discussion of buffers, and for the composition of standard buffer solutions called for in compendial tests and assays, see *Buffer Solutions* in the section *Solutions*. This referenced section is not intended to replace the use of the pH calibration buffers in *Table 2*.

¹ Commercially available buffer solutions for pH measurement system, ~~standardized~~
~~calibrated~~ ^{1S (USP39)}

by methods traceable to the National Institute of Standards and Technology (NIST) or other national authorities, labeled with a pH value accurate to 0.02 pH unit may be used. Solutions prepared from ACS reagent grade materials or other suitable materials, may be used provided the pH of the resultant solution is the same as that of the solution prepared from the NIST (or other national authorities) certified material. Buffer solutions that are greater than 12 should be used immediately, or should be prepared using freshly boiled water, and stored under conditions to minimize carbon dioxide absorption and ingress.

BRIEFING

〈 1035 〉 **Biological Indicators for Sterilization**, *USP 38* page 814. This chapter is proposed for omission from *USP*. A new general chapter, *Biological Indicators for Sterilization* 〈 1229.5 〉, has been proposed in *PF 41(2)* as part of the new series of chapters on sterilization. The chapters impacted are *Sterilization and Sterility Assurance of Compendial Articles* 〈 1211 〉, *Pharmaceutical Compounding—Sterile Preparations* 〈 797 〉, and *Sterility Testing—Validation of Isolator Systems* 〈 1208 〉. In these chapters, references to 〈 1035 〉 will be replaced by 〈 1229.5 〉.

The following Briefing list includes monographs and/or chapters that both reference the General Chapter under revision and require revision to keep references to the General Chapter accurate. Other monographs and/or chapters may also be listed, even where the reference to the General Chapter remains unchanged, as additional notice to stakeholders where there is believed to be potential for the change in the general chapter itself to affect pass-fail determinations for particular monograph articles.

- 〈 797 〉 *Pharmaceutical Compounding—Sterile Preparations*
- 〈 1208 〉 *Sterility Testing—Validation of Isolator Systems*
- 〈 1209 〉 *Sterilization—Chemical and Physicochemical Indicators and Integrators*
- 〈 1211 〉 *Sterilization and Sterility Assurance of Compendial Articles*

(GCM: Tirumalai.)

Correspondence Number—C157027

Comment deadline: July 31, 2015

Delete the following:

~~■ 〈 1035 〉 **BIOLOGICAL INDICATORS FOR STERILIZATION**~~

~~A biological indicator is broadly defined as a characterized preparation of a specific microorganism that provides a defined and stable resistance to a specific sterilization process.~~

Microorganisms widely recognized as suitable for biological indicators are spore-forming bacteria, because, with the exception of ionizing radiation processes, these microorganisms are significantly more resistant than normal microflora. A biological indicator can be used to assist in the performance qualification of the sterilization equipment and in the development and establishment of a validated sterilization process for a particular article. Biological indicators are used in processes that render a product sterile in its final package or container, as well as for the sterilization of equipment, materials, and packaging components used in aseptic processing. Biological indicators may also be used to monitor established sterilization cycles and in periodic revalidation of sterilization processes. Biological indicators may also be used to evaluate the capability of processes used to decontaminate isolators or aseptic clean room environments.

The principles and requirements for these applications are described under *Sterilization and Sterility Assurance of Compendial Articles* (1211).

TYPES OF BIOLOGICAL INDICATORS

There are at least three types of biological indicators. Each type of indicator incorporates a known species of a microorganism of known sterilization resistance to the sterilization mode. Some biological indicators may also contain two different species and concentrations of microorganisms.

One form of biological indicator includes spores that are added to a carrier (a disk or strip of filter paper, glass, plastic, or other materials) and packaged to maintain the integrity and viability of the inoculated carrier.

Carriers and primary packaging shall not contain any contamination (physical, chemical, or microbial) that would adversely affect the performance or the stability characteristics of the biological indicator. The carrier and primary packaging shall not be degraded by the specific sterilization process, which is used in a manner that will affect the performance of the biological indicator. The carrier should withstand transport in the primary and secondary packaging and handling at the point of use. The design of the carrier and primary packaging should minimize the loss of the original inoculum during transport, handling, and shelf life storage.

Another form of biological indicator is a spore suspension that is inoculated on or into representative units of the product to be sterilized. This represents an inoculated product; however, a simulated inoculated product may be used if it is not practical to inoculate the actual product. A simulated product is a preparation that differs in one or more ways from the actual product, but performs as the actual product using test conditions or during actual production sterilization processing. Spore suspensions with a known D value should be used to inoculate the actual or simulated product. If a simulated inoculated product is used, it must be demonstrated that it will not degrade the sterilization resistance of the bioindicator. The physical design of actual or simulated product can affect the resistance of spore suspensions that are inoculated on or into the products. In the case of liquid inoculated products, it is often advisable to determine both the D value and z value of the specific biological indicator microorganism in the specific liquid product. The population, D value, z value where applicable, and endpoint kill time of the inoculated actual or simulated product should be determined.

A third form of biological indicator is a self-contained indicator. A self-contained biological indicator is designed so that the primary package, intended for incubation following sterilization processing, contains the growth medium for recovery of the process-exposed microorganisms.

This form of biological indicator together with the self-contained growth medium can be considered a system. In the case of self-contained biological indicators, the entire system provides resistance to the sterilization process.

If the biological indicator is a paper strip or disk in a self-contained package that includes an available culture medium, the package design should be readily penetrable by the sterilizing agent. To allow for the time lag that may occur while the sterilizing agent reaches the contained microorganisms in the system, the D value, process endpoint kill time, and the survival time should be characterized for the system and not solely for the paper strip in the self-contained unit. Following the sterilizing treatment, the spore strip or disk is immersed in the self-contained medium by manipulation, which allows contact with the culture medium.

Self-contained biological indicators may also consist of a spore suspension in its own medium, and they often also contain a dye, which indicates positive or negative growth following incubation. Resistance of the self-contained system is dependent upon penetration of the sterilant into the package. Penetration may be controlled by the manufacturer through varying designs and composition of the self-contained biological indicator package, ampul, or container. Self-contained ampul biological indicators may be incubated directly following exposure to the sterilization process. The entire system is then incubated under the specified conditions. Growth or no growth of the treated spores is determined visually (either by observing a specified color change of an indicator incorporated in the medium or by turbidity) or by microscopic examination of the inoculated medium.

The self-contained system resistance characteristics must also comply with the labeling of the self-contained system and the relevant biological indicator monograph. The self-contained biological indicator system should withstand transport in the secondary packaging and handling at the point of use without breakage. The design of the self-contained system should be such to minimize the loss of the original inoculum of microorganisms during transport and handling. During or after the sterilization process, the materials used in the self-contained system shall not retain or release any substance that can inhibit the growth of low numbers of surviving indicator microorganism under culture conditions. Adequate steps must be taken to demonstrate that the recovery medium has retained its growth support characteristics after exposure to the sterilization process.

Preparation

All operations associated with the preparation of biological indicators are controlled by a documented quality system. Traceability is maintained for all materials and components incorporated in or coming into direct contact with the microorganism suspension, the inoculated carrier, or the biological indicator.

The preparation of stock spore suspensions of selected microorganisms used as biological indicators requires the development of appropriate procedures, including mass culturing, harvesting, purification, and maintenance of the spore suspensions. The stock suspension should contain predominantly dormant (nongerminating) spores that are held in a nonnutritive liquid.

The finished product (microbial suspension, inoculated carriers, or biological indicators) supplied by commercial manufacturers shall have no microorganisms, other than the test microorganism, present in sufficient numbers to adversely affect the product. The system to minimize the presence of microorganisms other than the biological indicator microorganism in the product will

be validated, monitored, and recorded.

Selection for Specific Sterilization Processes

The selection of a biological indicator requires a knowledge of the resistance of the biological indicator system to the specific sterilization process. It must be established that the biological indicator system provides a challenge to the sterilization process that exceeds the challenge of the natural microbial burden in or on the product.

The effective use of biological indicators for the cycle development, process, and product validation, and routine production monitoring of a sterilization process requires a thorough knowledge of the product being sterilized, along with its component parts (materials and packaging). Only the widely recognized biological indicators specified in the particular biological indicator monograph should be used in the development or validation of a sterilization process. This will ensure that the biological indicator selected provides a greater challenge to the sterilization process than the bioburden in or on the product. Some users may require biological indicators with characteristics that differ from those widely available commercially. In such cases, users may grow their own spore cultures for the express purpose of preparing in-house biological indicators for their specific use. In such a case, the user is well advised to use organisms already described in the scientific literature as indicator organisms, and the user must have the capability of determining D and z values for in-house biological indicators. When biological indicators are prepared in-house, users must confirm the population, purity, and shelf life of the biological indicator to ensure the validity of any test conducted using the in-house biological indicator. When a bioburden-based sterilization process design is used, data comparing the resistance of the biological indicator to that of bioburden are essential. Enumeration of the bioburden content of the articles being sterilized is also required. The process must result in a biologically verified lethality sufficient to achieve a probability of obtaining a nonsterile unit that is less than one in a million.

Alternatively, the overkill method may be used in the design of a sterilization process. In this case, specific assumptions are made regarding the resistance assumption used in establishing sterilization process lethality requirements. In general, all overkill processes are built upon the assumption that the bioburden is equal to one million organisms and that the organisms are highly resistant. Thus, to achieve the required probability of a nonsterile unit that is less than one in a million, a minimum 12-D process is required. A 12-D process is defined as a process that provides a lethality sufficient to result in a 12-log reduction, which is equivalent to 12 times a D value for organisms with sufficiently higher resistance than the mean resistance of bioburden. Because the bioburden is assumed to be one million, an overkill process will result in a probability of nonsterility at much less than 10^{-6} in actual practice. Overkill process design and evaluation may differ depending upon the sterilization process under test. The use of an overkill design and validation approach may minimize or obviate the need for bioburden enumeration and identification.

Moist Heat—For moist heat sterilization process, spores of suitable strains of *Bacillus stearothermophilus* are commercially available as biological indicators and frequently employed. Other heat-resistant spore-forming microorganisms such as *Clostridium sporogenes*, *Bacillus subtilis*, and *Bacillus coagulans* have also been used in the development and validation of moist heat sterilization processes.

Dry Heat—For dry heat sterilization, spores of *Bacillus subtilis* spp. are sometimes used to

validate the process. During the validation of dry heat sterilization processes, endotoxin depyrogenation studies are frequently conducted in lieu of microbial inactivation studies during the establishment of sterilization cycles because the inactivation rate of endotoxin is slower than the inactivation rate of *Bacillus subtilis* spores. In practice the reduction of endotoxin titer by three or more logs will result in a process that also achieves a probability of nonsterility substantially lower than 10^{-6} .

Ionizing Radiation—Spores of *Bacillus pumilus* have been used to monitor sterilization processes using ionizing radiation; however, this is a declining practice. Radiation dose setting methods that do not use biological indicators have been widely used to establish radiation processes. Furthermore, certain bioburden microorganisms can exhibit greater resistance to radiation than *Bacillus pumilus*.

Ethylene Oxide—For ethylene oxide sterilization, spores of a subspecies of *Bacillus subtilis* (*Bacillus subtilis* var. *niger*) are commonly used. The same biological indicator systems are generally used when 100% ethylene oxide or different ethylene oxide and carrier gas systems are used as sterilants.

Vapor-Phase Hydrogen Peroxide (VPHP)—This process has been shown to be an effective surface sterilant or decontaminant. VPHP is capable of achieving sterilization (probability of nonsterility of less than one in a million) when process conditions so dictate and if the target of sterilization is suitably configured. However, VPHP is also commonly used as a surface decontaminating agent in the treatment of sterility testing, biological and chemical containment, manufacturing isolators, and clean rooms.

Surface decontamination is a process that is distinct from sterilization of product contact materials, container closure systems, or product. It is a process designed to render an environment free of detectable or recoverable microorganisms. Biological indicators are widely used to verify the efficacy of the decontamination process. However, in the case of decontamination, a spore log reduction value of three to four is adequate because the goal is decontamination rather than sterilization.

Table 1. Typical Characteristics for Commercially Supplied Biological Indicator Systems

Sterilization Mode	Example of a Typical D value (minutes)	Range of D values for Selecting a Suitable Biological Indicator (minutes)	Limits for a Suitable Resistance (depending on the particular D value [minutes])	
			Survival Time	Kill Time
Dry heat ^a	1.9	Min. 1.0	Min. 4.0	10.0
160 ^c	-	Max. 3.0	Max. 14.0	32.0
Ethylene oxide ^b	-	-	-	-
600 mg per L	3.5	Min. 2.5	Min. 10.0	25.0
54 ^c	-	Max. 5.8	Max. 27.0	68.0

^a For 1.0×10^6 to 5.0×10^6 spores per carrier.

^b For 1.0×10^6 to 5.0×10^7 spores per carrier.

^c For 1.0×10^5 to 5.0×10^6 spores per carrier.

Sterilization Mode	Example of a Typical D value (minutes)	Range of D values for Selecting a Suitable Biological Indicator (minutes)	Limits for a Suitable Resistance (depending on the particular D value {minutes})	
			Survival Time	Kill Time
60% relative humidity	-	-	-	-
Moist heat ^c	1.9	Min. 1.5	Min. 4.5	13.5
121 ^e	-	Max. 3.0	Max. 14.0	32.0
^a For 1.0×10^6 to 5.0×10^6 spores per carrier. ^b For 1.0×10^6 to 5.0×10^7 spores per carrier. ^c For 1.0×10^5 to 5.0×10^6 spores per carrier.				

Bacillus stearothermophilus is the most prevalently used biological indicator for validating VPHP. Other microorganisms that may be useful as biological indicators in VPHP processes are spores of *Bacillus subtilis* and *Clostridium sporogenes*. Other microorganisms may be considered if their performance responses to VPHP are similar to those of the microorganisms cited above.

These spores may be inoculated on the surface of various gas impervious carrier systems having glass, metal, or plastic surfaces. Highly absorbent surfaces, such as fibrous substrates, or any other substrate that readily absorbs VPHP or moisture may adversely influence the VPHP concentration available for inactivation of inoculated microorganisms. Paper substrates are not used because VPHP will degrade cellulose based materials.

For representative characteristics of commercially supplied biological indicators, see *Table 1*.

The biological indicator may also be individually packaged in a suitable primary overwrap package that does not adversely affect the performance of the indicator, and is penetrable by VPHP. Spunbound polyolefin materials have proven to be well suited as an overwrap of biological indicators intended for use in evaluation of VPHP processes. The overwrap material may facilitate laboratory handling of the biological indicators following exposure to VPHP. Also, the use of an overwrap material to package VPHP biological indicators must be carefully assessed to ensure that, following VPHP exposure, residual hydrogen peroxide is not retained by the packaging material, possibly inducing bacteriostasis during the recovery steps. Microbial D values will be influenced by the presence of a biological indicator overwrap material relative to the rate of inactivation and the potential presence of residual VPHP. In cases where biological indicators (inoculated carriers) are being used without the primary package, stringent adherence to aseptic techniques is required.

PERFORMANCE EVALUATION

Manufacturer's Responsibility

The initial responsibility for determining and providing to the users the performance characteristics of a biological indicator³ lot resides with the manufacturer of biological indicators. The manufacturer should provide with each lot of biological indicators a certificate of analysis that attests to the validity of biological indicator performance claims cited on the

biological indicator package label or in the package insert of the label package. The manufacturer should define the sterilization process that the biological indicator will be used to evaluate. The characterization of each type of biological indicator, which provides the basis for label claims, should be performed initially by the manufacturer of the biological indicator using specialized and standardized apparatus under precisely defined conditions.⁴ The manufacturer should also provide information concerning the *D value*, the method by which the *D value* was determined, and microbial count and resistance stability of the biological indicator throughout the labeled shelf life of the indicator. Optimum storage conditions should be provided by the manufacturer, including temperature, relative humidity, and any other requirements for controlled storage. The data obtained from the various required performance assays should be cited in a package insert or on the label of the biological indicator package. The manufacturer should provide directions for use, including the medium and conditions to be used for the recovery of microorganisms after exposure to the sterilization process. Disposal instructions should also be provided by the manufacturer of the biological indicator.

User's Responsibility

Commercial Product—When biological indicators are purchased from a commercial source, their suitability for use in a specific sterilization process should be established through developmental sterilization studies unless existing data are available to support their use in the process. The user should establish in-house acceptance standards for biological indicator lots and consider rejection in the event the biological indicator lot does not meet the established in-house performance standards. A Certificate of Performance should be obtained for each lot of indicators, and the user should routinely perform audits of the manufacturer's facilities and procedures. If certificates are not obtained and audits have not been performed, or if the biological indicators are to be used outside of the manufacturer's label claims, verification and documentation of performance under conditions of use must exist.

Upon initial receipt of the biological indicator from a commercial supplier, the user should verify the purity and morphology of the purchased biological indicator microorganisms. Verification of at least the proper genus is desirable. Also, a microbial count to determine the mean count per biological indicator unit should be conducted. The manufacturer's comments relative to *D value* range, storage conditions, expiration dating, and stability of the biological indicator should be observed and noted. The user may consider conducting a *D value* assessment before acceptance of the lot. Laboratories that have the capability of performing *D value* assays could conduct a *D value* determination using one of the three methods cited in the general test chapter *Biological Indicators—Resistance Performance Tests* (55) and in the appropriate USP monographs for specific biological indicators. Particularly important is the verification of the *D value* and count stability of the biological indicator system if long-term storage is employed.

In the event the spore crop is maintained for longer than 12 months under documented storage conditions, both spore count and resistance analysis must be conducted, unless performance of an original parent crop has been validated for a longer storage period. The result of spore count and resistance assays should be within the range of acceptability established during initial acceptance of the spore crop lot.

Noncommercial Product—A user of biological indicator systems may elect to propagate microorganisms for developing in-house biological indicators to develop or validate sterilization processes. In the event a user becomes a "manufacturer" of biological indicators, biological indicator performance requirements must be met. If the biological indicator system is used for

the development of new sterilization processes or validation of existing processes, the same performance criteria described for commercial manufacturers of biological indicators must be followed.

Spore Crop Preparation

Because most biological indicators use microbial spores, accurate records of spore crop identification must be maintained by commercial and noncommercial biological indicator manufacturers. These records should include records pertaining to the source of the initial culture, identification, traceability to the parent spore crop, subculture frequency, media used for sporulation, changes in media preparation, any observation of crop contamination, and pre- and post-heat shock data. Records of usage of the spore crop and resistance to sterilization (namely, D values and z values where applicable) should also be maintained.

Instrumentation

The instrumentation used to evaluate the sterilization resistance of spore crops must be consistent with existing standards² related to the performance evaluation of biological indicator systems.

Equipment for the determination of D values of microorganisms exposed to VPHP should be able to closely control equipment operating parameters as described for other biological indicator systems under *Biological Indicators—Resistance Performance Tests* (55). Particularly important is the assurance of a consistently reproducible VPHP concentration, delivered within a finite time, and maintained within a specified concentration range or VPHP pressure range for a defined increment of time. Introduction of biological indicators into a stabilized concentration of VPHP conditions should be via a system that permits rapid entry and removal of the test units from the chamber. Also, the design of the test chamber should allow for the attainment of steady-state VPHP concentrations and pressure, or the use of a defined amount of cubic feet of free flowing VPHP at a standardized pressure and temperature. Currently, VPHP concentration measurement devices may not be widely used. Therefore, exposure conditions may need to be based on the maintenance of steady-state VPHP pressures or flow rates resulting from a known initial weight of hydrogen peroxide, admitted to the chamber in a defined unit of time. Using this information, together with the known fixed volume of the chamber environment, a calculation of the approximate VPHP concentration can be made. If conditions are maintained constant throughout each D value assessment run, comparisons of relative resistance among different biological indicator lots may be readily determined.

USE FOR IN-PROCESS VALIDATION

Regardless of the mode of sterilization, the amount of the initial population of the microorganisms, its resistance to sterilization, and the site of inoculation on or in the product can all influence the rate of biological indicator inactivation.

During product microbial challenges, various areas of the product should be inoculated with biological indicators. If, for example, a container with a closure system is sterilized, both the product solution and the closure should be challenged to ensure that sterilization equivalent to a 10^{-6} (one in a million probability of a nonsterile unit) sterilization assurance level (SAL) will be obtained in the solution as well as at the closure site.

~~One may need to determine through laboratory studies whether product components are more difficult to sterilize than, for example, a solution or drug within the product. Depending on the locations of the product components most difficult to sterilize, different process parameters may be involved in assuring microbial inactivation to an SAL of 10^{-6} . The product performance qualification phase should identify the most important process parameters for inactivation of microorganisms at the sites most difficult to sterilize. Once these critical processing parameters are determined, during sterilization in-process validation of the product, they should be operated at conditions less than the conditions stated in the sterilization process specifications. Biological indicator survival is predicated upon both resistance and population. Therefore, a 10^6 -biological indicator population is not always required to demonstrate a 10^{-6} SAL. The appropriate use for biological indicators is to employ them to confirm that the developed process parameters result in the desired SAL. In moist heat sterilization, the biological indicator is used to establish that physically measured lethality can be verified biologically. Biological indicators with substantive D values and populations substantially less than 10^6 are adequate to validate many sterilization and decontamination processes. It is important that the users be able to scientifically justify their selection of a biological indicator.~~

■ 1S (USP39)

[±] ~~See Apparatus under *Biological Indicators—Resistance Performance Tests* (55). These apparatuses have been designed to provide consistent physical conditions applicable to the characterization of biological indicators. The required performance characteristics are also indicated.~~

² ~~BIER/Steam Vessels, American National Standards, ANSI/AAMI ST45:1992.~~

BRIEFING

《 1058 》 **Analytical Instrument Qualification**, *USP 38* page 971. The General Chapters—Physical Analysis Expert Committee is proposing the following revisions to this general information chapter:

1. The *Introduction* is being modified to include the classification of instruments previously included as a separate section.
2. Several other modifications are introduced in *Qualification Phases* under *Analytical Instrument Qualification Process*.
3. Changes are also proposed under *Roles and Responsibilities*, *Software Validation*, *Change Control*, and *AIQ Documentation*.
4. The *Instrument Categories* section is being deleted.
5. A new *Glossary* is being added.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCPA: H. Pappa.)

Correspondence Number—C156065

Comment deadline: July 31, 2015

《 1058 》 ANALYTICAL INSTRUMENT QUALIFICATION

Change to read:

INTRODUCTION

A large variety of laboratory equipment, instruments, and computerized analytical systems, ranging from simple nitrogen evaporators to complex multiple-function technologies (see *Instrument Categories*), are used in the pharmaceutical industry to acquire data to help ensure that products are suitable for their intended use. An analyst's objective is to consistently obtain reliable and valid data suitable for the intended purpose. Depending on the applications, users validate their procedures, calibrate their instruments, and perform additional instrument checks, such as system suitability tests and analysis of in-process quality control check samples to help ensure that the acquired data are reliable. With the increasing sophistication and automation of analytical instruments, an increasing demand has been placed on users to qualify their instruments.

Unlike method validation and system suitability activities, analytical instrument qualification (AIQ) currently has no specific guidance or procedures. Competing opinions exist regarding instrument qualification and validation procedures and the roles and responsibilities of those who perform them. Consequently, various approaches have been used for instrument qualification, approaches that require varying amounts of resources and generate widely differing amounts of documentation. This chapter provides a scientific approach to AIQ and considers AIQ as one of the major components required for generating reliable and consistent data. Note that the amount of rigor applied to the qualification process will depend on the complexity and intended use of the instrumentation. This approach emphasizes AIQ's place in the overall process of obtaining reliable data from analytical instruments.

Validation versus Qualification

In this chapter, the term validation is used for manufacturing processes, analytical procedures, and software procedures and the term qualification is used for instruments. Thus, the phrase "analytical instrument qualification" (AIQ) is used for the process of ensuring that an instrument is suitable for its intended application.

■ A large variety of analytical instruments, ranging from simple apparatus to complex computerized systems are used in the pharmaceutical industry to acquire data to help ensure that products meet their specifications. The majority of these instruments combine a metrological function with software control. There are many ways of demonstrating that an instrument is qualified and under control and these can include qualification, calibration, validation, and maintenance. In order to assure "fitness for purpose" an integrated approach based upon a risk assessment is recommended. For the purposes of this chapter, the term "instrument" used in this chapter includes any apparatus, equipment, instrument, or instrument system used in the laboratory.

This chapter provides a scientific approach for carrying out analytical instrument qualification (AIQ); it is left to each laboratory to justify and document their specific approaches.

The instrument owner/user, and their management, are accountable for the qualification and validation work outlined in this general chapter.

This general chapter outlines the principles and frame work of risk-based instrument qualification to ensure fitness for the intended use in pharmacopeial analysis. The detailed operating parameters to be qualified are found in the respective general chapters for specific instrument types.

The risk assessment begins with the classification of the instrument to determine the extent of qualification needed to demonstrate fitness for purpose. Generally, the more complex that an instrument is, or higher criticality of the measurement, the greater the amount of work that is required to ensure that quality data will be generated. In addition, attention must be paid to ensuring that data integrity and security are maintained.

Instruments can generally be classified as belonging to Groups A, B, or C. It should be noted that the same type of instrument can fit into one or more categories depending on its intended use. Therefore, no specific examples are provided.

Group A includes the least complex, standard instruments that are used without measurement capability or user requirement for calibration such as a magnetic stirrer or vortex mixer. Proper function is assured by observation and no further qualification activities are needed for this group.

Group B includes instruments which may provide a measurement or a quantitative condition that can affect a measurement. Examples may include a pH meter or an oven. Proper function of instruments in this group may require only some elements of qualification, such as routine calibration, maintenance, or performance checks. The level of qualification will also depend on the criticality of the application. Generally these instruments may have firmware but not software that is changed by the user.

Group C comprises the majority of analytical instruments and normally includes a significant degree of computerization and complexity. All elements of qualification, including software validation, must be considered to assure proper functioning of instruments in this group.

■ 1S (USP39)

COMPONENTS OF DATA QUALITY

There are four critical components involved in the generation of reliable and consistent data (quality data). *Figure 1* shows these components as layered activities within a quality triangle. Each layer adds to the overall quality. Analytical instrument qualification forms the base for generating quality data. The other components essential for generating quality data are analytical method validation, system suitability tests, and quality control check samples. These quality components are described below.

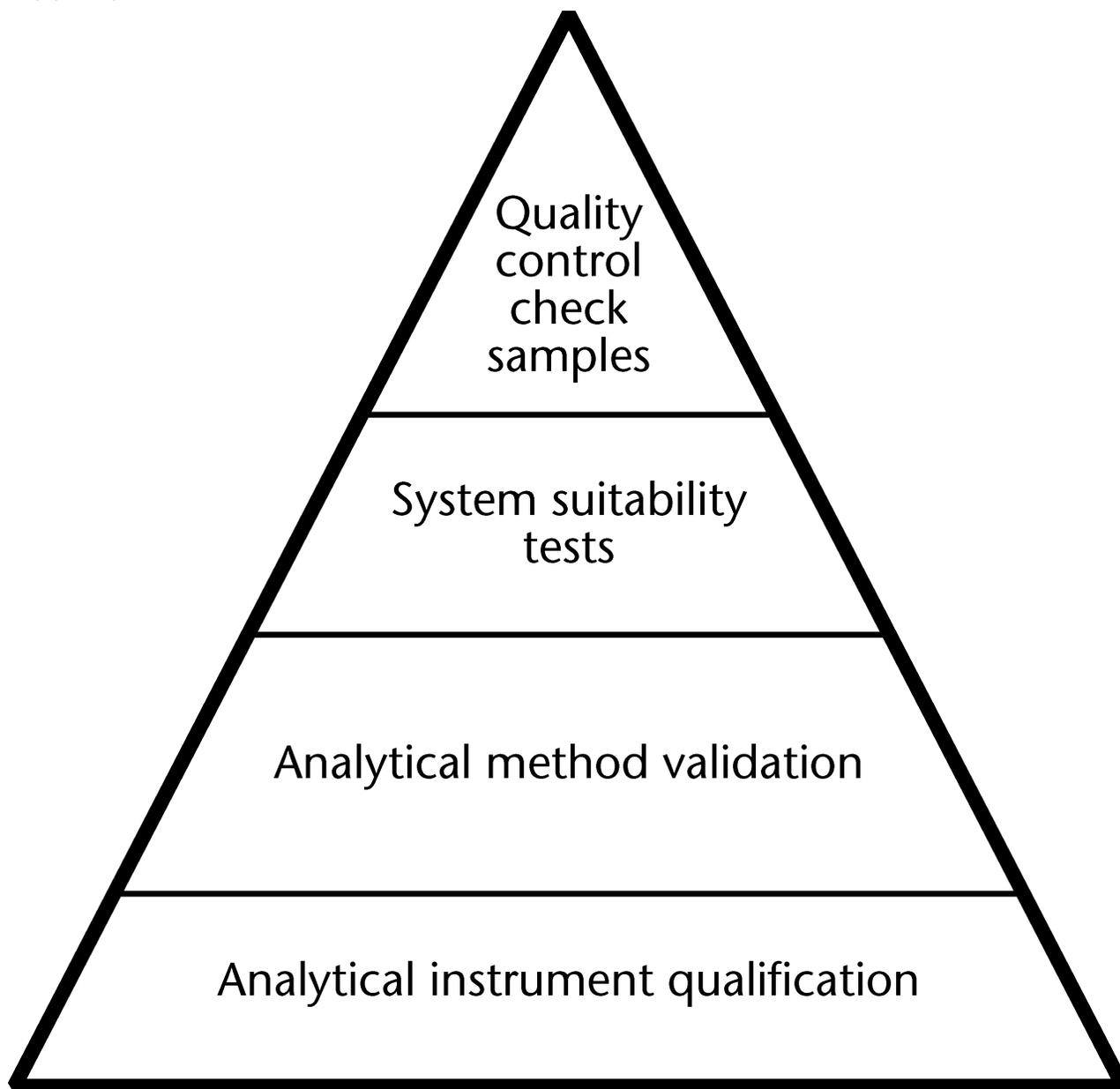


Figure 1. Components of data quality.

Analytical Instrument Qualification

AIQ is the collection of documented evidence that an instrument performs suitably for its intended purpose. Use of a qualified instrument in analyses contributes to confidence in the validity of generated data.

Analytical Method Validation

Analytical method validation is the collection of documented evidence that an analytical procedure is suitable for its intended use. Use of a validated procedure with qualified analytical instruments provides confidence that the procedure will generate test data of acceptable quality. Additional guidance on validation of compendial procedures may be found in the general information chapter *Validation of Compendial Procedures* (1225).

System Suitability Tests

System suitability tests verify that the system will perform in accordance with the criteria set forth in the procedure. These tests are performed along with the sample analyses to ensure that the system's performance is acceptable at the time of the test. USP general chapter *Chromatography* (621) presents a more detailed discussion of system suitability tests as related to chromatographic systems.

Quality Control Check Samples

Many analysts carry out their tests on instruments standardized using reference materials and/or calibration standards. Some analyses also require the inclusion of quality control check samples to provide an in-process or ongoing assurance of the test's suitable performance. In this manner, AIQ and analytical method validation contribute to the quality of analysis *before* analysts conduct the tests. System suitability tests and quality control checks help ensure the quality of analytical results *immediately before or during* sample analysis.

Change to read:

ANALYTICAL INSTRUMENT QUALIFICATION PROCESS

The following sections address in detail the AIQ process. The other three components of building quality into analytical data—analytical method validation, system suitability tests, and quality control check samples—are not within the scope of this chapter.

Qualification Phases

Instrument qualification is not a single continuous process, but instead results from several discrete activities

- over the life time of the instrument. ■ 1S (USP39)

For convenience, these activities can be grouped into four phases: design qualification (DQ), installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ).

Some AIQ activities cover more than one qualification phase, and analysts potentially could perform them during more than one of the phases (see *Table 1*). However, in many instances there is need for

- a ■ 1S (USP39)

specific order to the AIQ activities; for example, installation qualification must occur first in order to initiate other qualification activities. ~~The AIQ activities will be defined and documented.~~

- All AIQ activities should be defined and documented. ■ 1S (USP39)

- Routine analytical tests do not constitute OQ testing. OQ tests are specifically designed to verify the instrument's operation according to specifications in the user's environment

- as documented in the DQ, ■ 1S (USP39)

- and repeating the testing at regular intervals may not be required. However, when the instrument undergoes major repairs or modifications, relevant OQ and/or PQ tests should be repeated to verify whether the instrument continues to operate satisfactorily. If an instrument

is moved to another location, an assessment should be made of what, if any, OQ test should be repeated.

Table 1. Timing, Applicability, and Activities for Each Phase of Analytical Instrument Qualification^a

Design Qualification	Installation Qualification		Operational Qualification		Performance Qualification
Timing and Applicability					
Prior to purchase of a new model of instrument	At installation of each instrument (new, old, or existing unqualified)		After installation or major repair of each instrument		Periodically at specified intervals for each instrument
Activities					
Assurance of manufacturer's DQ	Description	↔	Fixed parameters		Preventive maintenance and repairs
Assurance of adequate support availability from manufacturer	Instrument delivery				Establish practices to address operation, calibration, maintenance, and change control
Instrument's fitness for use in laboratory	Utilities/facility	↔	Environment		
	Assembly and installation				
	Network and data storage	↔	Secure data storage, backup, and archive		
	Installation verification	↔	Instrument function tests ■ and software validation ■ 1S (USP39)	↔	Performance checks
<p>^a Activities under each phase are usually performed as given in the table. However, in some cases, it may be more appropriate to perform or combine a given activity with another phase. Such activities spanning more than one qualification phase are shown as connected by double arrows. If an activity listed under a given phase is performed under another phase, it is not necessary to repeat the activity under the phase where the activity is listed. Performing the activity is far more important than the phase under which the activity is performed.</p>					

DESIGN QUALIFICATION

Design qualification (DQ) is the documented collection of activities that define the functional and operational specifications of the instrument and criteria for selection of the vendor, based on the intended purpose of the instrument. Design qualification (DQ) may be performed not only by the instrument developer or manufacturer but also may be performed by the user. The manufacturer is generally responsible for robust design and maintaining information describing how the analytical instrument is manufactured (design specifications, functional requirements, etc.) and tested before shipment to users. Nonetheless, the user should ensure that commercial off-the-shelf (COTS) instruments are suitable for their intended application and that the manufacturer has adopted a quality system that provides for reliable equipment. Users

~~should also determine the manufacturer's capability for support installation, services, and training. This determination might be aided by the user's previous interaction with the manufacturer.~~

■ Design qualification (DQ) is the documented collection of activities that define the functional and operational specifications of the instrument, including the criteria for selection of the supplier, based on the intended purpose of the instrument. DQ states what the laboratory wants the instrument to do and shows that the selected one is suitable. Design qualification (DQ) may be performed by the instrument manufacturer or the user. It is expected that DQ requirements will be minimal for commercial, off-the-shelf (COTS) instruments. Verification that the instrument specifications meet the desired functional requirements may suffice.

The supplier is generally responsible for robust design and maintaining documentation describing how the analytical instrument and any associated controlling software are manufactured (for example, design specifications, functional requirements, etc.) and tested, sometimes called Factory Acceptance Tests (FAT). Nonetheless, the user should ensure that instruments are suitable for their intended application and that the supplier has adopted a quality system that provides for reliable instrumentation, software, and network connectivity. Users should also determine the supplier's capability to support installation, services, and training. This determination might be aided by the user's previous interaction with the supplier.

When use of an instrument changes or it is subject to a major upgrade, it may be necessary to review and/or update the user's DQ documentation.

■ 1S (USP39)

INSTALLATION QUALIFICATION

Installation qualification (IQ) is the documented collection of activities necessary to establish that an instrument is delivered as designed and specified, and is properly installed in the selected environment, and that this environment is suitable for the instrument. IQ applies to an instrument that is new or was pre-owned. ~~or to any instrument that exists on site but has not been previously qualified.~~

■ For any instrument that exists on site but has not been previously qualified, existing documents should be collated and a risk assessment should be undertaken to determine the best course of action. ■ 1S (USP39)

Relevant parts of IQ would also apply to a qualified instrument that has been transported to another location or is being reinstalled for other reasons, such as prolonged storage. The activities and documentation typically associated with IQ are as follows.

~~**Description**—Provide a description of the instrument or the collection of instrument components, including its manufacturer, model, serial number, software version, and location. Use drawings and flow charts where appropriate.~~

■ ■ 1S (USP39)

Instrument delivery: Ensure that the instrument, software, manuals, supplies, and any other instrument accessories arrive as specified ~~in the purchase order~~

■ by the user ■ 1S (USP39)

and that they are undamaged. For a pre-owned or existing instrument, manuals and documentation should be obtained.

■ **Description:** Document information about the instrument and all components, including supplier(s), model(s), serial number(s), software version(s), and location. ■ 1S (USP39)

Utilities/Facility/Environment: Verify that the installation site satisfactorily meets manufacturer

■ supplier ■ 1S (USP39)

-specified environmental requirements.

~~**Assembly and Installation**—Assemble and install the instrument, and perform any preliminary diagnostics and testing. Assembly and installation may be done by the manufacturer, vendor, specialized engineers, or qualified in-house personnel. Manufacturer-established installation tests and guides provide a valuable baseline reference for determining instrument acceptance. Any abnormal event observed during assembly and installation merits documenting. Installation packages purchased from the manufacturer or the vendor may, however, need to be supplemented with user-specific criteria.~~

■ **Assembly and installation:** Assemble and install the instrument, and perform any preliminary diagnostics and testing. Assembly and installation may be done by the supplier, service agents, specialized engineers, or qualified in-house personnel. Supplier-established installation tests provide a valuable baseline reference for determining instrument acceptance. Any abnormal event observed during assembly and installation merits documenting. IQ documentation packages purchased from a supplier should be reviewed to ensure that they are acceptable by the user before and after execution. ■ 1S (USP39)

~~**Network and Data Storage**—Some analytical systems require users to provide network connections and data storage capabilities at the installation site. When required, connect the instrument to the network, and check its functionality.~~

■ **Software installation, network, and data storage:** Some analytical systems require the installation of software onto a qualified computer and be connected to a network for communications and data storage at the installation site. IT involvement is often required with computerized laboratory systems. ■ 1S (USP39)

Installation verification: Perform the initial diagnostics and testing of the instrument after installation.

■ When required, connect the instrument to the network, and check its functionality.

Software configuration and/or customization: Any configuration or customization of instrument software should occur before the OQ and documented. Unless changes are needed for specific component tests, the OQ should be performed using the software configuration that will be used for routine analysis. ■ 1S (USP39)

OPERATIONAL QUALIFICATION

~~After a successful IQ, the instrument is ready for OQ testing. Operational qualification (OQ) is the documented collection of activities necessary to demonstrate that an instrument will function according to its operational specification in the selected environment. Testing activities in the OQ phase may consist of these test parameters.~~

■ Operational Qualification (OQ) is the documented collection of activities necessary to demonstrate that an instrument will function according to its operational specification testing in the selected environment. OQ demonstrates fitness of purpose for the user's ways of working, and should reflect the contents of the DQ document. Testing activities in the OQ phase may consist of these test parameters.

■ 1S (USP39)

Fixed parameters: These tests measure the instrument's non-changing parameters such as length, height, weight, voltage inputs, acceptable pressures, and loads. If the manufacturer-supplied specifications for these parameters satisfy the user, the test requirements may be waived. However, if the user wants to confirm the parameters, testing can be performed at the user's site. Fixed parameters do not change over the life of the instrument, and therefore never need redetermination

■ to be retested. ■ 1S (USP39)

[Note—These tests could also be performed during the IQ phase (see *Table 1*); if so, fixed parameters need not be re-determined as part of OQ testing.]

■ **Software functions:** Where applicable, OQ testing should include critical elements of the configured application software to show that the whole system works as intended. Functions to test would be functions applicable to data capture, analysis of data, and reporting results under actual conditions of use and security and access control and audit trail. The user can apply risk assessment methodologies and leverage the supplier's software testing to focus the OQ testing effort. ■ 1S (USP39)

Secure data storage, backup, and archiving: When applicable, test secure data handling such as storage, backup, audit trails, and archiving at the user's site according to written procedures.

Instrument function tests: Instrument functions required by the user should be tested to verify that the instrument operates as intended by the manufacturer. ~~Manufacturer-supplied~~

■ Supplier ■ 1S (USP39)

~~information is useful in identifying specifications for these parameters and in designing tests to evaluate the identified parameters. Users, or their qualified designees, should perform these tests to verify that the instrument meets manufacturer or user specifications in the user's environment.~~

■ verify that the instrument meets supplier or user specifications in the user's environment.

■ 1S (USP39)

The extent of OQ testing that an instrument undergoes depends on its intended applications, ~~Therefore, no specific OQ tests for any instrument or application are offered in this chapter.~~

■ therefore, no specific OQ tests for any instrument or application are offered in this chapter. Parameters to qualify are described in the general chapters pertaining to a specific analytical technique. ■ 1S (USP39)

OQ tests can be modular or holistic. Modular testing of individual components of a system may facilitate interchanging of such components without requalification. ~~Holistic tests, which involve the entire system, are also acceptable.~~

■ but requires a risk assessment to justify it. Holistic tests, which involve the entire system, demonstrate that the whole system works against user specifications in the DQ.

For OQ test suites purchased from a service provider or supplier, the user must review the material to assure themselves of the scientific soundness of the tests and compliance with applicable regulations. The user should review the documents before execution and approve the tests after execution to ensure completeness and accuracy of the completed document and the test data generated.

■ 1S (USP39)

PERFORMANCE QUALIFICATION

~~Performance qualification (PQ) is the documented collection of activities necessary to demonstrate that an instrument consistently performs according to the specifications defined by the user, and is appropriate for the intended use. After IQ and OQ have been performed, the instrument's continued suitability for its intended use is demonstrated through performance qualification. The PQ phase may include the following parameters.~~

■ Performance Qualification (PQ) is the documented collection of activities necessary to demonstrate that an instrument consistently performs according to the specifications defined by the user, and is appropriate for the intended use. The PQ verifies the fitness for purpose of the instrument under actual conditions of use. After IQ and OQ have been performed, the instrument's continued suitability for its intended use is demonstrated through continued performance qualification.

The user must define the PQ plans, including test procedures, acceptance criteria, and frequency. Preventive maintenance plans and documentation of repairs and other changes are also a necessary part of the overall instrument qualification.

PQ may include the following activities.

■ 1S (USP39)

Performance checks: ~~Set up a~~

■ A ■ 1S (USP39)

test or series of tests to verify the acceptable performance of the instrument for its intended use. PQ tests are usually based on the instrument's typical on-site applications and may consist of analyzing known components or standards. The tests should be based on good science and reflect the general intended use of the instrument. Some system suitability tests

or quality control checks that are performed concurrently with the test samples can be used to demonstrate that the instrument is performing suitably. PQ tests may resemble those performed during OQ, but the specifications for their results may be set differently if required. Nevertheless, user specifications for PQ tests should demonstrate trouble-free instrument operation for the intended applications. As is the case with OQ testing, PQ tests may be modular or holistic.

Testing frequency depends on the ruggedness of the instrument and the criticality of the tests performed. Testing may be unscheduled—for example, each time the instrument is used. It may also be scheduled for regular intervals. Experience with the instrument can influence this decision,

■ which should be documented. ■ 1S (USP39)

It may be useful to repeat the same PQ tests each time the instrument is used so that a history of the instrument's performance can be compiled. Alternatively, the instrument may be incorporated into an integrated support system to assure that it remains continually qualified. ~~Some system suitability tests or quality control checks that are performed concurrently with the test samples also imply that the instrument is performing suitably.~~

■ System suitability tests that are performed concurrently with the test preparations may also assure that the instrument is performing suitably. ■ 1S (USP39)

~~**Preventive maintenance and repairs:** When an instrument fails to meet PQ test specifications, it requires maintenance or repair. A periodic preventive maintenance may also be recommended for many instruments. The relevant PQ test(s) should be repeated after the needed maintenance or repair to ensure that the instrument remains qualified.~~

■ Periodic preventive maintenance activities are required for many instruments. This may include calibration. Document the PM plans, including procedures and frequency as part of the AIQ package. When an instrument fails to meet PQ criteria or otherwise malfunctions, the cause of the failure must be investigated and documented. The instrument may require maintenance or repair. The relevant OQ or PQ test(s) should be repeated after the needed maintenance or repair to ensure that the instrument remains qualified. ■ 1S (USP39)

~~**Practices for Operation, Calibration, Maintenance, and Change Control**—Establish practices to maintain and calibrate the instrument. Each maintenance and calibration activity should be documented.~~

■ **Practices for PQ, change control, and periodic review:** Each PQ, maintenance and calibration activity should be documented. Change control should be established to control changes to the instrument configuration, including firmware and software. Critical instruments should have a periodic review to ensure that the system is still under control. Typical areas for review can include qualification/validation status, currency of user procedures, change control records, correctness and completeness of records produced by the system, backup and recovery of electronic records, review and sign-off of test results.

The instrument owner/user and their management are responsible for this work, although portions can be carried out on his/her behalf by internal staff or external suppliers or service providers. ■ 1S (USP39)

Change to read:**ROLES AND RESPONSIBILITIES****Users**

~~Users are ultimately responsible for instrument operations and data quality. The user's group encompasses analysts, their supervisors, instrument specialists, and organization management.~~

■ Users are ultimately responsible for specifying their needs and ensuring that a selected instrument meets them and that data quality and integrity is maintained. The user's group encompasses analysts, their supervisors, instrument specialists, and organization management.

■ 1S (USP39)

Users should be adequately trained in the instrument's use, and their training records should be maintained as required by the regulations.

Users should also be responsible for qualifying their instruments because their training and expertise in the use of instruments make them the best-qualified group to design the instrument test(s) and specification(s) necessary for successful AIQ. ~~Consultants, equipment manufacturer or vendors, validation specialists, and quality assurance (QA) personnel can advise and assist as needed, but the final responsibility for qualifying instruments lies with the users. The users must also maintain the instrument in a qualified state by routinely performing PQ.~~

■ Consultants, instrument manufacturer or suppliers, validation specialists, and quality assurance (QA) personnel can advise and assist as needed, but the final responsibility for qualifying instruments and validating systems lies with the users. The users must also maintain the instrument in a qualified state by routinely performing PQ. ■ 1S (USP39)

Quality Unit

The role of the Quality Unit in AIQ remains the same as for any other regulated activity. Quality personnel are responsible for assuring that the AIQ process meets compliance requirements, that processes are being followed, and that the intended use of the ~~equipment is supported by~~

■ instrument is supported by complete, ■ 1S (USP39)

valid, and documented data.

The role of the Quality Unit in AIQ remains the same as for any other regulated activity. Quality personnel are responsible for assuring that the AIQ process meets compliance requirements, that processes are being followed, and that the intended use of the equipment is supported by valid and documented data.

Manufacturers,

■ **Suppliers, Service Agents, and Consultants** ■ 1S (USP39)

~~Manufacturers and developers are responsible for DQ when designing the instrument. They are also responsible for validation of relevant processes used in manufacturing and assembly of the~~

~~instrument. Manufacturers should test the assembled instruments before shipping them to users.~~

■ Manufacturers are responsible for designing and manufacturing the instrument, and ensuring the quality of relevant processes used in manufacturing and assembly of the instrument. Manufacturers should test the assembled instruments before shipping to users. To aid the user, vendors are responsible for developing meaningful specifications for the users to compare with their needs and aid selection as shown in *Figure 1*.

Where used, software should be developed and tested using a defined life cycle and have evidence of work performed to support major and minor revisions. Release notes should accompany each version of software released.

■ 1S (USP39)

Finally, it is desirable that ~~manufacturers and vendors~~

■ suppliers ■ 1S (USP39)

should notify all known users about hardware defects discovered after a product's release; offer user training, service, repair, and installation support; and invite user audits as necessary.

■ There should be a quality or technical agreement between the user organization and manufacturers, suppliers, service agents, or consultants who supply calibration, maintenance, qualification, or validation services that define the scope of work and the responsibilities between the two parties.

■ 1S (USP39)

Change to read:

SOFTWARE VALIDATION

■ There is an increasing inability to separate the hardware and software parts of modern analytical instruments. In many instances the software is needed to qualify the instrument and the instrument operation is essential when validating the software. Therefore to avoid overlapping and potential duplication, software validation and instrument qualification can be integrated into a single activity.

■ 1S (USP39)

~~Software used for analytical work can be classified into three categories: firmware; instrument control, data acquisition, and processing software; and stand-alone software. Although software validation is not the primary focus of this chapter, the following sections describe in which cases this activity is under the scope of the analytical instrument qualification.~~

■ Software used for analytical work can be classified into four categories: firmware; instrument control, data acquisition, and processing software; and standalone software. Although software validation is not the primary focus of this chapter, the following sections describe in which cases this activity is under the scope of the analytical instrument and system qualification.

Authoritative guidance for the validation of software used in analytical instruments are the Good Automated Manufacturing Practice (GAMP) guidelines and the GAMP Good Practice Guide on Risk Based Validation of Laboratory Computerized Systems.

■ 1S (USP39)

Firmware

Computerized analytical instruments contain integrated chips with low-level software (firmware). Such instruments will not function without properly operating firmware, and

■ in most cases ■ 1S (USP39)

users generally cannot alter firmware design or

■ ■ 1S (USP39)

function. Firmware is therefore considered a component of the instrument itself. Indeed, the qualification of hardware is not possible without operating it via its firmware.

Thus, when the hardware (that is, the analytical instrument) is qualified at the user's site, the integrated firmware is also essentially qualified. No separate on-site qualification of the firmware is needed. Whenever possible, the firmware version should be recorded as part of the IQ activities. Any changes made to firmware versions should be tracked through change control of the instrument (see *Change Control*, below).

■ In some instruments firmware can also be capable of fixed calculations on the acquired data. These calculations need to be defined and verified by the user. Some instruments have firmware that enable users to define programs for the instrument's operation, similarly these user defined programs need to be defined and verified to demonstrate that they are fit for intended purpose. Any user-defined programs should be placed under change control and, if possible, access restricted to authorized personnel.

■ 1S (USP39)

Instrument Control, Data Acquisition, and Processing Software

Software for instrument control, data acquisition, and processing for many of today's computerized instruments is loaded on a computer connected to the instrument. Operation of the instrument is then controlled via the software, leaving fewer operating controls on the instrument. Also, the software is needed for data acquisition and post-acquisition calculations. Thus, both hardware and software, their functions inextricably intertwined, are critical to providing analytical results.

~~The manufacturer should perform DQ, validate this software, and provide users with a summary of validation. At the user site, holistic qualification, which involves the entire instrument and software system, is more efficient than modular validation of the software alone. Thus, the user qualifies the instrument control, data acquisition, and processing software by qualifying the instrument according to the AIQ process.~~

■ The software in this group can be classified into three types: 1) non-configurable software (the software cannot be modified to change the business process), 2) configurable software (using tools from the supplier to modify the business process supported), and 3) configurable software with custom additions (configurable software above with custom software or macros to automate the business process).

The supplier of the system should develop and test the software according to a defined life cycle and provide users with a summary of the tests that were carried out. Ideally the software development should be carried out under a quality management system.

At the user site, integrated qualification of the instrument in conjunction with validation of the software involves the entire system. This is more efficient than separating instrument

qualification from validation of the software.

■ 1S (USP39)

Stand-Alone Software

~~An authoritative guide for validating stand-alone software, such as LIMS, is available.⁴ The validation process is administered by the software developer, who also specifies the development model appropriate for the software. Validation takes place in a series of activities planned and executed through various stages of the development cycle.~~

■ 1S (USP39)

Change to read:

CHANGE CONTROL

Changes to instruments, including software, become inevitable as ~~manufacturers~~

■ ~~suppliers~~ ■ 1S (USP39)

add new features and correct known defects. However, implementing all such changes may not always benefit users. Users should therefore adopt changes they deem useful or necessary and should also assess the effects of changes to determine what, if any, requalification is required. The change control process enables them to do this.

Change control may follow the DQ/IQ/OQ/PQ classification process. For DQ, evaluate the changed parameters, and determine whether need for the change warrants implementing it. If implementation of the change is needed, install the changes to the system during IQ. Evaluate which of the existing OQ and PQ tests need revision, deletion, or addition as a result of the installed change. Where the change calls for additions, deletions, or revisions to the OQ or PQ tests, follow the procedure outlined below.

Operational Qualification

Revise OQ tests as necessitated by the change. Perform the relevant tests affected by the change. This ensures the instrument's effective operation after the change is installed.

Performance Qualification

Revise PQ tests as necessitated by the change. Perform the PQ testing after installation of the change if similar testing is not already performed during OQ. In the future, perform the revised PQ testing.

For changes to firmware and to software for instrument control, data acquisition, and processing, change control is performed through DQ/IQ/OQ/PQ of the affected instrument. ~~Change control for stand-alone software requires user-site testing of changed functionality.~~

■ 1S (USP39)

Change to read:

AIQ DOCUMENTATION

Documents obtained during instrument qualification

■ qualification activities ■ 1S (USP39)

should be retained in an accessible manner. Where multiple instruments of one kind exist, documents common to all instruments and documents specific to an instrument may be stored separately. During change control, additional documents may supplement those obtained during the qualification process, and both sets of documents should be retained and maintained in a suitable manner that allows for appropriate protection and access.

Delete the following:

■

INSTRUMENT CATEGORIES

Modern laboratories typically include a suite of instruments and equipment varying from simple nitrogen evaporators to complex automated instruments. Therefore, applying a single set of principles to qualifying such dissimilar instruments would be scientifically inappropriate. Users are most capable of establishing the level of qualification needed for an instrument. On the basis of the level needed, it is convenient to categorize instruments into three groups: A, B, and C, as defined below. Examples of instruments in each group are provided. Note that the list of instruments provided here is for illustration only and is not meant to be exhaustive. It does not provide the exact category for an instrument at a user site. That category should be determined by users for their specific instruments or applications.

The exact grouping of an instrument must be determined by users for their specific requirements. Depending on individual user requirements, the same instrument may appropriately fall into one group for one user and another group for another user. Therefore, a careful selection of groups by users is highly encouraged.

Group A

Group A includes standard equipment with no measurement capability or usual requirement for calibration, where the manufacturer's specification of basic functionality is accepted as user requirements. Conformance of Group A equipment with user requirements may be verified and documented through visual observation of its operation. Examples of equipment in this group are nitrogen evaporators, magnetic stirrers, vortex mixers, and centrifuges.

Group B

Group B includes standard equipment and instruments providing measured values as well as equipment controlling physical parameters (such as temperature, pressure, or flow) that need calibration, where the user requirements are typically the same as the manufacturer's specification of functionality and operational limits. Conformance of Group B instruments or equipment to user requirements is determined according to the standard operating procedures for the instrument or equipment, and documented during IQ and OQ. Examples of instruments in this group are balances, melting point apparatus, light microscopes, pH meters, variable pipets, refractometers, thermometers, titrators, and viscometers. Examples of equipment in this group are muffle furnaces, ovens, refrigerator freezers, water baths, pumps, and dilutors.

Group C

Group C includes instruments and computerized analytical systems, where user requirements for

functionality, operational, and performance limits are specific for the analytical application. Conformance of Group C instruments to user requirements is determined by specific function tests and performance tests. Installing these instruments can be a complicated undertaking and may require the assistance of specialists. A full qualification process, as outlined in this document, should apply to these instruments. Examples of instruments in this group include the following:

- atomic absorption spectrometers
- differential scanning calorimeters
- dissolution apparatus
- electron microscopes
- flame absorption spectrometers
- high pressure liquid chromatographs
- mass spectrometers
- microplate readers
- thermal gravimetric analyzers
- X-ray fluorescence spectrometers
- X-ray powder diffractometers
- densitometers
- diode array detectors
- elemental analyzers
- gas chromatographs
- IR spectrometers
- near-IR spectrometers
- Raman spectrometers
- UV/Vis spectrometers
- inductively coupled plasma emission spectrometers

■ 1S (USP39)

Add the following:

■

GLOSSARY

The definitions of these terms may be different in other *USP* general chapters.

Software validation: Confirmation by examination and provision of objective evidence that software conforms to user needs and intended uses, and that the particular requirements implemented through software can be consistently fulfilled.

Qualification: Action of proving that any instrument works correctly and delivers the expected results; demonstration of fitness for purpose.

Calibration: The set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or values represented by a material measure or a reference material, and the corresponding values realized by standards.

Maintenance: Actions performed to keep an analytical instrument in a state of proper function so that it continues to operate within the boundaries set during qualification or validation.

Software configuration: Adapting software functions to a business process using tools

provided within the application by the supplier of the software.

Software customization: Changing the way software automates a business process by the addition of externally custom coded software modules using a recognized programming language or the development of macros within the application software.

Supplier: This term is used generically and can mean the manufacturer, a vendor, a service agent, or a consultant depending on the circumstances. ■1S (USP39)

†*General Principles of Software Validation: Final Guidance for Industry and FDA Staff*, U.S. Department of Health and Human Services, Food and Drug Administration, Rockville, MD, January 11, 2002. <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm085281.htm> (accessed December 2012.)

■ ■1S (USP39)

BRIEFING

《 1065 》 **Ion Chromatography**, *USP 38* page 1000 and *PF 40(3)* [May–June, 2014]. In response to comments received, the Chemical Analysis Expert Committee is proposing additional changes to this draft previously published in *PF 40(3)*. This revision is intended to align the text with current technology and terminology. Relevant sections in this chapter, particularly *Mobile Phases* and *Stationary Phases*, are extensively revised.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCCA: H. Pappa.)

Correspondence Number—C142380; C154452

Comment deadline: July 31, 2015

《 1065 》 ION CHROMATOGRAPHY

Change to read:

INTRODUCTION

Ion chromatography (IC) is a high-performance liquid chromatography (HPLC) instrumental technique used in *USP* test procedures such as identification tests, and

■ ■1S (USP39)

assays,

■ limit tests, and related impurity tests. ■1S (USP39)

to measure inorganic anions and cations, organic acids, carbohydrates, sugar alcohols, aminoglycosides, amino acids, ~~proteins, glycoproteins,~~

■ amines, phosphonates, ■1S (USP39)

and ~~potentially~~

■ ■1S (USP39)

other analytes.

■ Ion chromatography (IC) is a high-performance liquid chromatography (HPLC) instrumental technique used in *USP* test procedures such as identification tests, assays, and determination

of impurities, including limit test and quantitative tests. IC is used to measure anions and cations derived from organic or inorganic molecules, from small molecules to larger biomolecules. A not exhaustive list may include organic acids, carbohydrates, sugar alcohols, aminoglycosides, amino acids, amines, phosphonates, peptides, aminoglycosides, oligosaccharides, proteins, and glycoproteins. ■1S (USP39)

~~As dictated by the nature of the analyte,~~

■1S (USP39)

IC has been applied to all aspects of the manufacturing and disposition of pharmaceutical products, including characterization of active ingredients, excipients, degradation products, impurities, and process streams. ~~The following sample types are among those that have been analyzed: raw~~

■Raw ■1S (USP39)

~~materials, intermediates (including media and culture broths), bulk active ingredients, diluents, formulated products, production equipment cleaning solutions, and waste streams. The technique is especially valuable for ionic or ionizable (in the mobile phase) analytes that have little or no native UV absorbance. The ability to couple the ion-exchange separation with numerous detection strategies, e.g., pulsed amperometric detection (PAD), expands IC applications to instances where analyte-specific detection strategies can provide the required degree of sensitivity or specificity. Utilization of such strategies allows IC applications to be implemented on appropriately configured HPLC systems. Additionally, ion-exclusion separations and pulsed amperometric detection expand the range of application of IC to aliphatic organic acids as well as to nonionic analytes of significant pharmaceutical interest including alcohols, alditols, carbohydrates, and amino acids. The wide dynamic range of the methodology makes it applicable for the quantification of trace contaminants as well as major product components.~~

~~■process water, and waste streams may be analyzed using IC. ■1S (USP39)~~

Because

■The majority of IC methods use either anion- or cation-exchange chromatography coupled with suppressed conductivity detection. IC is especially valuable for ionic or ionizable (in the mobile phase) analytes that have little or no native UV absorbance. In addition to suppressed conductivity detection, the ion-exchange separation can be coupled to other detection strategies, including pulsed amperometric detection (PAD), UV/Vis absorbance detection, ■inductively coupled plasma mass spectrometric detection (ICPMS), ■1S (USP39)

~~and mass spectrometric detection, providing a wide range of analyte sensitivity and specificity. Ion-exclusion separations expand the range of IC applications to some nonionic analytes (e.g., alcohols) and provide a different selectivity for some analytes that can also be separated by ion exchange. The wide dynamic range of the majority of the IC detection methods makes IC applicable to the quantification of trace contaminants as well as major product components in the same run. ■1S (USP39)~~

~~IC typically uses dilute acids, alkalis,~~

■buffers, ■1S (USP39)

~~or salt solutions as the mobile phase, and does not use~~

■rarely uses ■1S (USP39)

~~an organic solvent, IC does not require the purchase of costly organic solvents and hazardous~~

■ reducing solvent cost and simplifying disposal of the waste effluent ■ 1S (USP39)

disposal of the waste effluent

■ logistics. ■ 1S (USP39)

The

■ In most cases, the effluent can be disposed of after appropriate neutralization (to a pH of ~7) ■ 1S (USP39)

effluent can be disposed of after appropriate neutralization (to a pH of ~7)

■ ■ 1S (USP39)

and, when necessary, after dilution with water.

IC allows separation using ion exchange, ion exclusion, or ion-pair approaches. IC separations are based on differences in charge density of the analyte species, which in turn depend on the valence and size of the individual ionic species to be measured. Separations are also performed on the basis of differences in the hydrophobic character of the ionic species. IC is typically performed at ambient temperature. As with other forms of HPLC, IC separations are based on varying capacity factors and typically follow the Knox equation. Ion chromatography is a technique complementary to the more commonly used reversed-phase and normal-phase HPLC and to atomic absorption and ion-coupled plasma (plasma spectrochemistry) techniques in pharmaceutical analysis.

■ IC is typically performed at or near ambient temperature. As with other forms of HPLC, IC separations are based on varying capacity factors and typically follow the Knox equation. IC is a technique often complementary to reversed-phase and normal-phase HPLC as well as atomic absorption and inductively-coupled plasma techniques in pharmaceutical analysis. ■ 1S (USP39)

Change to read:

APPARATUS

IC instruments closely resemble conventional HPLC instruments. Typical components include an autosampler, a high pressure pump, an injection valve with a sample loop of suitable size (typically 10

■ 5 ■ 1S (USP39)

–250 µL), a guard column, an analytical column, an optional

■ a ■ 1S (USP39)

■ an optional suppressor, or other forms

■ another type of post-column reaction system ■ 1S (USP39)

■ device, ■ 1S (USP39)

■ ■ 1S (USP39)

■ ■ 1S (USP39)

a flow-through detector, and a data system ranging in complexity from an integrator to a computerized data system (Figure 1).

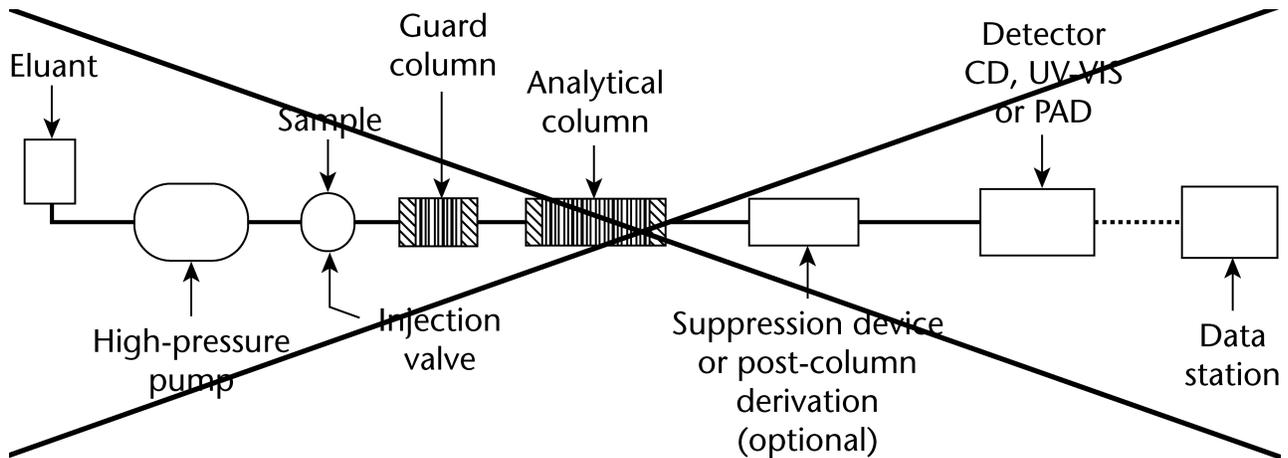


Figure 1. Components of a typical IC system illustrated schematically; CD = conductivity detector and PAD = pulsed amperometric detector.

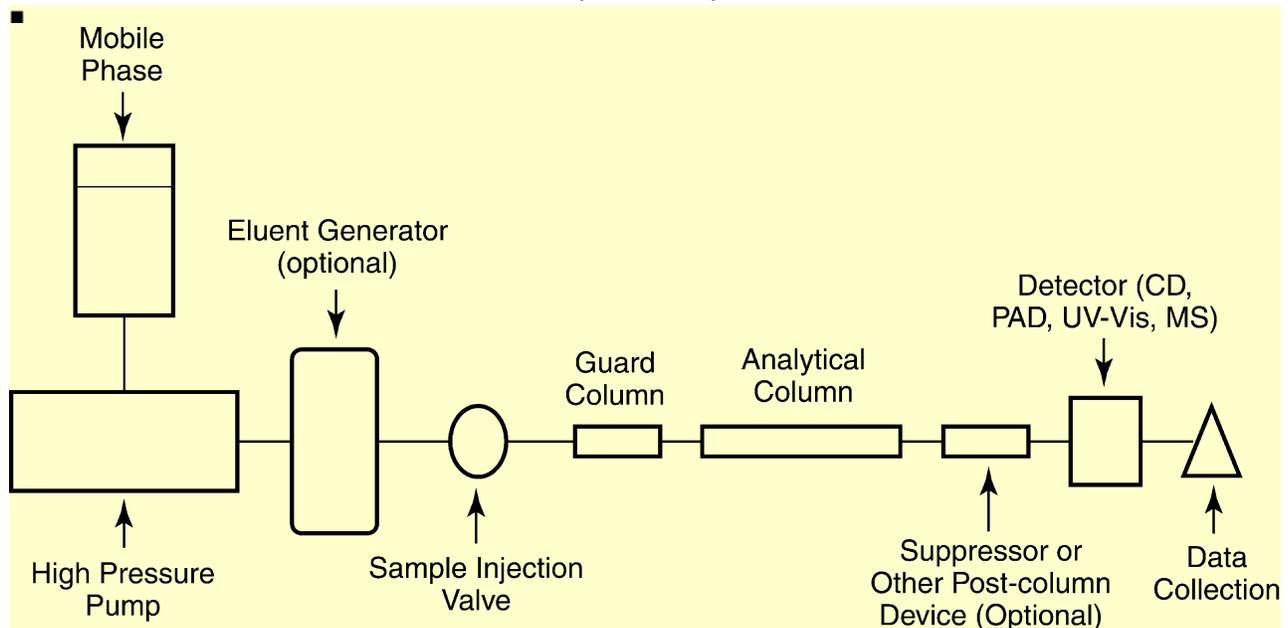


Figure 1. Components of a typical IC system illustrated schematically; CD = conductivity detector, PAD = pulsed amperometric detector, and MS = mass spectrometric detector.

[Note—Mobile phase is water when the optional *Eluent Generator* is used.]

■ 1S (USP39)

Because mobile phases generally consist of dilute acids, alkalis, or salt solutions, the components in contact with the mobile phase and the sample are typically made from inert materials, such as polyetheretherketone. Conventional HPLC systems also may be used provided that their components are compatible with the mobile phase and injected sample solutions. A metal-free system should be used for trace metal analysis. Following suitable preparation, the sample is introduced via the injection valve. After the optional chemical suppression or other post-column reaction on the column effluent, the analyte species are detected using conductivity, amperometry, UV/VIS, or other detection modes. Because IC uses a predominantly ionic mobile phase, a suppressor is often necessary prior to conductometric detection, although nonsuppressed conductometric detection has been successfully used in pharmaceutical analysis.

■ (PEEK). A conventional HPLC system can be used provided that its components are compatible

with the mobile phase and injected sample solutions, A

■ though a ■1S (USP39)

metal-free system should be used for trace metal analysis. Following suitable preparation, if needed, the sample is introduced via the injection valve. Without column effluent treatment or after chemical suppression or other post-column reaction, the analyte species are detected using conductivity, amperometry, UV/VIS, MS, or other detection mode.

■ ■1S (USP39)

Because IC uses a predominantly ionic mobile phase, a suppressor is typically necessary prior to conductometric detection when high sensitivity is needed, although nonsuppressed conductometric detection has been successfully used in pharmaceutical analysis. ■1S (USP39)

Stationary and Mobile Phases

As IC has developed and matured as an instrumental technique, the number of ion-exchange materials developed for IC has increased, facilitated by the understanding of the processes taking place at the surface of the stationary phase. In contrast to the silica-based column packing prevalent in classical HPLC, organic polymers are predominately used as support materials for IC. Such materials have a higher stability with respect to extremes in pH and in many cases are compatible with organic solvents. Typically, separation of anions requires the use of polymer-based anion exchangers and dilute bases as mobile phases. However, for cation separations, the stability over the entire pH range that is typical of organic polymers is not necessary, because dilute acids serve as mobile phases. Therefore, silica-based cation exchangers that exhibit a significantly higher chromatographic efficiency are commonly used for the separation of cations.

Depending on the separation mode (ion exchange, ion exclusion, or ion-pair), different types of stationary phases are used. For ion exchange, the stationary phase is either an anion or a cation exchanger. Typically, a strong cation exchanger is used for the ion-exclusion separation of organic acids, and a reversed-phase stationary phase is used when ion-pair is the separation mode. The ion-exchange capacity of a resin is defined as the number of ion-exchange sites per weight equivalent of the column packing and is typically expressed in terms of mEq per g of resin. With ion exchange, the retention times for the analyte ions increase with increasing ion-exchange capacity of the resin. This effect can be partly compensated for by using mobile phases of higher ionic strength. Styrene/divinylbenzene copolymers, polymethacrylate, and polyvinyl resins are the substrate materials used in the manufacturing process of the polymer-based ion exchangers. Organic polymers are functionalized directly at their surface, with the exception of latex-based ion exchangers, where the totally porous latex particle acts as an ion-exchange material. Surface-functionalized, "pellicular" substrates show a much higher chromatographic efficiency compared with the fully functionalized resins.

With ion exchange, a mobile phase consisting of mono- or divalent ionic species, alone or mixed at an optimum ratio, is used to accomplish the separation. In ion-exclusion methods, particularly for organic acids, the mobile phase consists of mineral acids to maintain organic acids in their undissociated forms. Often, the nature of the analyte dictates the mobile phase and the detection mode used. Typical mobile phases used in IC are described below in the section on detectors.

Mobile Phases

Nearly all IC separations require dilute acid

- acids ■ 1S (USP39)

or base

- bases diluted in high-purity water, generally with resistivity greater than 18 megohms-cm to prepare the ■ 1S (USP39)

mobile phases. The detection method, which is chosen for its sensitivity and selectivity for the analyte of interest, usually dictates the mobile phase choice.

- As in every chromatographic method the sensitivity dictates the detection method, and the selectivity dictates the mobile phase and column selection. ■ 1S (USP39)

For suppressed conductivity detection, the bases and acids used are suppressed to water or weakly dissociated species. The

- If the analytes are anions, the ■ 1S (USP39)

mobile phase bases used for the determination of anions

- as counterions ■ 1S (USP39)

by suppressed conductivity detection are sodium or potassium hydroxide, sodium carbonate, sodium bicarbonate, and less often sodium tetraborate. The

- If the analytes are cations, the ■ 1S (USP39)

mobile phase acids used for the determination of cations

- as counterions ■ 1S (USP39)

by suppressed conductivity detection are methanesulfonic acid, sulfuric acid, and less often hydrochloric acid.

- When determining low ion concentrations, appropriate trapping technology should be used to purify the mobile phase. ■ 1S (USP39)

When the detection chosen is

- is based on ■ 1S (USP39)

UV or visible absorbance, either with or without derivatization,

- ■ 1S (USP39)

then a wide variety of salt solutions may be used for

- into ■ 1S (USP39)

the mobile phase, ~~The typical mobile phases used for IC with nonsuppressed detection are~~
 ■ including ■ 1S (USP39)

phthalic acid and *p*-hydroxybenzoic acid for the determination of anions, and methanesulfonic acid for the determination of cations.

~~For nearly all IC methods using amperometric~~

■ Amperometric ■ 1S (USP39)

detection

■ uses ■ 1S (USP39)

~~either a strong acid or base solution is used for~~

■ as ■ 1S (USP39)

the mobile phase, although some methods can use a salt solution near neutral pH or an acid or base solution containing a salt. Most IC-MS methods use the same mobile phases used for suppressed conductivity, though volatile amines and volatile salt solutions have also been used. IC methods that use an ion-exclusion column use solutions of strong acids such as sulfuric acid as mobile phases. Organic solvents commonly used for HPLC are sometimes added to the mobile phase, typically at NMT 20% concentration. This addition is usually made to either modify selectivity or to enhance solubility of sample components that might otherwise contaminate the stationary phase.

■ Also, protein molecules may get a different surface charge distribution according to their structure and the pH of the mobile phase. In those cases, the pH, gradient of pH, ionic strength, or a combination of those parameters proved to be useful in the separation.

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Stationary Phases

~~Most~~

■ ■ 1S (USP39)

~~IC separations rely on ion exchange, and therefore most~~

■ ■ 1S (USP39)

stationary phases for IC are anion or cation exchangers, and less commonly phases containing both functionalities. ~~Silica-based stationary phases are less commonly used because of their incompatibility with extreme mobile phase pHs often needed in IC separations. The ion exchangers used for IC are typically constructed of polymers that allow them to be used over the entire pH range, or at a minimum, over an extended pH range compared to silica. Also, most~~

■ Stationary phases may be silica-based or polymer-based materials supporting ionic functional groups. However, due to the solubility of silica gel in water, particularly at alkaline pHs, the use of silica-based support is limited when the mobile phase is alkaline. In those cases, a polymeric support for IC is useful over an extended pH range. Most ■ 1S (USP39)

of the phases used today are constructed with highly cross-linked polymers, making them

compatible

- increasing their compatibility ■ 1S (USP39)

- with the organic solvents used for HPLC. Anion-exchange

- that sometimes are needed into the mobile phase. Polymeric anion-exchange ■ 1S (USP39)

- phases for IC are typically constructed starting with a

- from ■ 1S (USP39)

- polystyrene/divinylbenzene, ethylvinylbenzene/divinylbenzene, or polyvinyl alcohol polymeric substrate and polymethacrylate Packed columns (there are also IC columns prepared from porous polymeric monolithic backbones) have bead sizes that are as small as 4 μm , and typically no larger than 15 μm . The substrate bead can be nonporous or have pores as large as 2000 angstroms. Pore size is increased to increase capacity. To complete

- supports, with particles sizes ranging from 4 to 15 μm in diameter either non-porous or with pores up to 2000 \AA . To provide functionality to ■ 1S (USP39)

- the anion exchanger, the anion-exchange groups are attached to the substrate typically in one of two manners, electrostatically or covalently

- by condensation polymerization (a.k.a. a step-growth polymerization). ■ 1S (USP39)

- Pellicular phases use electrostatic bonding. The substrate is first sulfonated and then treated with small beads (<1 μm , the latex) that have bound anion-exchange groups. The latex beads are electrostatically bound to the substrate beads. Capacity and selectivity are varied by varying the latex size, its crosslinking, and the nature of the anion-exchange group. Condensation polymerization (also know as step-growth polymerization) also involves electrostatic bonding in distinct steps, the number of which allows control of both selectivity and capacity.

- ■ 1S (USP39)

- Cation-exchange phases for IC use the same polymeric substrates as the anion-exchange phases, but because the mobile phases for cation IC are acidic, silica can also be used. Most stationary phases for cation IC are grafted stationary phases, in which a polymer containing multiple cation-exchange groups is covalently bound to the substrate. Pellicular cation-exchange phases are less common. They are assembled with a second latex coating (i.e., an anion-exchange phase is treated with latex containing cation-exchange groups). For ion-exclusion chromatography, a porous strong cation-exchange stationary phase is used. Ion-pair ion-chromatography typically uses a polymer reversed-phase stationary phase.

- Ion-exclusion chromatography use a porous strong cation-exchange stationary phase, while ion-pair chromatography typically use a polymer reversed-phase stationary phase. ■ 1S (USP39)

- 1S (USP39)

Detectors

- **Detection** ■ 1S (USP39)

Conductivity detection is by far

- ■ 1S (USP39)

the most commonly employed mode of detection in IC, Although the original IC development work included the use of low capacity ion-exchange resins for efficient chromatographic separation and conductometric detection of ions in a chemically suppressed mobile phase, the advances in column technologies as well as instrumentation development allow the use of high-capacity ion-exchange today.

- especially

- for ■ 1S (USP39)

suppressed conductivity detection. ■ 1S (USP39)

In suppressed IC

■ conductivity detection, ■ 1S (USP39)

the background conductance of the ionic mobile phase is significantly reduced as it flows through the suppression device (suppressor). For example,

■ when ■ 1S (USP39)

diluted sodium hydroxide (10–50 mM) used as the mobile phase in IC of anions

■ flows through the suppressor, it ■ 1S (USP39)

is converted to water (poor conductivity) when the column effluent containing sodium hydroxide flows through the suppressor device present in an acidic form. The analyte ionic species in the column effluent are converted from their sodium or other metal salt forms to highly conducting acid forms (due to higher equivalent conductance of hydrogen ions compared to other cations):

■ water (H₂O), which shows very poor conductivity. ■ 1S (USP39)

Analogous reactions occur in the hydroxide form suppressor in IC of

■ using a suppressor for cations, ■ 1S (USP39)

where in

■ ■ 1S (USP39)

the acidic mobile phase is converted to water, and the analyte cations are converted to highly conducting hydroxide forms (due to higher equivalent conductance of hydroxide ions compared to other anions).

The reduced background conductance and the enhanced signal due to the ionic species result in an

■ a significantly ■ 1S (USP39)

enhanced signal-to-noise ratio for the conductometric detection of ions in suppressed IC. This results in reduced background noise and increasing sensitivity and reproducibility of the analysis. The commonly used chemical suppression devices fall into three broad categories. In the first type, the reactions occur across an ion-exchange membrane with the regenerant ions furnished by either a chemical or as products of electrolysis of water.

■ This results in reduced baseline noise while increasing the sensitivity and reproducibility of the analysis. Commonly used suppressors can be classified into two categories. In the first type, the reactions occur across an ion-exchange membrane with the regenerant ions furnished by either a chemical or as products of electrolysis of water. ■ 1S (USP39)

In the second type, the suppression reactions occur in a packed bed of high-exchange capacity resin

■ or monolith ■ 1S (USP39)

material, with regeneration either by a chemical or by electrolysis of water. In the third type, although not commonly used, the suppression reactions occur as the eluant stream mixes with the flowing stream of high capacity resin material.

For pharmaceutical analyses, suppressed conductometric detection may be used for detection of trace ions in high purity waters. The commonly used mobile phases for the separation of anions by suppressed IC include hydroxide ions or a mixture of bicarbonate and carbonate ions. The common mobile phases for separation of cations usually consist of mineral acids or methanesulfonic acid.

Ion chromatographic analyses also can be performed without chemical suppression, in which case the analytical column effluent flows directly to a conductivity detector. The typical eluants used in nonsuppressed IC are phthalic acid and *p*-hydroxybenzoic acid for the determination of anions and methanesulfonic acid for the determination of cations. The equivalent conductance values of chloride, sulfate, and other common anions are significantly greater than that of the eluant anion, and therefore, a positive peak is detected as the anions are carried through the detector. The equivalent conductance values of sodium, potassium, calcium, magnesium, and other common cations are significantly lower than that of the cation (H^+) in the eluant. In this instance, a negative peak is detected as the cations are carried through the detector.

Nonsuppressed IC is easier to perform, and it is a useful technique for determining ions of weak acids such as cyanide and sulfide, which are nonconductive after chemical suppression but show a higher baseline noise. Pharmaceutical analyses can be performed in the nonsuppressed mode because the quantification limits are usually in the upper mg per L to low percentage levels. While suppressor-based methodologies must often be implemented on the instrument systems specifically designed for this purpose, IC may be performed without the suppressor on an existing HPLC. This is possible because the commonly used eluants in IC include dilute bases or acids that are compatible for use on existing HPLC instruments. When this approach is considered, analysts are encouraged to consult the instrument manufacturer for applicability of the instrument for the IC analysis.

OTHER DETECTORS

Other commonly used detection modes in IC include pulsed amperometry, direct UV detection, or post-column derivatization followed by UV/VIS detection.

Pulsed amperometric detection mode (PAD)—PAD uses a specialized mode of the conventional amperometric technique. This type of detector is commonly used for the detection of electroactive species, e.g., organic compounds such as carbohydrates, sugar alcohols, amino acids, and organic sulfur species. In PAD, analytes are detected by an oxidative desorption process at the surface of an electrode located in the column effluent stream. Following the detection process, a series of potentials are applied for fixed time periods to clean the electrode surface. Unlike conventional amperometry that suffers from electrode surface fouling, a rapidly repeating sequence of different working potentials, referred to as waveform, helps the removal of the products of redox reactions from the electrode surface.

Direct and indirect UV detection—Direct UV detection is used for inorganic and organic ions that possess a UV chromophore. These include organic acids, bromide, iodide, nitrate, nitrite, thiosulfate, and cyano-metal complexes. Analogous to the inverse conductometric detection of cations, UV detection may also be performed indirectly. This method is called indirect photometric chromatography (IPC).

Photometric detection—Photometric detection involves chelation of the metal ions in column effluent with a color-forming reagent prior to detection with a visible wavelength. A classic example is the separation of metal ions in which the column effluent is chelated with 4-(2-pyridylazo)-resorcinol followed by detection at 510 to 530 nm.

■ Nonsuppressed (direct) conductivity detection is most often used for cation analysis and in situations that do not require high sensitivity, detecting concentrations in the range of mg/L.

~~Other commonly used IC detection techniques include pulsed/integrated amperometry, direct UV detection, mass spectrometry, and UV/VIS detection following post-column derivatization.~~

■ ■ 1S (USP39)

Pulsed/integrated amperometric detection (PAD/IPAD):

PAD and IPAD are modes of amperometric detection that apply more than one potential to the working electrode. These detection modes are commonly used for the detection of electroactive species, e.g., organic compounds such as carbohydrates, sugar alcohols, amino acids, amines, and organic sulfur species that can be easily oxidized. Analytes are detected by an oxidative desorption process at the surface of an electrode located in the column effluent stream. PAD uses one potential for detection while IPAD uses multiple potentials. The current generated during the fixed time periods these detection potentials are applied is integrated to yield charge. Following detection, a series of potentials are applied for fixed time periods to clean the electrode surface. Unlike conventional direct current amperometry that suffers from electrode surface fouling, this rapidly repeating sequence of potentials for detection and electrode cleaning, referred to as a waveform, allows detection and removal of the products of redox reactions from the working electrode surface.

Direct

■ ■ 1S (USP39)

UV detection: Direct UV detection is used for inorganic and organic ions that absorb UV light, typically at low wavelengths. These include organic acids, bromide, iodide, nitrate, nitrite, thiosulfate, and cyano-metal complexes.

■ Indirect UV detection uses eluents that strongly absorb in the visible or ultraviolet spectral region. A wavelength is selected where the eluent absorbs but the sample ions do not, and then negative peaks proportional to the analyte concentration are detected. In spectral detection after post-column reaction, some analytes are detected after the column effluent is combined with a reagent resulting in the formation of a compound that absorbs light at either a UV or visible wavelength. A classic example is the determination of metal ions, where the metal ions in the column effluent are chelated with 4-(2-pyridylazo)-resorcinol followed by detection at 510 and 530 nm.

■ 1S (USP39)

~~UV/visible detection after post-column reaction~~

~~—Some analytes can be detected after the column effluent is combined with a reagent that results in formation of a compound that absorbs light at either a UV or visible wavelength. A classic example is the separation of metal ions in which the metal ions in the column effluent are chelated with 4-(2-pyridylazo)-resorcinol followed by detection at 510–530 nm.~~

■ ■ 1S (USP39)

Mass spectrometry (MS):

Typically, analytes are detected after they have first passed through a suppressor to make the resulting effluent compatible with the mass spectrometer. Negative mode electrospray ionization is used for anions while the positive mode is used for cations. The suppressor effluent

is sometimes augmented with an organic solvent to improve ionization for increased sensitivity. Certain metal ions can be determined by an ion-exchange separation followed by ICP-MS.

■ 1S (USP39)

Change to read:

SAMPLE PREPARATION

Typically sample preparation for IC includes dilution or filtering through a 0.45- μm filter, or both. Under certain circumstances, samples may require removal of undesirable species through solid-phase extraction (SPE) techniques. For example, a highly alkaline sample can be neutralized by having it pass through an SPE cartridge packed with cation-exchange material in the acidic form.

■ As in many other analytical techniques, sample

■ Sample ■ 1S (USP39)

preparation may range from simple sample dissolution or dilution to the proper concentration, often followed by filtration, and in other cases more complex preparations including

■ need solid-phase extraction ■ 1S (USP39)

(SPE). approaches are needed.

■ ■ 1S (USP39)

If the solution is cloudy and/or contains particulates, then filtration through a syringe filter of 0.45- μm pore size that is suitable for IC is needed. Samples containing a high concentration of ions of the same charge as the target analyte may require a sample pretreatment to selectively remove the high concentration ion. ■ 1S (USP39)

Change to read:

PROCEDURE

Conductometric detection requires high purity water (generally, resistivity greater than 18 megohm-cm) and high purity chemicals for the preparation of the mobile phase. For ion-pair separation with UV detection, water and mobile phase components of low UV absorbance should be used.

For ion-exchange, the retention time of ions increases with a decrease in the ionic strength and valency (charge) of the mobile phase components. For example, at equimolar concentrations of sodium hydroxide or sodium carbonate mobile phase, capacity factors (K') for anions are smaller with sodium hydroxide as the mobile phase than with sodium carbonate as the mobile phase. Some mobile phases, such as sodium hydroxide, can absorb ambient carbon dioxide, resulting in its composition change and often in baseline artifacts. In this instance, care should be taken to prevent absorption of carbon dioxide by the sodium hydroxide mobile phase.

For ion-exclusion, capacity factors of organic acids increase with an increase in ionic strength

~~or concentration of mineral acids but decrease with the increase of the column temperature. Because permeation volume remains constant, these effects are usually small. Addition of a solvent such as acetonitrile shortens the retention of organic acids.~~

~~Like other HPLC techniques, IC systems are calibrated by plotting peak responses in comparison with known concentrations of a reference standard, using either an external or internal standardization procedure.~~

■ ~~Ion chromatography requires high purity water (generally, resistivity greater than 18 megohm-cm) for mobile phase preparation and any sample pretreatment.~~

■ ~~1S (USP39)~~

The choice of mobile phase is typically dictated by the choice of column, which in turn is chosen based on the selectivity for the analyte(s) compared to other ions of the same charge known or likely to be present. In situations where the other ions are in high concentration, a column with higher capacity is chosen to prevent column overload. This is especially important for many limit tests, where low concentration of a target analyte is in the presence of a large concentration of another ion of the same charge state. For anion IC, some mobile phases can be prepared from the solid or from commercial concentrates or ready-to-use solutions, e.g., sodium bicarbonate/carbonate. Other mobile phases should be prepared and handled with care, e.g., sodium hydroxide solutions, minimizing air exposure, and prepared from 50% sodium hydroxide solutions. Sodium hydroxide pellets and commercial dilute solutions contain large amounts of carbonate, thus altering the desired composition of the mobile phase. The acid solutions for cation IC are prepared by diluting high-purity concentrated acids. Alternately, carbonate/bicarbonate, hydroxide, and methanesulfonic acid mobile phases can be produced by an eluent generator. Most analyses will require the injection of 5–50 μL of sample solution, but larger volumes may be required for the analysis of low concentration analytes. As in other LC techniques, quantification is made by either internal or external standardization procedures, where the concentration is calculated by interpolation of the sample response into a calibration curve. IC methods are validated according to the recommendations described in *Validation of Compendial Procedures* $\langle 1225 \rangle$. ■ 1S (USP39)

BRIEFING

$\langle 1086 \rangle$ **Impurities in Drug Substances and Drug Products**, *USP 38* page 1063 and *PF* 40(3) [May–June 2014]. This revision is proposed on the basis of public comments received on the previous publication in *PF*. As part of an ongoing monograph modernization initiative, USP is updating this general chapter and proposing a new chapter, *Organic Impurities in Drug Substances and Drug Products* $\langle 476 \rangle$, which addresses organic impurities testing for articles with monographs in relevant USP compendia. This chapter has been updated to align it with current scientific and regulatory standards and to help ensure the appropriate control of organic impurities and degradation products in drug substances and drug products. In addition to providing updated general guidelines, this chapter introduces definitions and a decision tree for addressing impurities associated with drug substances and drug products. These new resources should assist the user who may have questions related to implementation of $\langle 476 \rangle$. Over time, *Ordinary Impurities* $\langle 466 \rangle$ may be used less frequently and may be withdrawn.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCPA: A. Hernandez-Cardoso.)
Correspondence Number—C157667

Comment deadline: July 31, 2015

1086 IMPURITIES IN DRUG SUBSTANCES AND DRUG PRODUCTS

Change to read:

INTRODUCTION

This general information chapter is intended to provide common terminology for impurities and degradation products that may be present in compendial drug substances and drug products. Impurities or degradation products in drug substances can arise during the manufacturing process or during storage of the drug substance. The degradation products in drug products can arise from drug substances or reaction products of the drug substance with the environment, with an excipient, or an immediate container closure system. Biological and biotechnological products, fermentation products and semisynthetic products derived therefrom, and radiopharmaceutical products are not covered in this chapter.

Communications about impurities and degradation products in compendial articles may be improved by including in this Pharmacopeia the definitions of terms and the contexts in which these terms are used. (See *Definitions* below.) There has been much activity and discussion in recent years about the definition of terms. Certain industry-wide concerns about terminology and context deserve widespread publication and ready retrievability and are included here. See section 5.60, *Impurities and Foreign Substances* in section 5, *Monograph Components* under *General Notices and Requirements*, as well as the general chapter *Ordinary Impurities* (466). Some other general chapters added over the years have also addressed topics of purity or impurity as these have come into focus or as analytical methodology has become available. Analytical aspects are enlarged upon in the chapter *Validation of Compendial Procedures* (1225).

Purity or impurity measurements for drug products present a challenge to Pharmacopeial standards setting. Where degradation of a drug product over time is at issue, the same analytical methods that are stability indicating are also purity indicating. Resolution of the active ingredient(s) from the excipients necessary to the preparation presents the same qualitative problem. Thus, many monographs for Pharmacopeial preparations feature chromatographic assays. Where more significant impurities are known, some monographs set forth specific limit tests. In general, however, this Pharmacopeia does not repeat impurity tests in subsequent preparations where these appear in the monographs of drug substances and where these impurities are not expected to increase. It is presumed that adequate retention specimens are in storage for the exact batch of drug substances used in any specific lot of a drug product. Whenever analysis of an official article raises a question of the official attributes of any of the drug substances used, subsequent analysis of retention specimens is in order.

DRUG SUBSTANCE

~~**Classification of Impurities**—Impurities can be classified into the following categories:~~

- ~~1. Organic impurities (process and drug-related)~~
- ~~2. Inorganic impurities~~
- ~~3. Residual solvents~~

~~Organic impurities can arise during the manufacturing process and/or storage of the drug substance. They can be identified or unidentified, volatile or nonvolatile, and include the following:~~

- ~~1. Starting materials~~
- ~~2. Byproducts~~
- ~~3. Intermediates~~
- ~~4. Degradation products~~
- ~~5. Reagents, ligands, and catalysts~~
- ~~6. Geometric and stereoisomers~~

~~Inorganic impurities can result from the manufacturing process. They are normally known and identified and include the following:~~

- ~~1. Reagents, ligands, and catalysts~~
- ~~2. Heavy metals or other residual metals~~
- ~~3. Inorganic salts~~
- ~~4. Other materials (e.g. filter aids, charcoal)~~

~~Residual solvents are organic liquids used as vehicles for the preparation of solutions or suspensions in the synthesis of a drug substance. Because these are generally of known toxicity, the selection of appropriate controls is easily accomplished (see *Residual Solvents* (467)).~~

~~Concepts for setting impurity or degradation product limits in drug substances are based on chemistry and safety concerns. As such, limits for organic and inorganic impurities and residual solvents should be established for drug substances. The basic tenet for setting limits is that levels of impurities or degradation products in a drug substance must be controlled throughout its development to ensure its safety and quality for use in a drug product.~~

~~Documented evidence that the analytical procedure used to evaluate impurities or degradation products is validated and suitable for the detection and quantification of impurities or degradation products should be established.~~

~~**DRUG PRODUCT**~~

~~The specification for a drug product should include a list of degradation products expected to occur during manufacture of the commercial product and under recommended storage conditions. Stability studies, knowledge of degradation pathways, product development studies, and laboratory studies should be used to characterize the degradation profile. The selection of degradation products in the drug product specification should be based on the degradation products found in batches manufactured by the proposed commercial process.~~

~~This rationale should include a discussion of the degradation profiles observed in the safety and~~

clinical development batches and in stability studies, together with a consideration of the degradation profile of batches manufactured by the proposed commercial process. For degradation products known to be unusually potent or to produce toxic or unexpected pharmacological effects, the quantitation/detection limit of the analytical procedures should be commensurate with the level at which the degradation products should be controlled.

For drug products the concept for setting degradation product limits is based on sound scientific judgment as applied to available data on the safety and stability of the drug product, data that may include the degradation pathways of the drug substance, the manufacturing process, known excipient interactions, any safety assessment studies, stability studies conducted under the recommended storage conditions, and ancillary studies that may provide additional information on the stability profile of the drug product. Impurities that are not degradation products (e.g., process impurities from the drug substance) are often not controlled in the drug product, as they are typically controlled in the drug substance and these impurities are not expected to increase over time. Additional guidance for setting limits can be found in various ICH and FDA guidance documents, as well as in the USP monograph submission guidelines.

Documented evidence that the analytical procedure used to evaluate impurities or degradation products is validated and suitable for the detection and quantification of impurities or degradation products should be established.

Drug products should contain levels of residual solvents no higher than can be supported by safety data (see *Residual Solvents* (467)).

DEFINITIONS

Concomitant Components—Concomitant components are characteristic of many drug substances and are not considered to be impurities in the Pharmacopeial sense. Limits on contents, or specified ranges, or defined mixtures are set forth for concomitant components in this Pharmacopeia. Examples of concomitant components are geometric and optical isomers (or racemates) and antibiotics that are mixtures. Any component that can be considered a toxic impurity because of significant undesirable biological effect is not considered to be a concomitant component.

Degradation Product—An impurity resulting from a chemical change in the drug substance brought about during manufacture and/or storage of the drug product by the effect of, for example, light, temperature, pH, water, or by reaction with an excipient and/or the immediate container closure system.

Foreign Substances (Extraneous Contaminants)—An impurity that arises from any source extraneous to the manufacturing process and that is introduced by contamination or adulteration. These impurities cannot be anticipated when monograph tests and assays are selected. The presence of objectionable foreign substances not revealed by monograph tests and assays constitutes a variance from the official standard. Examples of foreign substances include ephedrine in Ipecac or a pesticide in an oral liquid analgesic. Allowance is made in this Pharmacopeia for the detection of foreign substances by unofficial methods. (See section 5.60, *Impurities and Foreign Substances* in section 5, *Monograph Components under General Notices and Requirements*.)

Identified Impurities and Identified Degradation Products—Impurities or degradation products for which structural characterizations have been achieved.

Impurity—Any component of a drug substance that is not the chemical entity defined as the drug substance and in addition, for a drug product, any component that is not a formulation ingredient.

Inorganic Impurities—Inorganic impurities can result from the manufacturing process (e.g., residual metals, inorganic salts, filter aids, etc.). Inorganic impurities are typically controlled by tests such as *Heavy Metals* (231) and *Residue on Ignition* (281). Information found in *Plasma Spectrochemistry* (730) and *Ion Chromatography* (1065) may also be of value.

Intermediate—A material that is produced during steps of the synthesis of a drug substance and that undergoes further chemical transformation before it becomes a drug substance. The intermediate is often isolated during the process.

Ordinary Impurities—Some monographs make reference to ordinary impurities. For more details see *Ordinary Impurities* (466).

Other impurities—See section 5. *Monograph Components under General Notices and Requirements*.

Polymorphs—Different crystalline forms of the same drug substance. These can include solvation or hydration products (also known as pseudopolymorphs) and amorphous forms. Although polymorphs are not impurities by definition, an understanding of the crystalline forms, hydration or solvation states, or amorphous nature is critical to the overall characterization of the drug substance.

Process Contaminants—Process contaminants are identified or unidentified substances (excluding related substances and water), including reagents, catalysts, other inorganic impurities (e.g., heavy metals, chloride, or sulfate); and may also include foreign substances (extraneous contaminants). These contaminants may be introduced during manufacturing or handling procedures.

Reagent—A substance other than a starting material, intermediate, or solvent that is used in the manufacture of a drug substance.

Related Substances—Related substances are structurally related to a drug substance. These substances may be (a) identified or unidentified impurities arising from the synthesis manufacturing process, such as starting materials, intermediates, or by-products, and do not increase on storage, or (b) identified or unidentified degradation products that result from drug substance or drug product manufacturing processes or arise during storage of a material.

Residual Solvents—An organic liquid used as a vehicle for the preparation of solutions or suspensions in the synthesis of a drug substance (see *Residual Solvents* (467)).

Specified Impurities and Specified Degradation Products—Previously referred to as Signal Impurities, specified impurities or specified degradation products are impurities or degradation products that are individually listed and limited with specific acceptance criteria in individual monographs as applicable. Specified impurities or specified degradation products can be identified or unidentified.

Starting Material—A material that is used in the synthesis of a drug substance and is incorporated as an element into the structure of an intermediate and/or of the drug substance. Starting materials are often commercially available and have well-defined chemical and physical properties and structure.

Stereomeric Impurity—A compound with the same 2-dimensional chemical structure as the drug substance but differs in the 3-dimensional orientation of substituents at chiral centers within that structure. In those cases where all chiral centers are in the opposite orientation, the impurity is an enantiomer (enantiomeric impurity). Determinations of impurities in this category often require special chiral chromatographic approaches. Diastereomeric or epimeric impurities occur when only some of the chiral centers are present in the opposite orientation.

Toxic Impurities—Toxic impurities have significant undesirable biological activity, even as minor components, and require individual identification and quantification by specific tests. These impurities may arise out of the synthesis, preparation, or degradation of compendial articles. Based on validation data, individualized tests and specifications are selected. These feature comparison to a Reference Standard of the impurity, if available. It is incumbent on the manufacturer to provide data that would support the classification of such impurities as toxic impurities.

Unidentified Impurities and Unidentified Degradation Products—Impurities or degradation products for which structural characterizations have not been achieved and that are identified solely by qualitative analytical properties (e.g., chromatographic retention times).

Unspecified Impurities and Unspecified Degradation Products—Impurities or degradation products that are limited by general acceptance criteria but not individually listed with their own specific acceptance criteria in individual monographs.

INTRODUCTION

This general information chapter provides guidance on the control of organic impurities in drug substances and drug products (generally referred to as impurities in this chapter). Impurities are critical quality attributes of drug substances and drug products because they have the potential to affect safety and efficacy of the product. Therefore, all drug substances and drug products are subject to control of organic impurities.

The regulatory and compendial standards for the control of impurities continue to evolve due to advancements in analytical science, technology, and toxicology. A threshold-based approach described in the International Conference on Harmonization (ICH) Q3A and Q3B guidances may be used for the control of organic impurities in drug substances or drug products generated during the manufacturing process or storage. The organic impurities to be controlled in the drug substance are the process impurities and degradation products. The organic impurities to be controlled in the drug product are those resulting from the degradation of the drug substance or from the interaction of the drug substance with excipients and/or the primary container closure. Drug substance process impurities need not be controlled in the drug product unless they are also degradation products.

For marketed products, the manufacturers are responsible for controlling organic impurities in accordance with current regulatory standards. Manufacturers should consider the chemical characteristics and safety aspects of impurities when they identify and classify impurities in a

drug substance or drug product. Organic impurities should be included in the specifications for drug substances and drug products with appropriate tests, methods, and acceptance criteria. Analytical procedures for the detection and quantitation of impurities should be verified or validated. (For additional information, see *Validation of Compendial Procedures* (1225)). For impurities that are known or suspected to be highly toxic (e.g., genotoxic) or that produce undesired pharmacological effects, the quantitation/detection limit of the analytical procedures should be commensurate with the acceptance criteria to ensure patient safety.

This chapter covers drug substances and drug products marketed in the United States based on approval by the Food and Drug Administration (FDA) either via New Drug Applications (NDAs) or Abbreviated New Drug Applications (ANDAs) or through the FDA Over-the-counter (OTC) monograph system.

This chapter does not cover veterinary products, biological/biotechnological products, peptides, oligonucleotides, fermentation products and semisynthetic products derived from them, polymorphic forms, radiopharmaceuticals, herbal products, and crude products of animal or plant origin. In addition, impurities present in the drug product originating from excipients or leached from the container-closure system, inorganic/elemental impurities, and residual solvents are out of the scope of this chapter.

If a new impurity is uncovered or when the level of a known impurity increases as compared to an existing monograph, the manufacturer is responsible for evaluating impact on the safety and efficacy of the drug. If an individual monograph is inadequate to control an impurity, the manufacturer is responsible for developing and validating appropriate analytical procedures, establishing acceptance criteria, and communicating with USP.

(Definitions of key terms used in this chapter can be found in *Appendix 1: Definitions*. Additional sources of guidance on impurities in drug substances and drug products may be found in *Appendix 2: Additional Sources of Information and Guidance*.)

ORGANIC IMPURITIES IN DRUG SUBSTANCES

Organic impurities in drug substances arising from the manufacturing process and/or storage should be controlled. The organic impurities to be controlled in the drug substance are the process impurities (starting materials, byproducts, intermediates, reagents, ligands, and catalysts) and degradation products. They can be identified or unidentified. Impurities that increase over time on storage are considered degradation products and should be monitored throughout the retest period.

Drug substances manufactured by alternative processes (e.g. different starting materials, synthetic pathways, and/or purification steps) should be evaluated to determine if the differences affect the impurity profile listed in the existing monograph.

A threshold-based approach as described in ICH Q3A (R2 or current version) may be used for the reporting, identification, and/or qualification of impurities in drug substances. Because toxicity is a dose-related phenomenon, the thresholds are set based on the amount of drug substance administered per day (see *Table 1*). Higher thresholds may be applied if scientifically justified. Lower thresholds may be applied if the impurity is unusually toxic.

Table 1. Drug Substance Impurity Thresholds

	Impurity Thresholds	
	≤2 g	≥2 g
Maximum daily dose	≤2 g	≥2 g
Reporting	0.05%	0.03%
Identification	0.10% (1.0 mg) ^a	0.05%
Qualification	0.15% (1.0 mg) ^a	0.05%
^a The total daily intake in parentheses applies if it is lower than the calculated value.		

Acceptance criteria shall be set for all impurities present at or above the qualification thresholds at release and through the retest period. Principles of setting acceptance criteria for impurities in drug substance are discussed in ICH and FDA guidances for NDAs and ANDAs. The acceptance criteria shall be based on applicable guidances or other acceptable scientific means, with safety as the primary consideration and not solely based on process capability.

ORGANIC IMPURITIES IN DRUG PRODUCTS

Organic impurities in drug products arising from the manufacturing process and/or storage of the drug product should be controlled. The organic impurities to be controlled in the drug product are only the degradation products resulting from the degradation of the drug substance or from the interaction of the drug substance with excipients and/or the primary container closure. They can be identified or unidentified. Drug substance process impurities need not be monitored or specified in drug products unless they are also degradation products. Principles of setting acceptance criteria for degradation products in drug products are discussed in ICH and FDA guidances for NDAs and ANDAs.

A threshold-based approach as described in ICH Q3B (R2 or current version) may be used for the reporting, identification, and/or qualification of impurities in drug products. Because the toxicity is a dose-related phenomenon, the thresholds are based on the amount of drug substance administered per day (see *Table 2*). Higher thresholds may be applied if scientifically justified. Lower thresholds may be applied if the degradation product is unusually toxic.

Table 2. Drug Product Degradation Thresholds

	Degradation Product Thresholds					
	<1 mg	1–10 mg	>10–100 mg	>100 mg–1 g	>1–2 g	>2 g
Maximum daily dose	<1 mg	1–10 mg	>10–100 mg	>100 mg–1 g	>1–2 g	>2 g
Reporting	0.1%	0.1%	0.1%	0.1%	0.05%	0.05%
Identification	1.0% or 5 µg TDI ^a	0.5% or 20 µg TDI ^a	0.2% or 2 mg TDI ^a	0.2% or 2 mg TDI ^a	0.2% or 2 mg TDI ^a	0.10%
Qualification	1.0% or 50 µg TDI ^a	1.0% or 50 µg TDI ^a	0.5% or 200 µg TDI ^a	0.2% or 3 mg TDI ^a	0.2% or 3 mg TDI ^a	0.15%
^a Whichever is lower, calculated value or Total Daily Intake (TDI).						

Acceptance criteria shall be set for all impurities present at or above the qualification thresholds at release and through the shelf life. Principles of setting acceptance criteria for impurities in drug products are discussed in ICH and FDA guidances for NDAs and ANDAs. The acceptance criteria shall be based on applicable guidances or other acceptable scientific

means, with safety as the primary consideration and not solely based on process capability.

In cases of complex impurity profiles, it may not be feasible to resolve each impurity individually or detect them and quantify them using a single analytical procedure. In such cases, manufacturers can consider the use of multiple analytical procedures to test for impurities. Also, limits may be established based on combinations of impurities, as appropriate.

Similar principles may be applied to set thresholds and acceptance criteria for degradation products in FDA OTC monograph drug products, which are not discussed in ICH or FDA guidances. Degradation products in these drugs may also need to be reported, identified, and/or qualified. Higher thresholds than those shown in *Table 2* may be justified based on historical information such as manufacturing experience with the commercial process, stability, and safety of the drug. Lower thresholds may be applied if the degradation product is known or suspected to be highly toxic or produce undesired pharmacological effects that are unusually toxic. Factors such as quantitative structure-activity relationship (QSAR) of the drug substance, route of administration, the likely consumption patterns such as duration of treatment and the patient population, and historical safety of the drug may be considered in justifying the acceptance criteria.

Measurement of degradation products can be challenging for products containing multiple drug substances and complex formulations. The use of placebo products as controls in stability studies may aid in the deconvolution of chemical changes that could be related to excipients rather than the drug substance. For drug products that contain multiple drug substances, it is recommended to quantify unidentified degradation products based on the drug substance present in the lowest amount in the formulation (which is the most conservative approach for setting impurity control).

ORGANIC IMPURITIES DECISION TREE

The decision tree shown in *Figure 1* provides guidance for impurities in drug substances and drug products.

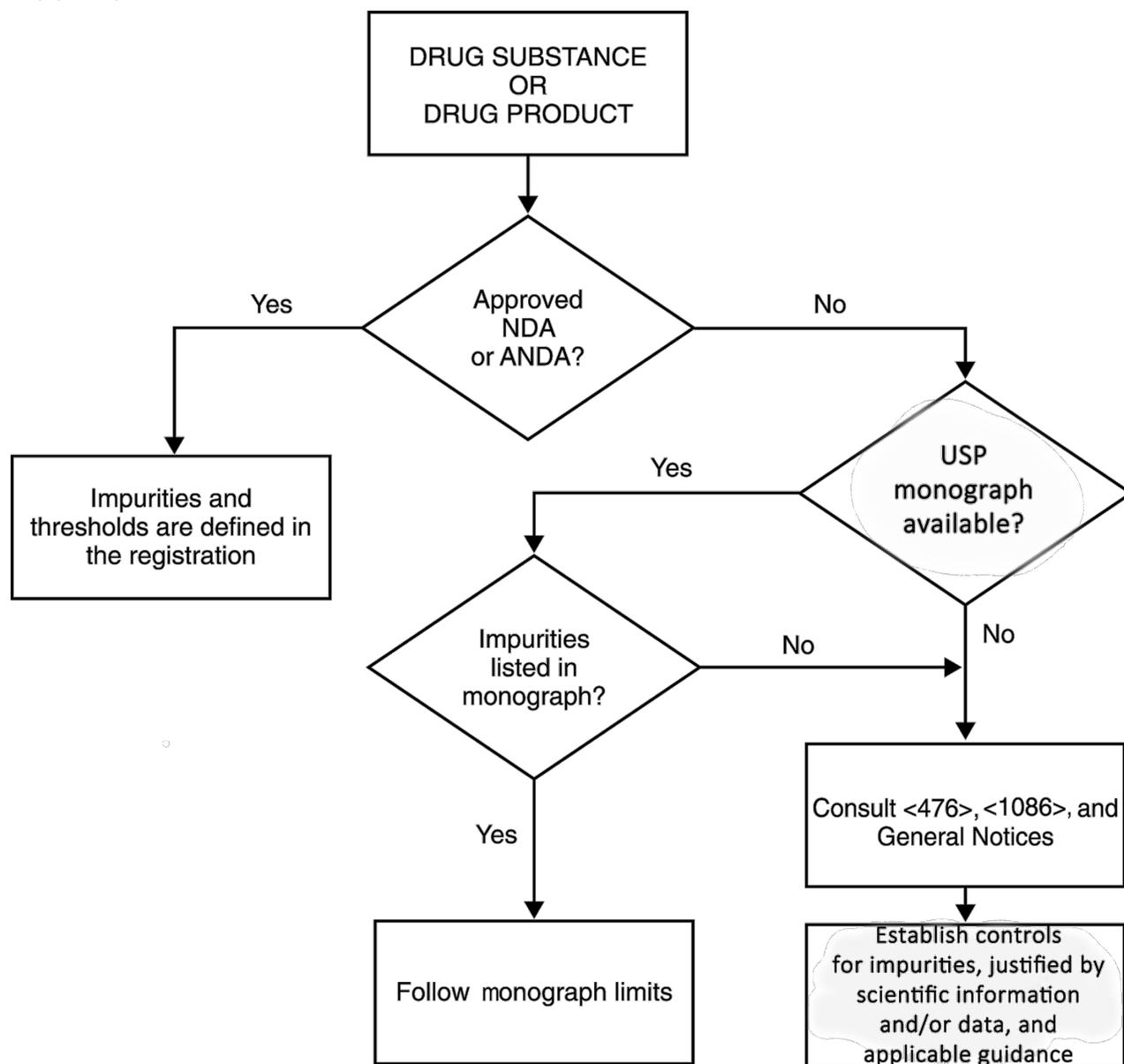


Figure 1. Decision tree for control of organic impurities in drug substances and drug products.

Note that all impurities specific to a given drug product formulation may or may not be included in the *USP* monograph. If the impurity is listed in the monograph, follow monograph limits. If the impurity is not listed, consult *Organic Impurities in Drug Substances and Drug Products* (476) and this chapter for guidance.

APPENDIX 1: DEFINITIONS

Degradation product: An impurity resulting from a chemical change in the drug substance brought about during manufacture or storage of the drug product by the effect of, for example, light, temperature, pH, or water, or by reaction with an excipient or the primary container-closure system.

Identified impurity/degradation product: An impurity or degradation product for which structural characterization has been established.

Identification threshold: A limit above which an impurity should be identified.

Impurity: For a drug substance, any component of the drug substance that is not the chemical entity that is defined as the drug substance; for a drug product, any component of a drug product that is not the drug substance or an excipient in the drug product.

Qualification: The process of acquiring and evaluating data that establish the biological safety of an individual impurity or a given impurity profile at the level(s) specified.

Qualification threshold: A limit above which an impurity should be qualified.

Reporting threshold: A limit above which an impurity should be reported.

Specified impurity/degradation product: An impurity or degradation product that is individually listed and limited with a specific acceptance criterion in the drug substance or drug product specification. A specified impurity or specified degradation product can be either identified or unidentified.

Unidentified impurity/degradation product: An impurity or degradation product for which a structural characterization has not been achieved and that is defined solely by qualitative analytical properties (e.g., chromatographic retention time).

Unspecified impurity/degradation product: An impurity or degradation product that is limited by a general acceptance criterion, but not individually listed with its own specific acceptance criterion, in the drug substance or drug product specification.

APPENDIX 2: ADDITIONAL SOURCES OF INFORMATION AND GUIDANCE

- ICH. Q3A(R2) Impurities in New Drug Substances. 2006.
http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3A_R2/St
- ICH. Q3B(R2) Impurities in New Drug Products. 2006.
http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3B_R2/St
- FDA. Guidance for industry, NDAs: Impurities in Drug Substances. 2000.
<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidance>
- FDA. Guidance for industry, ANDAs: Impurities in Drug Products. 2010.
<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidance>
- *Organic Impurities in Drug Substances and Drug Products* 〈 476 〉. USP. In: *Pharmacopeial Forum* 40(3) [May–June, 2014]. Rockville (MD): United States Pharmacopeial Convention; 2014. <http://www.usppf.com>
- Consumer Healthcare Products Association. *Your Health at Hand Book: Guide to OTC Active Ingredients in the United States*. 2010.
http://www.yourhealthathand.org/images/uploads/Your_Health_at_Hand_Book.pdf. Accessed 24 January 2014.

■ 1S (USP39)

BRIEFING

〈 1209 〉 **Sterilization—Chemical and Physicochemical Indicators and Integrators**, *USP* 38 page 1424. This chapter is proposed for omission from *USP*. A new general chapter, *Physicochemical Integrators and Indicators for Sterilization* 〈 1229.9 〉, has been proposed in

PF 41(2) as part of the new series of chapters on sterilization. The chapter impacted by this omission is *Sterilization of Compendial Articles* 〈 1229 〉. The reference to 〈 1209 〉 in this chapter needs to be replaced by 〈 1229.9 〉.

The following Briefing list includes monographs and/or chapters that both reference the General Chapter under revision and require revision to keep references to the General Chapter accurate. Other monographs and/or chapters may also be listed, even where the reference to the General Chapter remains unchanged, as additional notice to stakeholders where there is believed to be potential for the change in the general chapter itself to affect pass-fail determinations for particular monograph articles.

- 〈 1229 〉 *Sterilization of Compendial Articles*

(GCM: R. Tirumalai.)

Correspondence Number—C157028

Comment deadline: July 31, 2015

Delete the following:

■ 〈 1209 〉 ~~STERILIZATION—CHEMICAL AND PHYSICOCHEMICAL INDICATORS AND INTEGRATORS~~

INTRODUCTION

The Federal Code of Regulations, Part 211 on Good Manufacturing Practices for Finished Pharmaceuticals in section 211.165 states: "There shall be appropriate laboratory testing, as necessary, of each batch of drug product required to be free of objectionable microorganisms." This statement has been interpreted to mean that an alternate laboratory control test for sterility is required for any batch load of a product that is parametrically released. An appropriate laboratory test for each batch may be a biological indicator, which is included in each batch of product that is terminally sterilized (see *Biological Indicators for Sterilization* 〈 1035 〉), or a physicochemical indicator or integrator. This requirement may also be met by a primary product release system that includes the documented recording of thermometric measurements systems that are calibrated with a NIST traceability system and that demonstrate a $\pm 0.5^\circ$ performance capability.

The presence of this chapter in the *USP* does not mean that chemical indicators and integrators are primary release requirements for parametric released product. The recorded and documented measurements from established thermometric measurement systems and associated process controllers (that have been calibrated and used during initial and periodic validation studies, as well as in routine production) can be considered to be primary, product-release systems for parametric release.

PERFORMANCE

Performance standards within lots and between lots of physicochemical indicators or integrators from a given manufacturer should be consistent. They should not interact physically or chemically with any container or product when placed adjacent to the product for sterilization

in the sterilizer load, and should not alter the strength, quality, or purity of the sterilized article. The safety of personnel handling the physicochemical indicators or integrators should also be assessed, and, if need be, appropriate precautions should be taken.

Similar to biological indicators, chemical indicators are considered Class II devices and require the indicator manufacturer to obtain a device 510K approval prior to commercial use.

PHYSICOCHEMICAL INDICATORS

Recorded process engineering data can be supplemented by the presence in each sterilized batch of a physicochemical indicator. A physicochemical indicator is defined as a device that responds in a measurable fashion to one or more critical sterilization parameters.

A number of different kinds of indicators dependent on chemical or physicochemical means have been developed for monitoring sterilization cycles. Some products are used in a sterilization apparatus for monitoring whether the contents have been exposed to a selected factor (i.e., temperature) of the particular sterilization cycle, but may not show the duration or intensity of such exposure. Chemical and physicochemical indicators are used to monitor a physical parameter of a sterilization apparatus and can be placed on the outside of the packages of articles to be sterilized, or they may be distributed within the sterilizer load. In the latter case, one can evaluate to some extent the effect of the packaging material and configuration of the load on the selected parameter.

PHYSICOCHEMICAL INTEGRATORS

A physicochemical integrator is defined as a device that responds to a sterilization process critical parameter, which results in a measurable or quantifiable value that can be correlated to some standard of microbial lethality. Physicochemical integrators have been designed to broadly match the predictable inactivation of those spore preparations in biological indicators that have high and defined resistance to the sterilizing agent.

The manufacturers of physicochemical integrators should provide data to demonstrate that the labeled performance characteristics tests of the integrators are met. Users of physicochemical integrators should verify that specific measured values directly correlate to successful microbial lethality in a validated sterilization cycle.

A physicochemical integrator indicates whether or not the critical combination of physical parameters of a validated sterilization cycle has been met or exceeded. The integrator is not generally used as a substitute for a biological indicator in the development and validation of sterilization cycles. An indication by a physicochemical integrator that the critical combination of physical parameters of a stated sterilization cycle has been achieved should not be considered equivalent to the inactivation of spores of a variety of biological indicators. However, the physicochemical integrator can detect whether the sterilization process has been continued for too long, at too high a temperature or gas concentration, or has been overexposed to radiation.

The interval between the lower range and the upper range of time, or any other designated set of parameters, resembles the survival time and kill time window characteristics of a biological indicator. This interval should not be wider than that desirable for the designated parameter, but may be narrower if the manufacturer is able to achieve consistent performance over a

narrower range. Even where a sterilization apparatus with consistent performance is used, cases may occur where the determined performance characteristics of the integrator differ from the label claims. This could represent a difference between the user's apparatus performance and the manufacturer's apparatus used for verifying the label claims. Closer conformity to the label claims may also be shown with any highly developed apparatus, such as a BIER vessel.¹⁷² Hence, the integrator requires its own precautions in use and has appropriate interpretive criteria within its performance characteristics. Tests for performance characteristics of physicochemical integrators include determination under applicable defined conditions of (a) the maximum time of exposure at which none of the specimens indicates that adequate exposure to the cycle has occurred, and (b) a minimum time of exposure at which all specimens show that adequate exposure to the cycle has occurred. An intermediate time of exposure, where about half the number of specimens show adequate exposure, could indicate an approaching exposure endpoint for the physicochemical integrator.

Because an indicator reflects only the interaction of the physical parameters of sterilization, it will not be affected by some of the factors that may influence the resistance of the microbial load on the products to be sterilized (e.g., progeny resistance, spore population, inoculum substrate, oil, salts, proteins, or residues or configurations), all of which may protect a contaminated area from penetration by the sterilizing agent. (Hence, the inappropriateness of these devices for cycle development.) There are other factors, however, that may affect a biological indicator that could also affect a physicochemical integrator (e.g., interfering configuration of a pack in which the integrator was placed, variations in the applied timing or temperature control, or failure of the apparatus to reach the set temperature or meet other requirements).

Defective performance of the sterilization apparatus generally can be ascertained from gauges and from records of temperature, pressure, time of exposure, and gas concentration, whichever are applicable. The integrator can only indicate inadequate, adequate, or excessive exposure to a combination of critical sterilization parameters. Where an integrator shows inadequate exposure to the sterilization parameters, it is necessary to ascertain whether the gauges and recordings reflect accurately the sterilization conditions within the sterilization chamber. Variations between sterilization vessels, which might affect the efficiency of a selected sterilization cycle, might be detectable by parallel exposure of several integrators in a number of locations in each sterilizer load.

Physicochemical integrators for steam sterilization are designed to react predictably to a particular combination of physical sterilization parameters: temperature, steam pressure, and time of exposure. Deviation to some extent of one or more of these critical parameters, not compensated by modification of other parameters, causes the integrator to indicate failure to reach the preset integrated limits.

Physicochemical integrators for ethylene oxide sterilization are designed with similar general principles as the integrators for steam sterilization, but to react predictably to the particular combination of the physical sterilization parameters: humidity, temperature, sterilizing gas concentration, and time of exposure. Deviation to some extent of one or more of these critical parameters, not compensated by modification of other parameters, causes the integrator to indicate failure to reach the preset integrated limits.

Physicochemical integrators have been designed to match broadly the predictable inactivation of spore preparations that have a high and defined resistance to the sterilizing agent. For

steam sterilization, a strain of *Bacillus stearothermophilus* is used (see *Biological Indicator for Steam Sterilization, Paper Carrier*), and for ethylene oxide sterilization, a strain of *Bacillus subtilis*, subspecies *niger*, is used (see *Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier*). Since there are no standard preparations of these strains, the performance characteristics of these physicochemical integrators must be interpreted in relation to a specific validated sterilization cycle.

Performance standards within lots and between lots of physicochemical integrators from a given manufacturer should be consistent. The integrators should not interact physically or chemically with container or product when placed adjacent to the product for sterilization in the sterilizer load, and shall not alter the strength, quality, or purity of the sterilized article beyond official requirements. Users should obtain information from integrator manufacturers on whether or not sterilization in the presence of physicochemical integrators may affect particular articles to be sterilized. In addition, safety in using these integrators should be verified.

Moist Heat Sterilization

The use of steam sterilization physicochemical integrators to supplement the information obtained through physical assessment of the critical operating parameters should be part of parametric release of moist heat sterilized products. These should be designed to enable the assurance that the lethality delivery specified for the process has been met or exceeded. The consistency of the performance of physicochemical integrators for moist heat sterilization should be ensured through the testing of performance characteristics that include testing of the performance of the indicator or integrator system at various pre-set selected moist heat process conditions.

Critical elements of this type of physicochemical integrator would include an organic compound sensitive to the combination of temperature and steam, a polymeric material penetrable by saturated steam, and a wicking device under the polymeric material that is in contact with the organic compound. As steam passes through the polymeric material, the organic compound melts in a predictable fashion in steps dependent on the steam temperature during the cycle. The liquefied material travels along the wick for a distance that can be measured on a scale. This organic compound has a stated melting temperature range. Some integrators may, for example, have melting ranges of 132.2 ° to 134.5 ° or of 137.0 ° to 142.0 °. Other melting ranges could also be specified so as to indicate the sterilization parameters to which it has actually been exposed. The combination of the sterilization parameters, applied for the exposure time required for a stated moist heat sterilization cycle, is indicated on the front of the article by the linear travel of the melt.

These types of physicochemical integrators can also be used for so-called "flash" moist heat sterilization cycles in which the successive steps of the sterilization process are rapidly carried out in such a manner as to achieve the required lethality for the validated process. Other types of physicochemical integrators for moist heat sterilization can be used if they are also calibrated against a specified validated moist heat sterilization cycle.

Ethylene Oxide Sterilization

Physicochemical integrators for ethylene oxide sterilization should be designed to match broadly the predictable inactivation of spore preparations that have a high and defined resistance to ethylene oxide sterilization. The inactivation of spores of a strain of *Bacillus subtilis*, subspecies *niger*, can be used as a model, although other spores of relevant microorganisms can also be

used. The critical elements of physicochemical integrators for ethylene oxide sterilization are a base with an organic compound along a linear indicator strip sensitive to a combination of temperature, humidity, and sterilizing gas concentration. Where the organic compound is exposed to a sterilizing gas mixture at a specific temperature and humidity, a chemical reaction triggers the appearance of a color along the linear indicator bar. This is dependent on the time of exposure under the conditions of ethylene oxide sterilization in a predictable fashion. The absence of fading or of decolorization of the indicator bar for a stated period after the sterilization cycle has been completed would confirm adequate humidification in the cycle. The integrator should be capable of detecting deviations from the prescribed parameters of temperature, sterilizing gas concentration, humidity, and time of exposure that may affect sterilization. It does not show the required reactions if exposed to reduced amounts of gas concentration, temperature, and humidity, even if exposed for prolonged periods.

Other types of physicochemical integrators for ethylene oxide sterilization based on different principles or mechanisms of integration of critical parameters of sterilization could be used if they are also calibrated against a specified validated ethylene oxide sterilization cycle.

The consistency of performance of physicochemical integrators for ethylene oxide sterilization has to be ensured through the testing of performance characteristics at various pre-set selected times for a given ethylene oxide sterilization cycle. ■ 1S (USP39)

[±] Standard for BIER/Steam Vessels, 27 March 1981, Association for the Advancement of Medical Instrumentation (AAMI), 3330 Washington Boulevard, Suite 400, Arlington, VA 22201-4598.

² Standard for BIER/EO Vessels, 27 March 1992, Association for the Advancement of Medical Instrumentation (AAMI), 3330 Washington Boulevard, Suite 400, Arlington, VA 22201-4598.

BRIEFING

《 2251 》 **Adulteration of Dietary Supplements with Drugs and Drug Analogs.** This new general chapter provides tools for detection of dietary supplement adulteration with extraneously added synthetic compounds. The illegal addition of synthetic substances to products marketed as dietary supplements constitutes a significant threat to consumer health, considering that these products, administered without medical supervision, may contain toxic constituents or substances whose safety has never been examined, and whose interaction with medications may be unpredictable or lethal. The proposed chapter suggests multiple methods for detection of adulteration. It is advisable to use several screening techniques to maximize the potential for adulteration detection, because no single methodology is universally applicable. Presently, the chapter targets supplements adulterated with phosphodiesterase type 5 inhibitors; subsequent revisions will include methodologies specific to analysis of adulterated weight loss and sports performance enhancement products. It is anticipated that this chapter will be updated regularly.

(GCCA: A. Bzhelyansky.)

Correspondence Number—C144928

Comment deadline: July 31, 2015

Add the following:

■ 《 2251 》 **ADULTERATION OF DIETARY SUPPLEMENTS WITH DRUGS AND DRUG ANALOGS**

INTRODUCTION

The illegal addition of undeclared synthetic compounds to products marketed as dietary supplements¹ (DS) is a serious problem. This fraud is practiced to impart therapeutic effects that cannot be achieved by the supplement constituents alone. Increasingly, synthetic intermediates and structural analogs of the pharmaceuticals and drugs that have been discontinued or withdrawn from the market due to unsatisfactory safety profiles are being used as adulterants. Multiple adulterating compounds may be added to a single DS, frequently in erratic amounts.

The proposed test methodologies facilitate screening of DS for synthetic adulterants. No individual technique is capable of addressing all potential analytes; thus, a combination of orthogonal approaches adds certainty to the analytical outcome. Mass spectrometric techniques provide strong substantiation of the analytical findings. In some cases, e.g., with hormonal drugs, the amounts of physiologically relevant adulterants may be so low that GC-MS or LC-MS may be the only fitting analytical options.

The express purpose of assembling the procedures recommended herein is their utility in DS screening. The level of evidence achievable by application of one or several of the recommended procedures is ultimately dictated by the specific requirements of the end-user. It should be noted that structural elucidation and quantitative assessment of adulterants are beyond the scope of this chapter.

This chapter is meant to be updated regularly, thereby counteracting the efforts of adulterators, as novel analogs are being developed, new concealment methodologies for the adulterants are introduced, or improvements to the methods of analysis are realized.

DIETARY SUPPLEMENT ADULTERATION CATEGORIES

The following major categories of adulterated DS are recognized:

- **Sexual Enhancement:** This category is also referred to as the Erectile Dysfunction (ED) category. It encompasses a functionally coherent group of adulterants, including several approved drugs, their numerous approved and unapproved analogs, synthetic intermediates, and derivatives. Their functionality is manifested by selective inhibition of phosphodiesterase type 5 enzyme (PDE5), which hydrolyzes cyclic guanosine 3,5-monophosphate (cGMP); this group of drugs is frequently identified as PDE5 inhibitors. Screening methods for DS adulterated with ED drugs are presented in *Appendix A*.
- **Weight Loss (WL):** This category comprises a functionally and chemically diverse collection of compounds that include stimulants, laxatives, diuretics, anorexiant, and psychoactive drugs. Although stimulants constitute an important segment of WL adulterants, the oral anorexiant sibutramine dominates this category, frequently in combination with phenolphthalein, a laxative. Methods for analysis of DS adulterated with WL drugs will be addressed in Appendix B (to come).
- **Sports Performance Enhancement (SPE):** These compounds constitute the third major category of adulteration. Professional and amateur athletes are targeted with designer anabolic steroids and stimulants, which are systematically banned by the World Anti-Doping Agency. Functional and structural diversity, synthetic proclivity of the adulterators, and the generally small amounts of the infringing substances required to elicit a therapeutic effect make this category especially challenging to address. These supplements are customarily formulated in protein- and fat-rich matrices, thereby further complicating detection. For these reasons, GC- and LC-MSⁿ techniques constitute primary analytical methodologies within this category. Analysis

of DS adulterated with SPE drugs will be addressed in Appendix C (to come).

DIETARY SUPPLEMENT BULK POWDERS AND DOSAGE FORMS

DS may be subjected to adulteration either as bulk raw material or at any stage of the finished product manufacturing. Analysts should be mindful of the adulterants admixed to the finished dosage ingredients, including DS matrix or excipients, as well as components. In the latter, synthetic compounds may be embedded into the capsule shell body. This underscores the need for deliberate adjustment to the laboratory procedures that typically focus on the capsule contents alone. Appropriate sampling practices for powders and finished dosage forms should be exercised, particularly when only a limited amount of sample is available.

RECOMMENDED ANALYTICAL METHODOLOGIES

Analysis of DS for adulteration may be broadly categorized into targeted and nontargeted methods. The distinction between these types may be subtle, and a minor adjustment to the methodology will transform a nontargeted method into a targeted method.

Targeted

These techniques are warranted when the analytes are known. An example of a targeted approach would be monitoring a chromatographic run at a particular wavelength (or mass), and quantifying the analyte that appears within a pre-defined retention time window. Targeted analysis is conceptually straightforward, because it relies on pre-existing knowledge of the analyte and allows optimization of test methodology for its reliable detection. The targeted approach also is a rarity in the adulterant detection paradigm, where the nature of the analyte may be anticipated only tentatively, and variable amounts of multiple adulterants belonging to several functional categories are commonplace.

Nontargeted

These methods are better suited to a broad-spectrum detection requirement presented by adulterated DS. Nontargeted screening trades precise knowledge of the analyte identity, along with specificity and accuracy, for a wider detection scope. Examples of nontargeted chromatographic screening include acquisition of photodiode array data and full mass-spectral scanning following a chromatographic separation. The procedures in this chapter are written with an eye toward applying all techniques in a nontargeted mode, even the ones considered to be inherently targeted, thereby facilitating detection of a suspect adulterant even in the absence of a matching reference compound.

It is generally recommended to apply a broadly nontargeted methodology first, followed by a targeted procedure. It is crucial to clearly define the end-purpose of analysis, and only then decide on the appropriate instrumentation and assemble a logical testing strategy from the procedures provided. Thus, application of a nontargeted screening method may satisfy the requirements of a DS manufacturer for the purposes of monitoring bulk raw materials. Conversely, a laboratory requiring a higher level of evidence to enact an enforcement action may opt for a two-step procedure: a preliminary screen, followed by confirmatory analysis of suspect samples.

USP Reference Standards recommended for adulterants screening are listed at the end of each relevant Appendix. However, considering the rate of propagation of structural analogs and proliferation of newly developed "designer" molecules, establishing and maintaining an all-inclusive catalog of reference materials for potential adulterants is challenging and impractical. Several commercial sources of the compounds of interest exist.² Please note that mention of the external reference materials suppliers does not in any way constitute their endorsement, and neither does the listing of reagents, supplies, and instrumentation.

APPENDIX

- **Appendix A. Screening Methodologies for PDE5 Inhibitors**

- 1. HPLC with Photodiode Array Detection**

Solution A: 0.1% Formic acid in water

Solution B: 0.1% Formic acid in acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
15	5	95
23	5	95
24	95	5
31	95	5

Diluent: Acetonitrile and water (50:50)

Standard solution: 100 µg/mL of each USP Sildenafil Citrate RS, USP Tadalafil RS, or USP Vardenafil Hydrochloride RS in *Diluent*

Sample solution: Combine one-fifth of the dosage unit, 10–20 mg of bulk material, or a small fragment of the capsule shell (3 mm × 3 mm) with 10 mL of *Diluent*, sonicate for 30 min, and pass through a 0.2-µm PTFE syringe filter.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Photodiode array, 200–400 nm

Analytical wavelength: 290 nm

Column: 2.1-mm × 15-cm; 5-µm packing L1³

Column temperature: 40°

Flow rate: 0.2 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Theoretical plates: NLT 3000 for any of the standard peaks

Tailing factor: NMT 1.5 for any of the standard peaks

Analysis

Sample: *Sample solution*

Examine UV spectra of the prominent peaks for similarity to those in the *Sample solution* or other known PDE5 inhibitor compounds (*Figure 1* and *Table 5*). Typical retention times of several PDE5 inhibitors are provided in *Table 5*. Retention time matching may provide some corroborating information; however, numerous analytes may elute within the same retention time window.

- 2. HPLC with Mass-Spectrometric Detection**

Ideally, a mass-spectrometric detector is connected in sequence to the UV-Vis detector. The settings below apply to an ion-trap mass spectrometer. Other MS detectors are suitable; however, it is advisable to use spectrometers that possess MS/MS capability.

Solution A: 0.1% Formic acid in water

Solution B: 0.1% Formic acid in acetonitrile

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	95	5
15	5	95
23	5	95
24	95	5
31	95	5

Diluent: Acetonitrile and water (50:50)

Standard solution: 5 µg/mL of each USP Sildenafil Citrate RS, USP Tadalafil RS, or USP Vardenafil Hydrochloride RS in *Diluent*

Sample solution: Combine one-fifth portion of the dosage unit, 10–20 mg of bulk material, or a small fragment of the capsule shell (3 mm × 3 mm) with 10 mL of *Diluent*, sonicate for 30 min, and pass through the 0.2-µm PTFE syringe filter. Dilute the filtrate 20-fold with *Diluent* before injection.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 290 nm

Column: 2.1-mm × 15-cm; 5-µm packing L1⁴

Column temperature: 40°

Flow rate: 0.2 mL/min

Injection volume: 1 µL

Mass spectrometric system⁵

(See *Mass Spectrometry* { 736. })

Ionization: ESI

Polarity: Positive or negative

Sheath gas: 35 mL/min

Sweep gas: 5 mL/min

Capillary temperature: 300°

Source voltage: 5 kV

Collision: 45 meV

Scanning: *m/z* 110–1050 and dependent scan on the most intense ion

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between vardenafil and sildenafil peaks

Tailing factor: NMT 1.5 for any of the standard peaks

Analysis

Sample: *Sample solution*

Compare mass-to-charge ratios of the molecular ions $[M + H]^+$ or $[M - H]^-$ and fragments to those of the *Standard solutions* or other known analytes listed in *Table 4*. Typical retention times of several common PDE5 inhibitors are provided in *Table 5*.

3. High Performance Thin-Layer Chromatography (HPTLC) with Visual, UV, and/or MS Detection

Standard solution: A 0.2-mg/mL composite of each USP Sildenafil Citrate RS, USP Tadalafil RS, and USP Vardenafil Hydrochloride RS in methanol, with sonication if necessary. Additional reference standards may be available commercially.

Sample solution: Comminute 1 dosage unit, including the capsule shell and tablet coating, or about 500 mg of raw material; combine with 10 mL of methanol, and subject to ultrasonication for 30 min. Centrifuge or filter the solution, and use the supernatant. [Note—Upon development, if the chromatographic bands appear too saturated and UV densitometric spectra are distorted, dilute the *Sample solution* 10-fold with methanol.]

Developing solvent system: *tert*-Butyl methyl ether, methanol, and 28.0% (w/w) ammonium hydroxide (20:2:1). [Note—Strength of ammonium hydroxide was found to be crucial for adequate method performance. It is therefore advisable to establish the titer of higher-concentration ammonia⁶ and to adjust the latter to exactly 28.0% immediately before the experiment.]

Chromatographic system

(See *Chromatography* 〈 621 〉, *Thin-Layer Chromatography*.)

Mode: HPTLC

Adsorbent: Chromatographic silica gel with an average particle size of 5 μm

Application volume: 3 μL , as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 47% using a suitable device in the presence of a saturated solution of potassium isothiocyanate.

Temperature: Ambient

Saturation: 20 min, with paper

Developing distance: 62 mm

Derivatization reagent: none

Drying: 5 min in a stream of cold air

Detection 1: Visual, under illumination with 254- and 365-nm UV light

Detection 2: UV spectrometry (scanning densitometer), 190–550 nm

Detection 3: Mass spectrometry, m/z 110–1050

Mass spectrometric system⁷

(See *Mass Spectrometry* 〈 736 〉.)

Ionization: ESI

Polarity: Positive, negative, or rapid switching

Desolvation gas (N₂): 300 L/h

Cone gas (N₂): 80 L/h

Temperatures

ESI probe: 105^o

Desolvation: 150^o

Capillary voltage: 3.0 kV

Cone voltage: 50 V

Scanning: m/z 110–1050

System suitability

Sample: *Standard solution*

Suitability requirements: The R_F values of sildenafil, vardenafil, and tadalafil bands are 0.28 ± 0.03 , 0.32 ± 0.03 , and 0.46 ± 0.03 , respectively.

Analysis: Inspect the plate under short-wave (254-nm) and long-wave (365-nm) UV light. PDE5 inhibitors appear as dark bands against the fluorescent background at 254 nm and typically exhibit different shades of blue fluorescence at 365 nm. Note the similarities in R_f values between the bands in the *Standard solution* and *Sample solution*; these may be informative, but co-elutions are common. Relative intensities of the bands permit approximation of the amounts. Using scanning densitometry, obtain UV spectra of the prominent bands in the *Sample solution*, and compare them to those of the PDE5 inhibitors in the *Sample solution* and those provided in *Table 5* and *Figure 1*. Mass-spectrometric interface, if available, may facilitate more definitive assignment of the analyte bands: compare mass-to-charge ratios of the molecular ions $[M+H]^+$ or $[M-H]^-$ and fragments to those of the common adulterants listed in *Table 4*.

4. Ambient Ionization Mass Spectrometry

Diluent: Acetonitrile and water (50:50), with 0.1% formic acid

Standard solution: A composite solution containing 20 $\mu\text{g/mL}$ each of USP Sildenafil Citrate RS, USP Tadalafil RS, and USP Vardenafil Hydrochloride RS in *Diluent*. Additional reference materials may be available commercially.

Sample solution: Grind the entire dosage form, including capsule shell and tablet coating, to a fine powder. Weigh about 50 mg of the resulting powder, or about 50 mg of bulk material, and combine with 5 mL of *Diluent*. Cap tightly, subject to ultrasonication for 2 min, and vortex thoroughly. Centrifuge or filter the resulting solution, and dilute an aliquot of the supernatant or filtrate 100-fold with *Diluent*.

Mass spectrometric system

(See *Applications of Mass Spectrometry* (1736), *Ambient Ionization Procedures*.)

Ionization: Ambient with thermal desorption

Mode: Thermal profile

Polarity: Positive, negative, or rapid switching

Gas temperature: 150 $^\circ$, 250 $^\circ$, 350 $^\circ$, and 450 $^\circ$

Scanning: m/z 110–1050

System suitability: Deposit 3- μL aliquots of the *Standard solution* onto the disposable sample cards. Set the compatible mass spectrometer for a 30-s acquisition from 110 to 1050 Da. Using one sample card for each temperature setting, acquire mass spectra at each of the following desorption gas temperatures: 150 $^\circ$, 250 $^\circ$, 350 $^\circ$, and 450 $^\circ$ in positive ionization mode. Switch polarity, and re-acquire spectra at the same four temperatures in the negative ionization mode. [Note—If the mass spectrometer permits rapid polarity switching, both positive and negative spectra may be acquired

simultaneously using a single sample.] Confirm that the $[M+H]^+$ or $[M-H]^-$ ions of sildenafil, tadalafil and vardenafil are observed as listed in *Table 4*.

Analysis: Deposit 3- μL aliquots of the *Sample solution* onto the disposable sample cards, and follow the procedure outlined above for the *Standard solution*. In the event that a single ion dominates the mass spectrum at every temperature setting, dilute the *Sample solutions* 10-fold with *Diluent* and re-analyze. Compare mass-to-charge ratios of the molecular ions $[M+H]^+$ or $[M-H]^-$ and fragments to those of the known analytes listed in *Table 4*.

5. NMR Spectroscopy—Low-Field and High-Field

(See *Nuclear Magnetic Resonance Spectroscopy* (761), *Qualitative and Quantitative*

NMR Analysis.)

[Note—Deuterated acetonitrile (CD_3CN) should be NLT 99.8 atom % D, and should contain 0.05% tetramethylsilane (TMS) as a chemical shift reference. Use of solvents in sealed ampules is recommended. NMR tubes should be suitable for use at the selected magnetic field strength.]

Standard solutions: Dissolve 10 mg of USP Sildenafil Citrate RS, USP Tadalafil RS, or USP Vardenafil Hydrochloride RS in separate 1-mL aliquots of CD_3CN , and transfer 700- μL aliquots of the resulting solutions into individual NMR tubes. Additional reference materials are available commercially.

Sample solution: Grind the entire dosage form, including capsule shell and tablet coating, to a fine powder. Transfer 100–200 mg of the ground material, or an equivalent amount of bulk raw material powder, into a 5-mL sealable glass vial. Add 1 mL of CD_3CN , vortex thoroughly, and allow the solids to settle. Transfer about 700 μL of the supernatant to an NMR tube, taking care to minimize transfer of solids.

Instrument performance qualification

(See *Nuclear Magnetic Resonance Spectroscopy* 〈 761 〉.)

Magnetic field strength: NLT 42.5 MHz (^1H operating frequency)

Data collection: Use the parameters specified in *Table 3*; perform 90° pulse width calibration before the analysis according to the recommendations of the equipment manufacturer.

Table 3

Parameter	^1H -NMR Qualitative Measurement
Pulse program	Single pulse ^1H
Spectral width	14 ppm (–1 to 13 ppm)
Transmitter offset	Center of spectral width
Relaxation delay	5–10 s
Acquisition time	2–5 s
Number of data points per FID ^a	NLT 16,000
Temperature	25 $^\circ$
^a Free induction decay.	

System suitability: Acquire a ^1H spectrum of the *Sample solution* using the settings outlined in *Data collection*. Record a sufficient number of scans to ensure that signal-to-noise ratio of the TMS signal is NLT 10.

Analysis: Acquire a ^1H spectrum of the *Sample solution* under the conditions outlined in *Data collection*. Record a sufficient number of scans to ensure that the signal-to-noise ratio of the TMS signal is NLT 10. Reference all acquired spectra to the ^1H signal of TMS (0 ppm). Measure and record the chemical shift and multiplicity of the NMR signals in the spectra of the *Standard solutions* and *Sample solution*. Compare the ^1H NMR spectrum of the *Sample solution* to those of the *Standard solutions*, paying particular attention to the aromatic region (5–9 ppm). Determine whether the chemical shift and multiplicity of the NMR signals in the *Sample solution* exhibit sufficient similarity to those found in the *Standard solutions*.

6. Bioassay⁸

PDE5 enzyme⁹ stock solution: Prepare a concentration of approximately 3000 Units/ μL . If necessary, dilute with 40 mM Tris-HCl, pH 8.0; 110 mM NaCl, 2.2 mM KCl; 3 mM DTT; and 20% glycerol. Vortex gently to mix.

PDE5 working solution (100 Units per 6.5 μL): Combine 400 μL PDE-Glo™ Reaction Buffer 5X, 10 μL of *PDE5 enzyme stock solution*, and 1590 μL purified water. Vortex gently to mix.

cGMP solution: Combine 400 μL of PDE-Glo™ Reaction Buffer 5X, 40 μL of 1-mM *cGMP stock solution*, and 1560 μL of purified water. Mix thoroughly by vortexing.

100-mM IBMX stock solution in DMSO: Prepare a 22.2-mg/mL solution of 3-isobutyl-1-methylxanthine (IBMX) in DMSO, e.g., dissolve 100 mg of IBMX in 4.5 mL of DMSO. Mix thoroughly by vortexing.

Reaction buffer: Combine 400 μL of PDE-Glo™ Reaction Buffer 5X and 1600 μL of purified water. Mix thoroughly by vortexing.

Reaction buffer with 4% DMSO: Combine 400 μL of PDE-Glo™ Reaction Buffer 5X, 80 μL of DMSO and 1520 μL of purified water. Mix thoroughly by vortexing.

Termination buffer: Combine 400 μL of PDE-Glo™ Termination Buffer 5X, 40 μL of *100-mM IBMX stock solution in DMSO*, and 1560 μL of purified water. Mix thoroughly by vortexing.

Detection buffer: Combine 400 μL of PDE-Glo™ Detection Buffer 5X, 16 μL of Protein Kinase A Solution, and 1584 μL of purified water. Vortex gently to mix.

Kinase-Glo™ reagent: Add 10 mL of Kinase-Glo™ Buffer to the vial of Kinase-Glo™ Substrate, and vortex gently.

Standard solution (400 nM): Dissolve 5 mg of USP Sildenafil Citrate RS in 3.0 mL of DMSO to obtain 2.5-mM stock solution. Combine a 10- μL aliquot of the resulting solution with 240 μL of DMSO, and mix thoroughly (100 μM). Combine a 10- μL aliquot of the resulting solution with 90 μL of DMSO, and mix thoroughly (10 μM). Combine a 10- μL aliquot of the resulting solution with 240 μL of *Reaction buffer*, and mix thoroughly (400 nM).

Control solution: Combine 10 μL of DMSO with 240 μL of *Reaction buffer*, and mix thoroughly by vortexing.

Sample solution: Grind the entire dosage form, including capsule shell and tablet coating, to a fine powder. Transfer 100 mg of the ground material into a 5-mL polypropylene vial. Add 3.0 mL of DMSO, and vortex for 60 s. Allow solids to settle, combine 50 μL of the clear supernatant with 200 μL of DMSO, and mix thoroughly by vortexing. Combine a 10- μL aliquot of the resulting solution with 90 μL of DMSO, and mix thoroughly by vortexing. Combine a 10- μL aliquot of the resulting solution with 240 μL of *Reaction buffer*, and mix thoroughly by vortexing.

Analysis

1. Dispense 12.5- μL aliquots of *Standard solution (400 nM)*, *Control solution*, and *Sample solution* into microplate wells, in triplicate. Use a white, flat-bottom, opaque polystyrene, nontreated, 96-well microtiter plate.¹⁰[Note—Do not use treated plates, black plates, or clear plates.]
2. Add 6.5 μL of *PDE5 working solution* to each well. Incubate for 5 min.
3. Add 6.0 μL of *cGMP solution* to effect a 5- μM cGMP concentration in a 25- μL volume. Incubate for 30 min.
4. Add 12.5 μL of *Termination buffer*. Incubate for 5 min.
5. Add 12.5 μL of *Detection buffer*. Incubate for 20 min.
6. Add 50 μL of *Kinase-Glo™ reagent*. Incubate for 10 min.
7. Record luminescence at 560 nm with a microplate luminometer at 0.5 s/well.

31	Imidazosagatriazinone (Desulfovardenafil)	139756-21-1	C ₁₇ H ₂₀ N ₄ O ₂	312.1586	313	—	313, 284, 256, 169, 151
32	Isopiperazinonafil	—	C ₂₅ H ₃₄ N ₆ O ₄	482.2642	483	—	—
						481	453, 422, 379, 336, 325, 311, 309
33	Lodenafil carbonate	398507-55-6	C ₄₇ H ₆₂ N ₁₂ O ₁₁ S ₂	1034.4102	1035	—	518, 487, b , <i>377, 311^c</i>
34	Mirodenafil	862189-95-5	C ₂₆ H ₃₇ N ₅ O ₅ S	531.6698	532	—	488, 404, 362, 296, 268
35	Mutaproduenafil	1387577-30-1	C ₂₇ H ₃₅ N ₉ O ₅ S ₂	629.7635	630.2279	—	489, 377, 142, 113
36	<i>N</i> -Butylnortadalafil	171596-31-9	C ₂₅ H ₂₅ N ₃ O ₄	431.18451	432.25	—	310, b , <i>282, 197, 169^c</i>
37	<i>N</i> -Desethylvardenafil	448184-46-1	C ₂₁ H ₂₈ N ₆ O ₄ S	460.18927	461.20	—	392, 377, 376, 329, 313, 312, 299, 284, 283, 151
38	<i>N</i> -Desmethylsildenafil	139755-82-1	C ₂₁ H ₂₈ N ₆ O ₄ S	460.18927	461.19	—	377, 313, 311, 299, 283
39	Nitrodenafil	147676-99-1	C ₁₇ H ₁₉ N ₅ O ₄	357.3647	358	—	307, 289, 261, 217, 176, 154, 136, 107, 89
40	Nitroso-prodenafil	1266755-08-1	C ₂₇ H ₃₅ N ₉ O ₅ S ₂	629.2203	630	—	142
41	<i>N</i> -Octylnortadalafil (Octylnortadalafil)	1173706-35-8	C ₂₉ H ₃₃ N ₃ O ₄	487.2471	488	—	366, 227
42	Noracetildenafil (Demethylhongdenafil)	949091-38-7	C ₂₄ H ₃₂ N ₆ O ₃	452.25359	453.26	—	425, 406, 396, 380, 367, 355, 353, 339, 325, 324, 313, 297, 296, 253
43	Norneosildenafil (Piperidino sildenafil)	371959-09-0	C ₂₂ H ₂₉ N ₅ O ₄ S	459.19403	460.20	—	432, 377, 329, 311, 299, 283
44	Norneovardenafil	358390-39-3	C ₁₈ H ₂₀ N ₄ O ₄	356.1485	357	—	329, 307, 289, 176, 154, 136, 107, 99
45	Nortadalafil (Demethyltadalafil)	171596-36-4	C ₂₁ H ₁₇ N ₃ O ₄	375.1219	376	374.1138	262, 234, 233, 232
46	Oxohongdenafil	—	C ₂₅ H ₃₂ N ₆ O ₄	480.2485	481	—	451, 396, 354, 339, 312, 297, 289
47	Piperazinonafil (Piperazonafil, Dihydroacetildenafil)	1335201-04-1	C ₂₅ H ₃₄ N ₆ O ₄	482.2642	483	—	—
						481	453, 435, 348, 336, 321, 311, 309, 282, 267
48	Piperidino acetildenafil (Piperiacetildenafil)	147676-50-4	C ₂₄ H ₃₁ N ₅ O ₃	437.2427	438	—	410, 408, 355, 353, 341, 325, 297, 288
49	Piperidinovardenafil (Piperidenafil, Pseudovardenafil)	224788-34-5	C ₂₂ H ₂₉ N ₅ O ₄ S	459.19403	460.20	—	432, 403, 391, 377, 349, 329, 312, 311, 301, 299, 284, 283, 270, 256, 169, 151
50	Propoxyphenyl aildenafil	—	C ₂₄ H ₃₄ N ₆ O ₄ S	502.2362	503	—	252
51	Propoxyphenyl hydroxyhomosildenafil (Methylhydroxyhomosildenafil)	139755-87-6	C ₂₄ H ₃₄ N ₆ O ₅ S	518.2311	519	—	501, 475, 391, 331, 325, 299, 283, 129, 112, 99
52	Propoxyphenyl sildenafil	877777-10-1	C ₂₃ H ₃₂ N ₆ O ₄ S	488.2205	489.2272	—	447, 391, 325, 299, 283, 100
53	Propoxyphenyl thioaildenafil (Propoxyphenyl thiomethisosildenafil)	856190-49-3	C ₂₄ H ₃₄ N ₆ O ₃ S ₂	518.2133	519	—	260
54	Propoxyphenyl thiohydroxyhomosildenafil	479073-90-0	C ₂₄ H ₃₄ N ₆ O ₄ S ₂	534.2083	535.2150	—	517, 359, 341, 315, 299, 271, 129, 112, 99
55	Sildenafil	139755-83-2	C ₂₂ H ₃₀ N ₆ O ₄ S	474.20492	475.21	—	447, 418, 391, 377, 374, 346, 329, 311, 297, 283, 255, 163, 160, 100
						473.45	445, 310, 282
56	(<i>S,R</i>)-Aminotadalafil ((+)- <i>trans</i> -Aminotadalafil)	—	C ₂₁ H ₁₈ N ₄ O ₄	390.1328	391	—	No data
57	Tadalafil (Tildenafil)	171596-29-5	C ₂₂ H ₁₉ N ₃ O ₄	389.13756	390	—	302, 268, 262, 250, 240, 197, 169, 135
						388.1288	262, 234, 233, 232
58	Thioaildenafil (Sulfoaildenafil, Thiomethisosildenafil, Sulfodimethyl sildenafil, Dimethylthiosildenafil)	856190-47-1	C ₂₃ H ₃₂ N ₆ O ₃ S ₂	504.19773	505.21	—	448, 393, 327, 299, 113, 99
59	Thiohomosildenafil (Sulfohomosildenafil, Homosildenafil thione)	479073-80-8	C ₂₃ H ₃₂ N ₆ O ₃ S ₂	504.19773	505.21	—	477, 421, 393, 357, 355, 343, 327, 315, 299, 271, 113, 99
60	Thioquinapiperil (KF31327)	220060-39-9	C ₂₄ H ₂₈ N ₆ O ₅	448.2045	449	—	363, 246, 225, 204, 121
61	Thiosildenafil (Sulfosildenafil, Sildenafil thione)	479073-79-5	C ₂₂ H ₃₀ N ₆ O ₃ S ₂	490.18208	491.19	—	407, 393, 343, 341, 327, 315, 313, 299, 283, 271, 163, 99
62	Udenafil	268203-93-6	C ₂₅ H ₃₆ N ₆ O ₄ S	516.2519	517.260	—	474, 418, 347, 325, 299, 283
63	Vardenafil	224785-90-4	C ₂₃ H ₃₂ N ₆ O ₄ S	488.22057	489.2274	—	461, 420, 377, 376, 375, 346, 339, 329, 312, 299, 284, 283, 169, 151, 123, 99
						487.33	459, 310, 282
64	Xanthoanthrafil (Benzamidenafil)	1020251-53-9	C ₁₉ H ₂₃ N ₃ O ₆	389.15869	390.31	—	344, 252, 223, 151, b , <i>107, 91^c</i>

^a Compiled from peer-reviewed literature, and communications with USP collaborators. See corresponding chemical structures in Figure 2.

^b Bold, fragment subjected to MS³ fragmentation.

^c Italics, MS³ fragments derived from the parent fragment (**bold**).

Table 5. UV Absorbance Maxima and Retention Time Data for Select PDE5 Inhibitors^a

#	Name	CAS Number	Chemical Formula	UV Absorbance Maxima (nm)	UV Absorbance Spectrum Type (Figure 1)	Retention Time (min) ^b	Relative Retention Time with Respect to Sildenafil
1	(—)- <i>trans</i> -Tadalafil (ent-Tadalafil)	629652-72-8	C ₂₂ H ₁₉ N ₃ O ₄	231, 282, 289	—	—	—

2	Acetaminotadalafil	1446144-71-3	C ₂₃ H ₂₀ N ₄ O ₅	202, 222, 282	b	14.3	1.1
3	Acetil acid		C ₁₈ H ₂₀ N ₄ O ₄	230, 260, 285	—	—	—
4	Acetildenafil (Hongdenafil)	831217-01-7	C ₂₅ H ₃₄ N ₆ O ₃	234, 282	e	12.5	1.0
5	Acetylvardenafil	1261351-28-3	C ₂₅ H ₃₄ N ₆ O ₃	218, 246, 268(s)c	d	11.4	0.9
6	Aildenafil (Dimethylsildenafil, Methisosildenafil)	496835-35-9	C ₂₃ H ₃₂ N ₆ O ₄ S	226, 294	a	13.3	1.0
7	Aminotadalafil	385769-84-6	C ₂₁ H ₁₈ N ₄ O ₄	200, 220, 284, 290(s)	b	14.2	1.1
8	Avanafil	330784-47-9	C ₂₃ H ₂₆ N ₇ O ₃ Cl	198, 244	f	13.0	1.0
9	Benzylsildenafil		C ₂₈ H ₃₄ N ₆ O ₄ S	291	—	—	—
10	Carbodenafil (Fondenafil)	944241-52-5	C ₂₄ H ₃₂ N ₆ O ₃	295	—	—	—
11	Chlorodenafil	1058653-74-9	C ₁₉ H ₂₁ ClN ₄ O ₃	211, 235, 279	—	—	—
12	Chloropretadalafil	171489-59-1	C ₂₂ H ₁₉ ClN ₂ O ₅	204, 222, 284	b	17.4	1.3
13	Cinnamildenafil		C ₃₂ H ₃₈ N ₆ O ₃	239	—	—	—
14	Cyclopentynafil	1173706-34-7	C ₂₆ H ₃₆ N ₆ O ₄ S	218, 290	—	—	—
15	Depiperazinothiosildenafil		C ₁₇ H ₂₀ N ₄ O ₄ S ₂	295, 354	—	—	—
16	Descarbonsildenafil	1393816-99-3	C ₂₁ H ₃₀ N ₆ O ₄ S	225, 295	—	—	—
17	Desmethylcarbodenafil		C ₂₃ H ₃₁ N ₆ O ₃	226, 296	a	11.9	0.9
18	Dimethylacetildenafil		C ₂₅ H ₃₄ O ₃ N ₆	233, 276	—	—	—
19	Dioxo-acetildenafil		C ₂₅ H ₃₀ N ₆ O ₅	No data	—	—	—
20	Dithio-desmethylcarbodenafil	1333233-46-7	C ₂₃ H ₃₀ N ₆ O ₅ S ₂	258, 285, 356	—	—	—
21	Gendenafil	147676-66-2	C ₁₉ H ₂₂ N ₄ O ₃	232, 274	f	16.8	1.3
22	Gisadenafil	334826-98-1	C ₂₃ H ₃₃ N ₇ O ₅ S	No data	—	—	—
23	Homosildenafil	642928-07-2	C ₂₃ H ₃₂ N ₆ O ₄ S	226, 292	a	13.3	1.0
24	Hydroxyacetildenafil (Hydroxyhongdenafil)	147676-56-0	C ₂₅ H ₃₄ N ₆ O ₄	234, 280	e	12.2	0.9
25	Hydroxychlorodenafil	1391054-00-4	C ₁₉ H ₂₃ ClN ₄ O ₃	212, 303	—	—	—
26	Hydroxyhomosildenafil	139755-85-4	C ₂₃ H ₃₂ N ₆ O ₅ S	226, 296	a	12.9	1.0
27	2-Hydroxypropylnortadalafil	1353020-85-5	C ₂₄ H ₂₃ N ₃ O ₅	222, 284	—	—	—
28	Hydroxythiohomosildenafil (hydroxyhomosildenafil thione, sulfohydroxyhomosildenafil)	479073-82-0	C ₂₃ H ₃₂ N ₆ O ₄ S ₂	228, 296, 352	c	15.0	1.2
29	Hydroxythiovardenafil	912576-30-8	C ₂₃ H ₃₂ N ₆ O ₄ S ₂	203, 235, 316	—	—	—
30	Hydroxyvardenafil	224785-98-2	C ₂₃ H ₃₂ N ₆ O ₅ S	216	—	—	—
31	Imidazosagatriazinone (Desulfovardenafil)	139756-21-1	C ₁₇ H ₂₀ N ₄ O ₂	212, 253	—	—	—
32	Isopiperazinonafil		C ₂₅ H ₃₄ N ₆ O ₄	221, 290	—	—	—
33	Lodenafil carbonate	398507-55-6	C ₄₇ H ₆₂ N ₁₂ O ₁₁ S ₂	226, 296	a	15.4	1.2
34	Mirodenafil	862189-95-5	C ₂₆ H ₃₇ N ₅ O ₅ S	216, 248	f	14.5	1.1
35	Mutaproduenafil	1387577-30-1	C ₂₇ H ₃₅ N ₉ O ₅ S ₂	218, 240, 283, 297, 335	—	—	—
36	N-Butylnortadalafil	171596-31-9	C ₂₅ H ₂₅ N ₃ O ₄	222, 284	—	—	—
37	N-Desethylvardenafil	448184-46-1	C ₂₁ H ₂₈ N ₆ O ₄ S	226, 246(s)	d	11.9	0.9
38	N-Desmethylsildenafil	139755-82-1	C ₂₁ H ₂₈ N ₆ O ₄ S	224, 294	a	12.9	1.0
39	Nitrodenafil	147676-99-1	C ₁₇ H ₁₉ N ₅ O ₄	212, 298	—	—	—
40	Nitroso-prodenafil	1266755-08-1	C ₂₇ H ₃₅ N ₉ O ₅ S ₂	241, 301	—	—	—
41	N-Octylnortadalafil (Octylnortadalafil)	1173706-35-8	C ₂₉ H ₃₃ N ₃ O ₄	281	—	—	—
42	Noracetildenafil (Demethylhongdenafil)	949091-38-7	C ₂₄ H ₃₂ N ₆ O ₃	234, 280	e	12.3	0.9
43	Norneosildenafil (Piperidino sildenafil)	371959-09-0	C ₂₂ H ₂₉ N ₅ O ₄ S	226, 300	a	18.7	1.4
44	Norneovardenafil	358390-39-3	C ₁₈ H ₂₀ N ₄ O ₄	215, 241	—	—	—
45	Nortadalafil (Demethyltadalafil)	171596-36-4	C ₂₁ H ₁₇ N ₃ O ₄	No data	—	—	—
46	Oxohongdenafil		C ₂₅ H ₃₂ N ₆ O ₄	481	—	—	—
47	Piperazinonafil (Piperazonifil, Dihydroacetildenafil)	1335201-04-1	C ₂₅ H ₃₄ N ₆ O ₄	221, 290	—	—	—
48	Piperidino acetildenafil (Piperiacetildenafil)	147676-50-4	C ₂₄ H ₃₁ N ₅ O ₃	234, 284	e	13.1	1.0
49	Piperidinovardenafil (Piperidenafil, Pseudovardenafil)	224788-34-5	C ₂₂ H ₂₉ N ₅ O ₄ S	224, 246(s)	d	16.5	1.3
50	Propoxyphenyl aildenafil		C ₂₄ H ₃₄ N ₆ O ₄ S	215, 225, 295	—	—	—

51	Propoxyphenyl hydroxyhomosildenafil (Methylhydroxyhomosildenafil)	139755-87-6	C ₂₄ H ₃₄ N ₆ O ₅ S	226, 294	a	13.4	1.0
52	Propoxyphenyl sildenafil	877777-10-1	C ₂₃ H ₃₂ N ₆ O ₄ S	226, 292	a	1.4	1.0
53	Propoxyphenyl thioaildenafil (Propoxyphenyl thiomethisosildenafil)	856190-49-3	C ₂₄ H ₃₄ N ₆ O ₃ S ₂	227, 295, 355	—	—	—
54	Propoxyphenyl thiohydroxyhomosildenafil	479073-90-0	C ₂₄ H ₃₄ N ₆ O ₄ S ₂	227, 295, 353	—	—	—
55	Sildenafil	139755-83-2	C ₂₂ H ₃₀ N ₆ O ₄ S	224, 294	a	13.0	1.0
56	(S,R)-Aminotadalafil ((+)-trans-Aminotadalafil)		C ₂₁ H ₁₈ N ₄ O ₄	225, 283	—	—	—
57	Tadalafil (Tildenafil)	171596-29-5	C ₂₂ H ₁₉ N ₃ O ₄	200, 222, 284, 292(s)	b	15.0	1.2
58	Thioaildenafil (Sulfoaildenafil, Thiomethisosildenafil, Sulfodimethyl sildenafil, Dimethylthiosildenafil)	856190-47-1	C ₂₃ H ₃₂ N ₆ O ₃ S ₂	228, 250(s), 296, 352, 366(s)	c	15.5	1.2
59	Thiohomosildenafil (Sulfohomosildenafil, Homosildenafil thione)	479073-80-8	C ₂₃ H ₃₂ N ₆ O ₃ S ₂	228, 248(s), 296, 354, 370(s)	c	14.4	1.1
60	Thioquinapiperifil (KF31327)	220060-39-9	C ₂₄ H ₂₈ N ₆ O ₅	211, 268, 363	—	—	—
61	Thiosildenafil (Sulfosildenafil, Sildenafil thione)	479073-79-5	C ₂₂ H ₃₀ N ₆ O ₃ S ₂	228, 250(s), 296, 356, 368(s)	c	15.2	1.2
62	Udenafil	268203-93-6	C ₂₅ H ₃₆ N ₆ O ₄ S	228, 298	f	13.6	1.0
63	Vardenafil	224785-90-4	C ₂₃ H ₃₂ N ₆ O ₄ S	226, 252(s)	d	12.1	0.9
64	Xanthoanthrafil (Benzamidenafil)	1020251-53-9	C ₁₉ H ₂₃ N ₃ O ₆	202, 228, 278, 390	f	15.3	1.2
<p>^a Compiled from peer-reviewed literature, and contributed by the USP collaborators. See corresponding chemical structures in <i>Figure 2</i>.</p> <p>^b Retention times derived from the experiments conducted as described in <i>HPLC with Photodiode Array Detection</i> and in <i>HPLC with Mass Spectrometric Detection</i> methods.</p> <p>^c (s) denotes a shoulder.</p>							

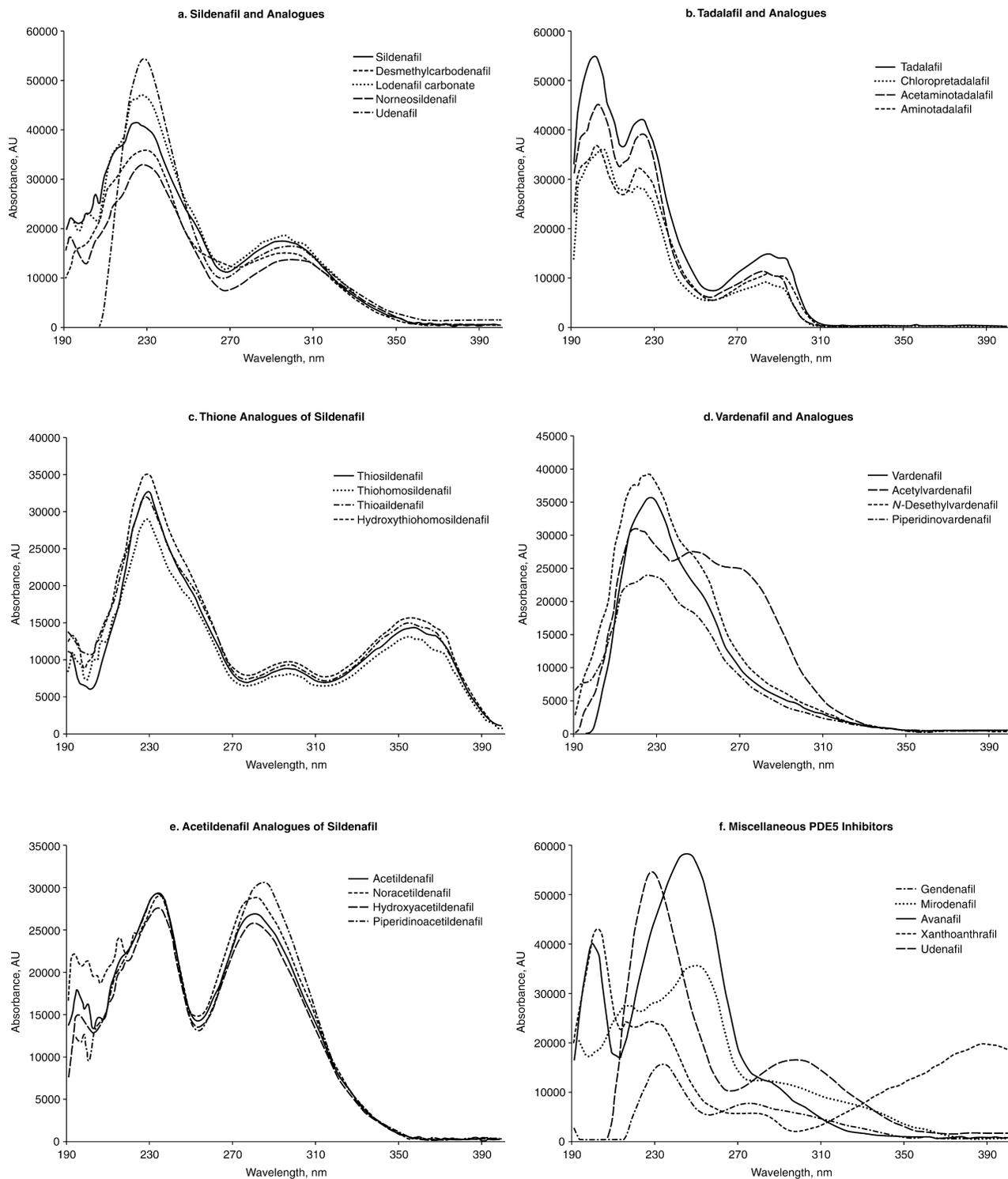
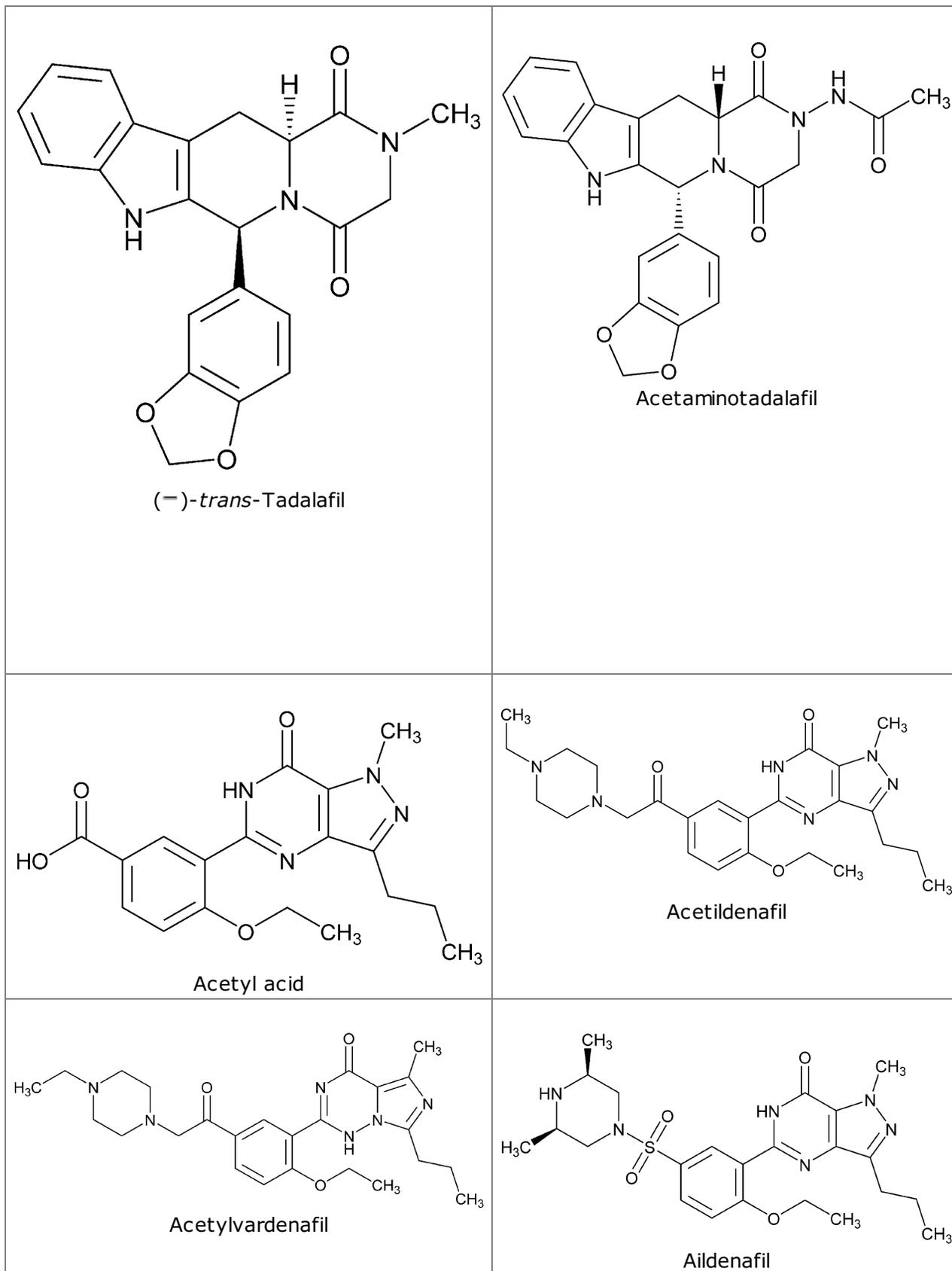
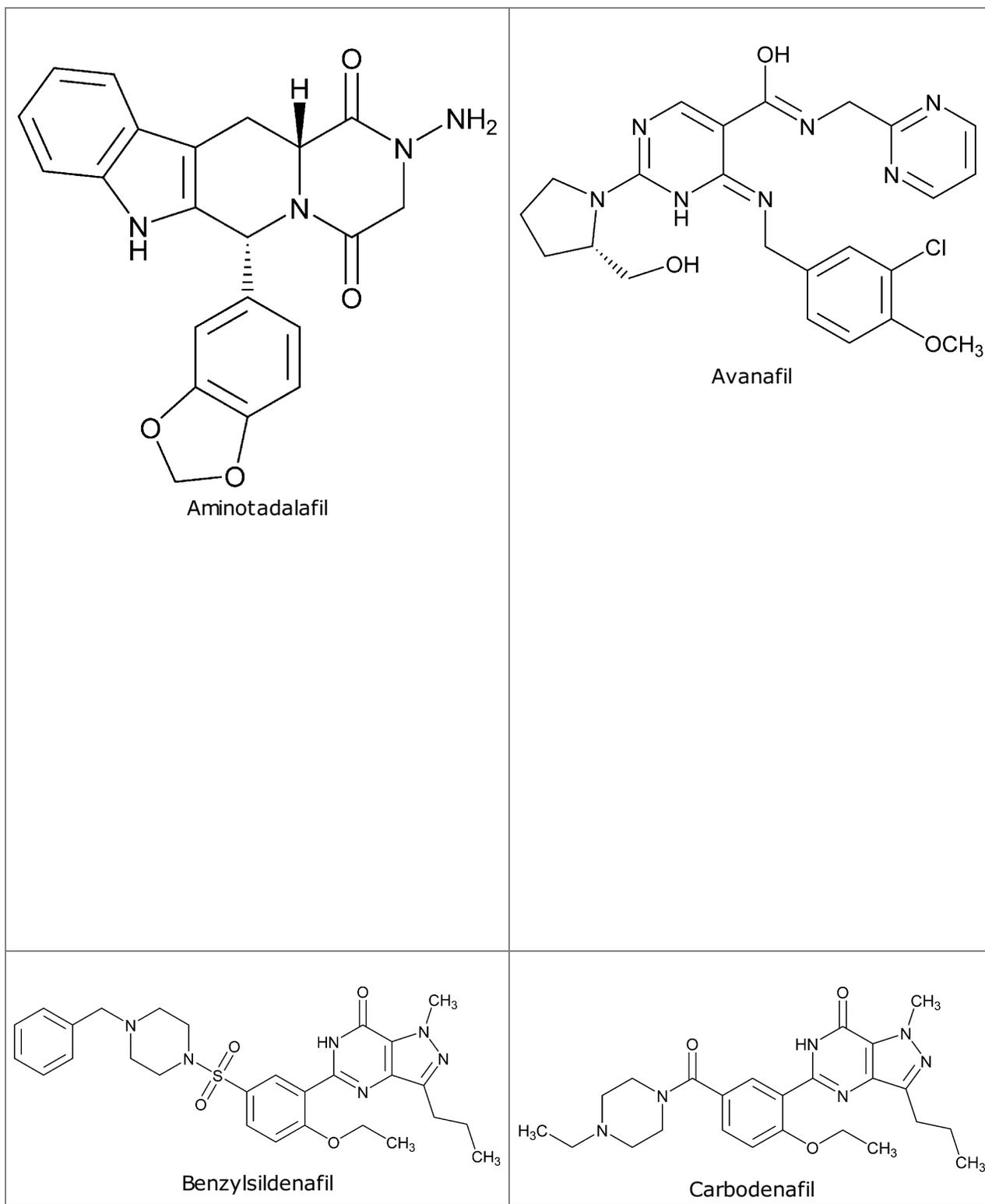
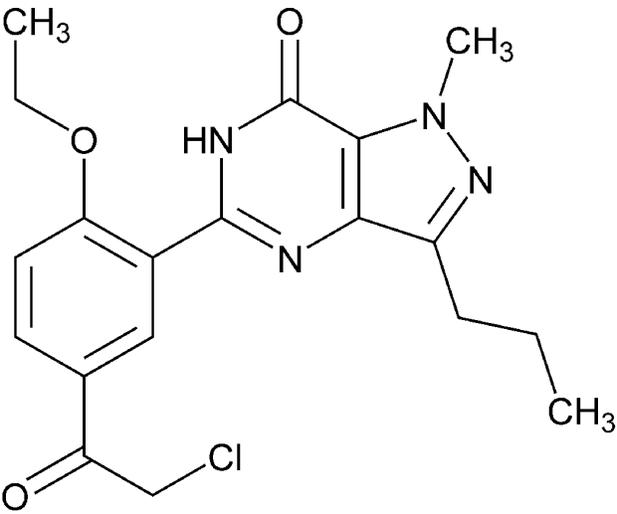
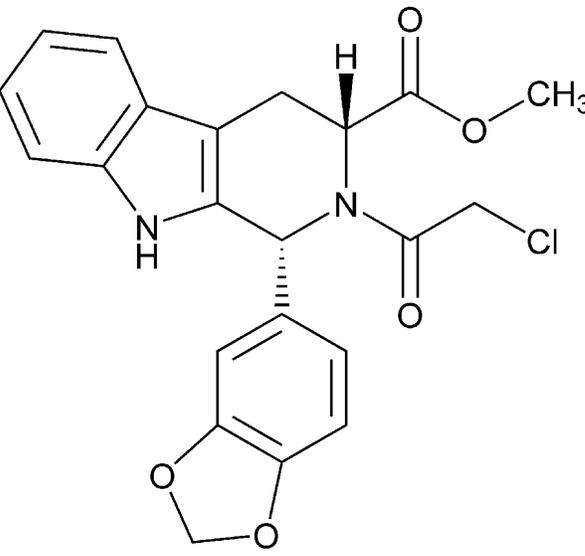
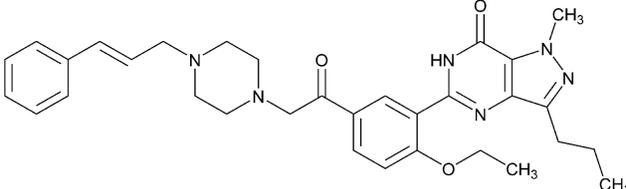
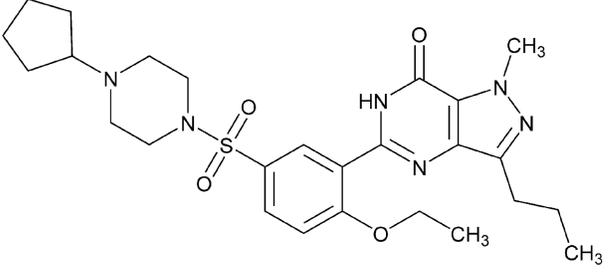
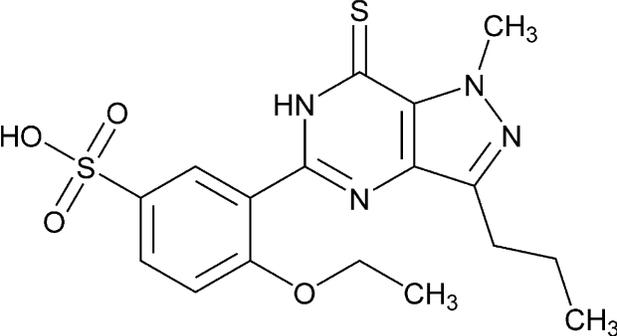
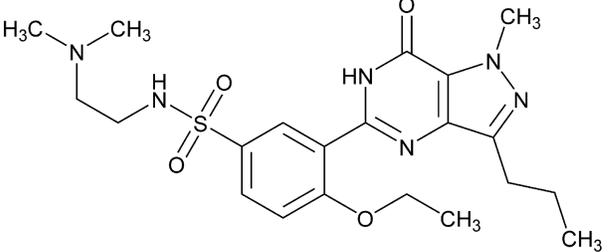


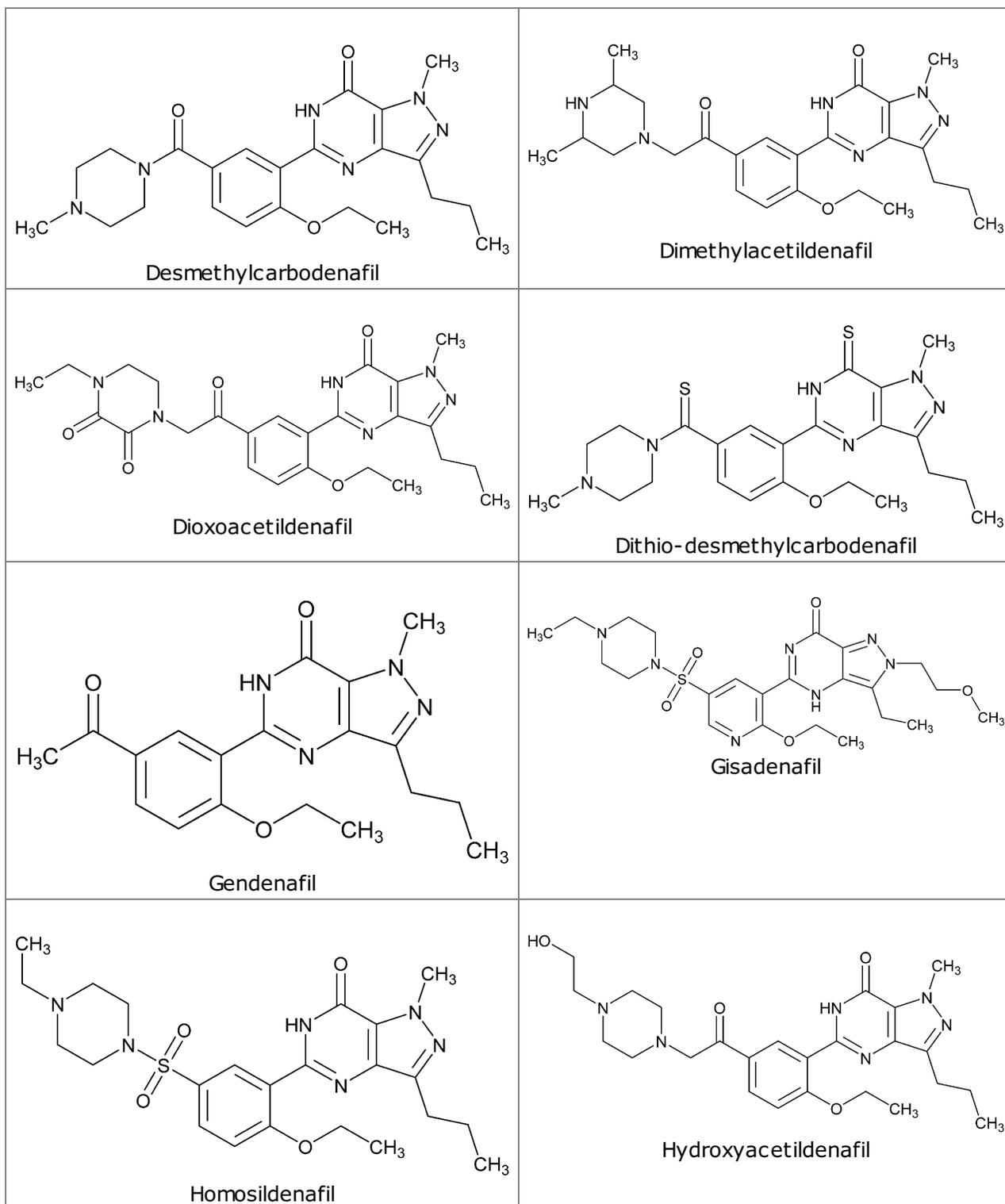
Figure 1. UV absorbance spectra of select PDE 5 inhibitors.¹¹

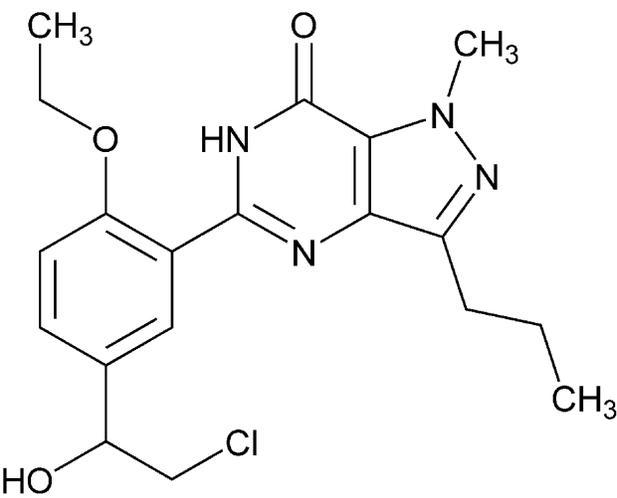
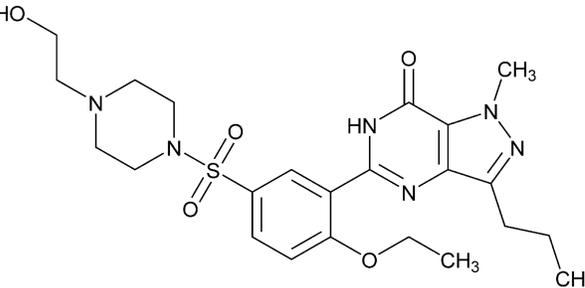
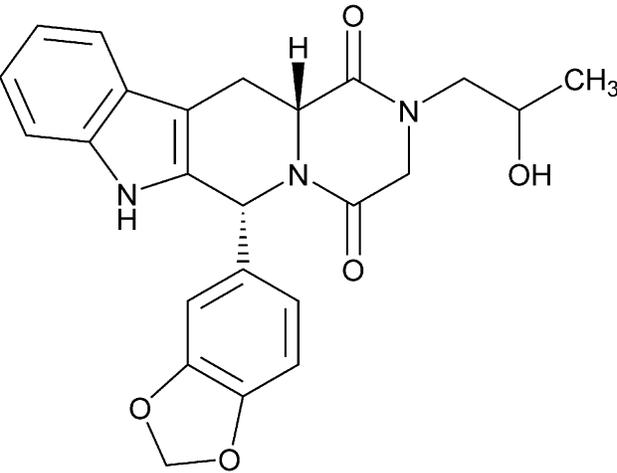
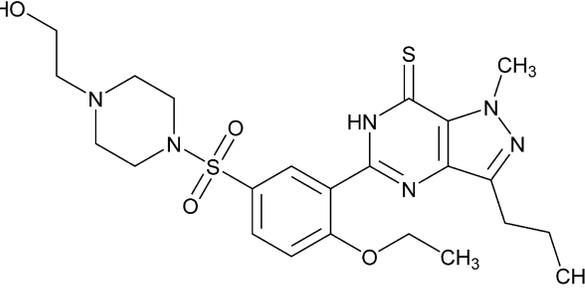
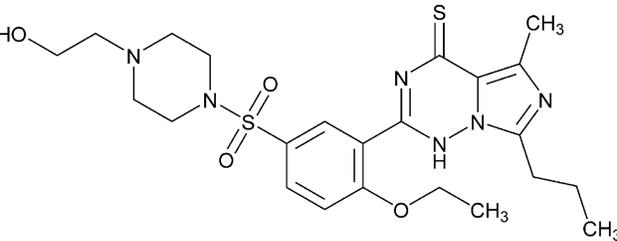
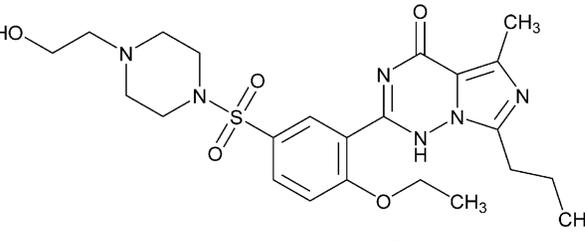
Figure 2. Chemical structures of select PDE 5 inhibitors.¹²

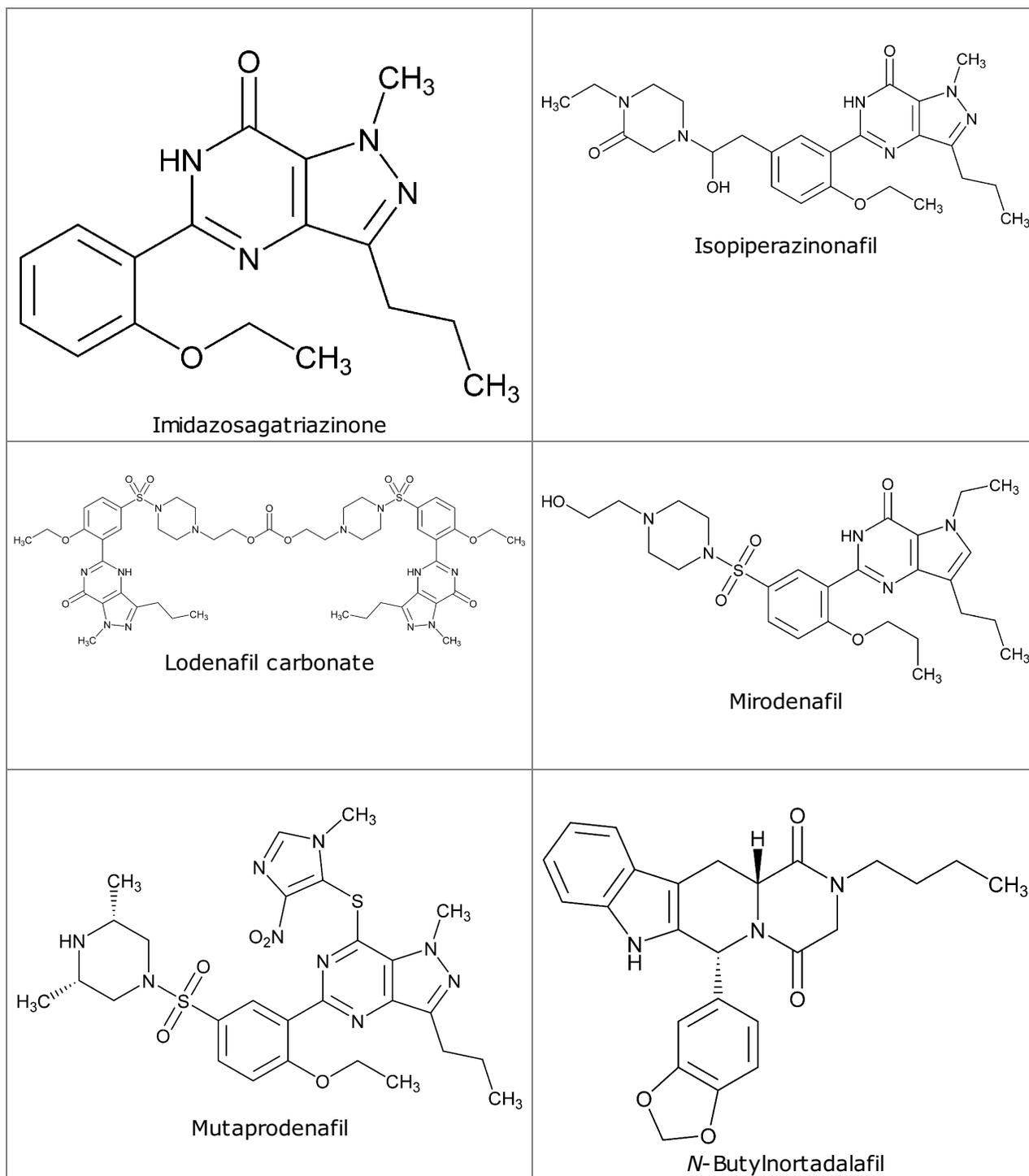


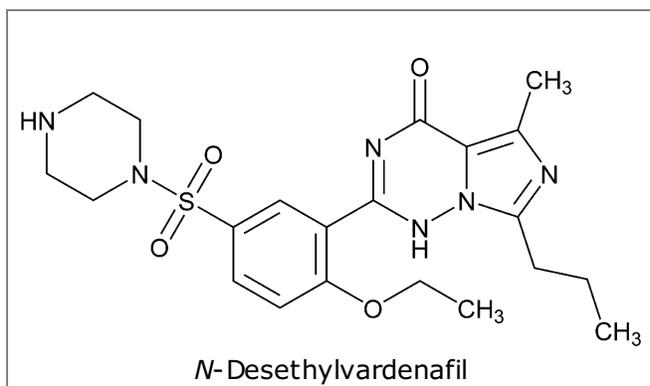
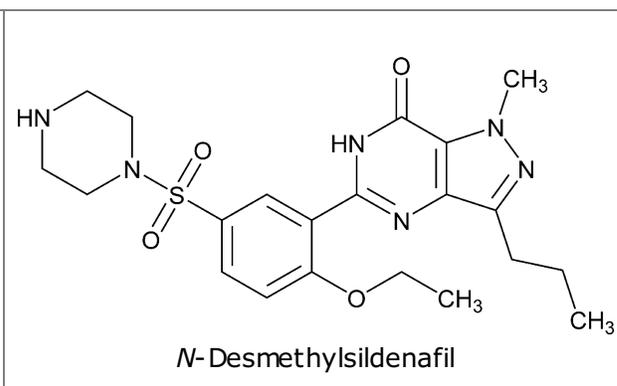
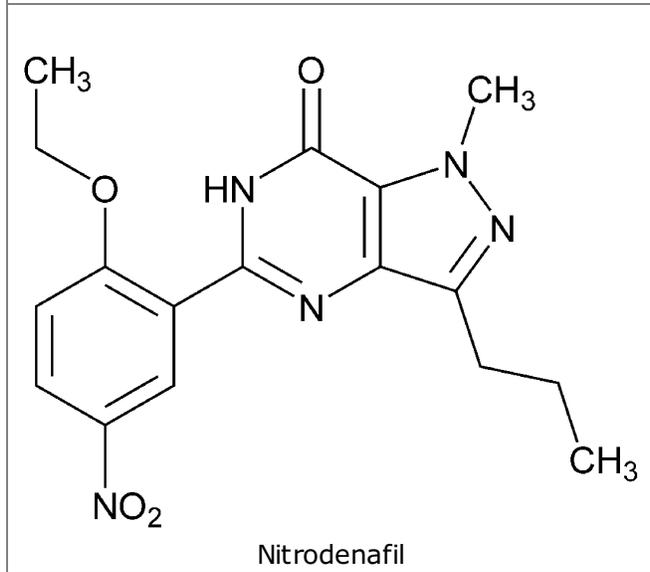
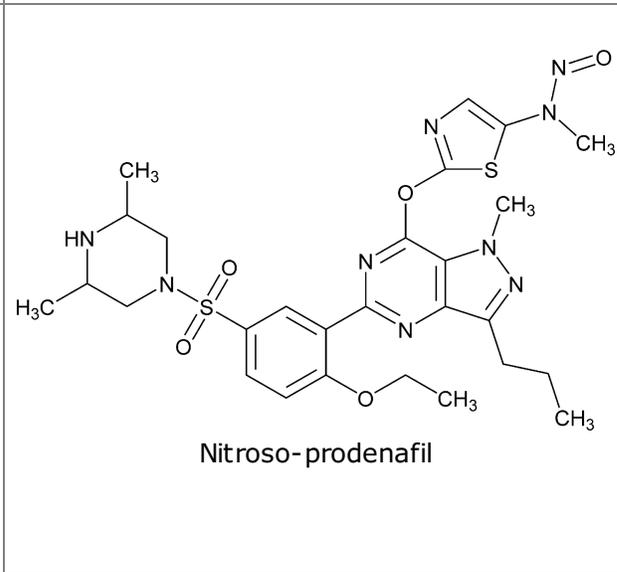
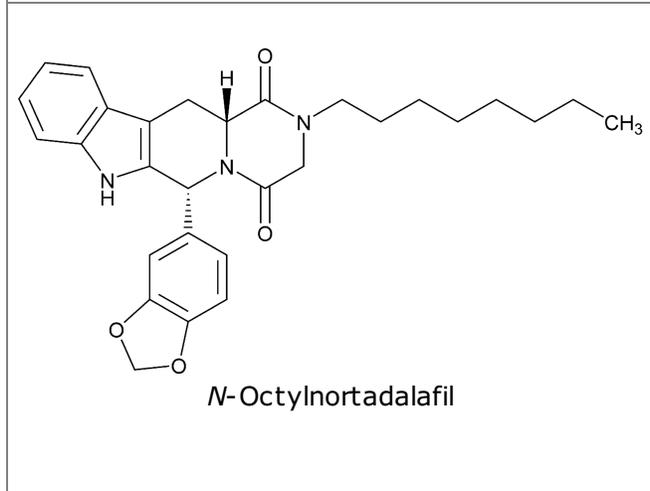
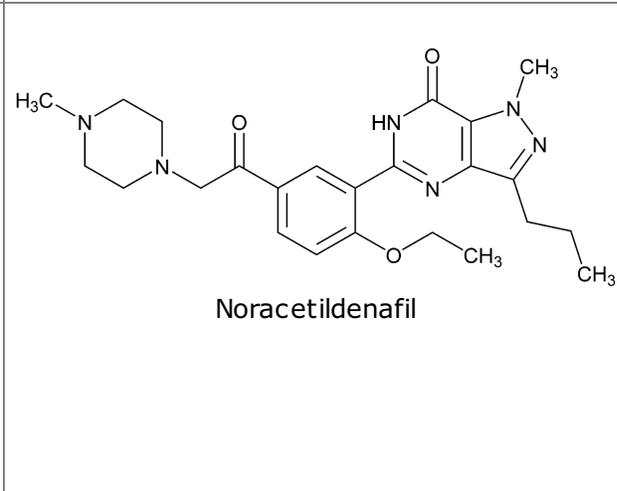


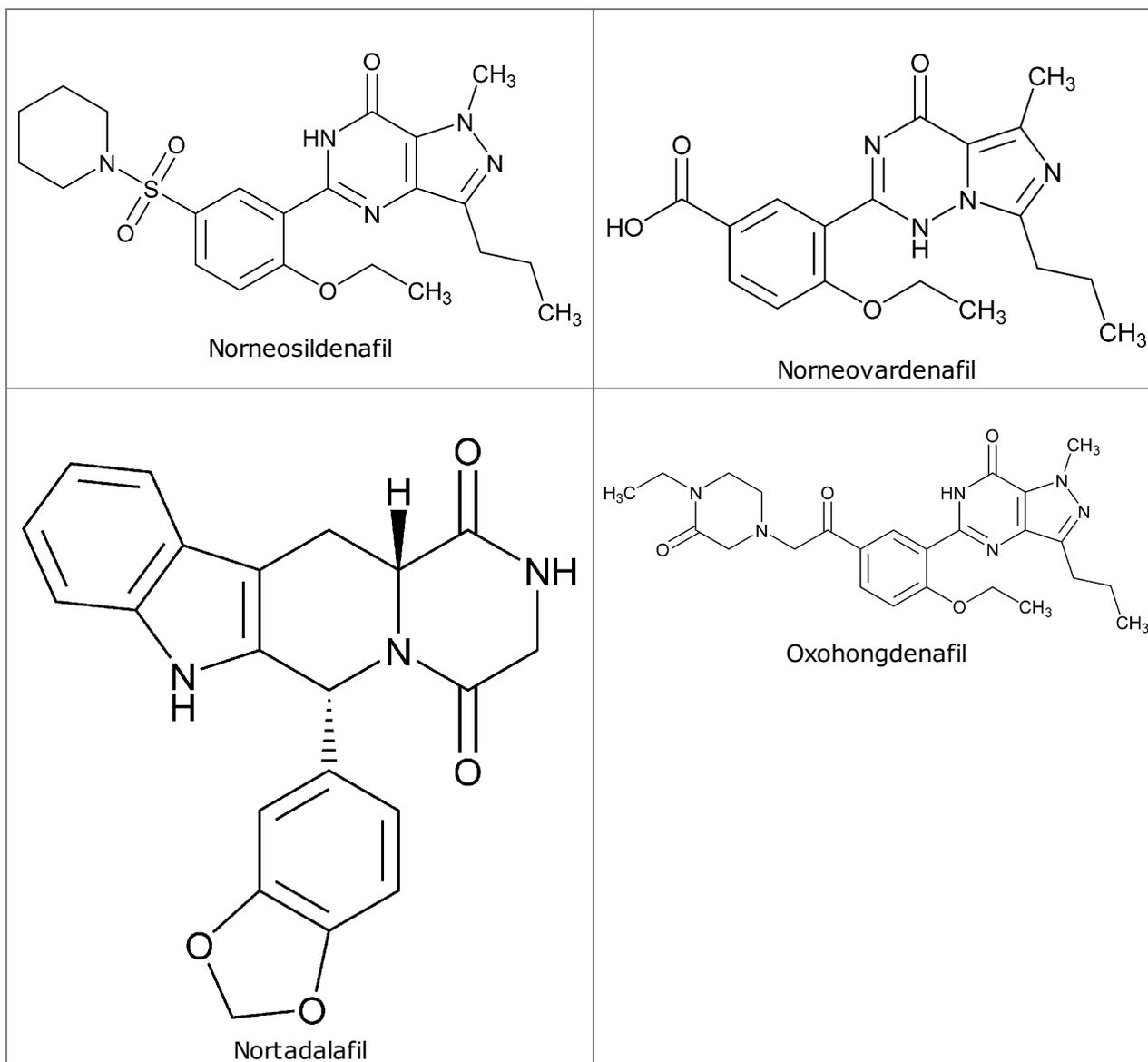
 <p>Chemical structure of Chlorodenafil: A 6,7-dihydro-1H-benzotriazolopyrimidin-2(1H)-one core substituted with a propyl group at position 4, a 4-(chloroacetyl)phenoxy group at position 7, and a 4-(methoxymethyl)phenoxy group at position 8.</p> <p>Chlorodenafil</p>	 <p>Chemical structure of Chloropretadalafil: A 6,7-dihydro-1H-benzotriazolopyrimidin-2(1H)-one core substituted with a propyl group at position 4, a 2-(chloroacetyl)phenyl group at position 7, and a 1-(2-(chloroacetyl)phenyl)pyrrolidine-2-carboxylate group at position 8.</p> <p>Chloropretadalafil</p>
 <p>Chemical structure of Cinnamyldenafil: A 6,7-dihydro-1H-benzotriazolopyrimidin-2(1H)-one core substituted with a propyl group at position 4, a 4-(cinnamylamino)phenoxy group at position 7, and a 4-(ethoxymethyl)phenoxy group at position 8.</p> <p>Cinnamyldenafil</p>	 <p>Chemical structure of Cyclopentynafil: A 6,7-dihydro-1H-benzotriazolopyrimidin-2(1H)-one core substituted with a propyl group at position 4, a 4-(cyclopentylamino)sulfonylphenoxy group at position 7, and a 4-(ethoxymethyl)phenoxy group at position 8.</p> <p>Cyclopentynafil</p>
 <p>Chemical structure of Depiperazinothiosildenafil: A 6,7-dihydro-1H-benzotriazolopyrimidin-2(1H)-one core substituted with a propyl group at position 4, a 4-(ethoxymethyl)phenoxy group at position 7, and a 4-(piperidin-1-ylthio)phenoxy group at position 8.</p> <p>Depiperazinothiosildenafil</p>	 <p>Chemical structure of Descarbosildenafil: A 6,7-dihydro-1H-benzotriazolopyrimidin-2(1H)-one core substituted with a propyl group at position 4, a 4-(ethoxymethyl)phenoxy group at position 7, and a 4-(dimethylamino)sulfonylphenoxy group at position 8.</p> <p>Descarbosildenafil</p>

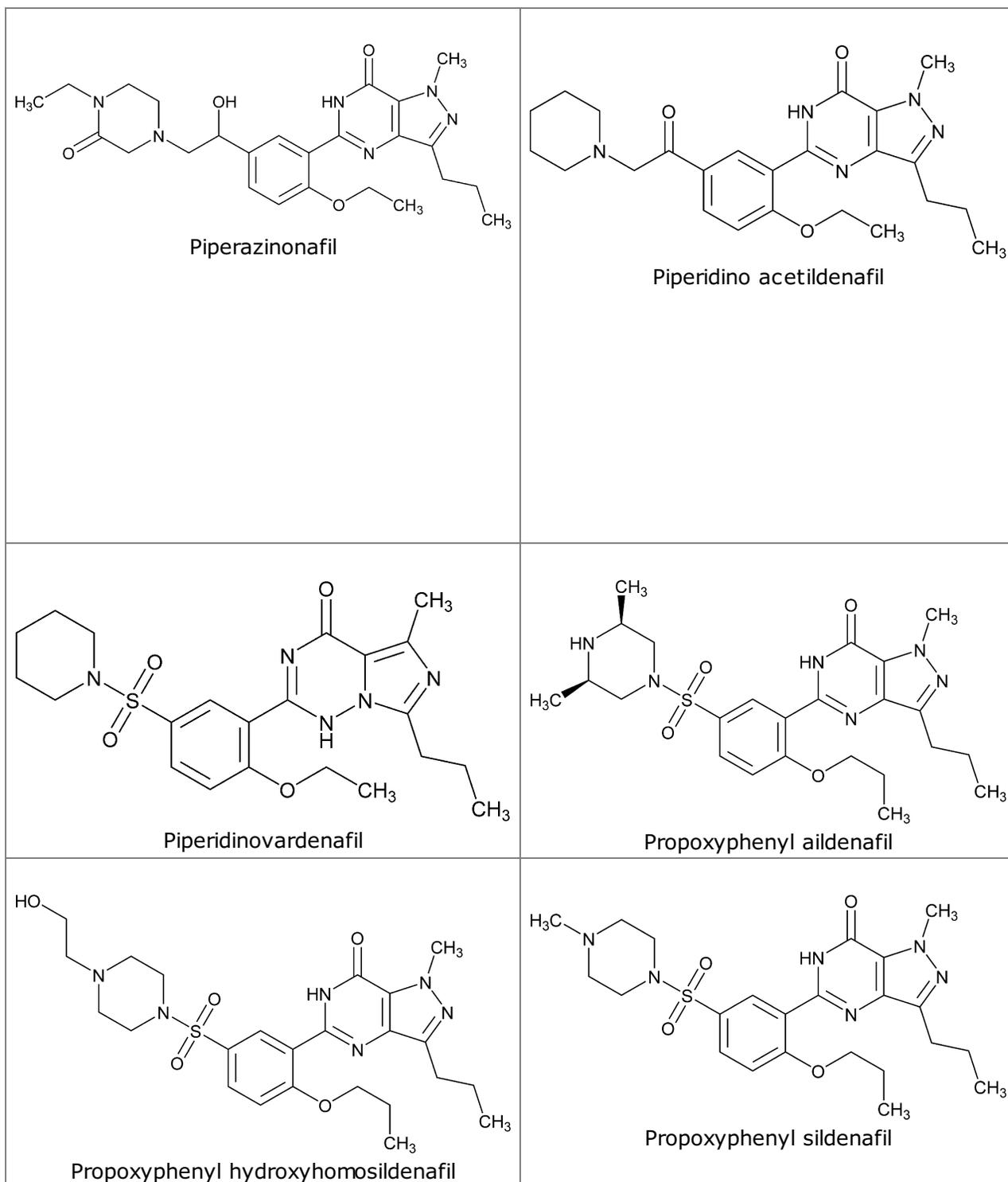


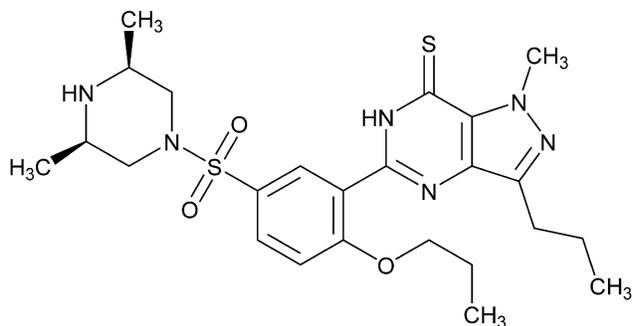
 <p>Chemical structure of Hydroxychlorodenafil: A 6-hydroxy-1,2,3,4-tetrahydro-1H-benzotriazin-4(1H)-one core substituted with a 4-(2-chloroethyl)phenoxy group at position 2, a methyl group on the triazine ring, and a propyl group at position 4.</p> <p>Hydroxychlorodenafil</p>	 <p>Chemical structure of Hydroxyhomosildenafil: A 6-hydroxy-1,2,3,4-tetrahydro-1H-benzotriazin-4(1H)-one core substituted with a propyl group at position 4, a 4-(2-hydroxyethyl)piperazine-1-sulfonyl group at position 2, and an ethoxy group at position 6.</p> <p>Hydroxyhomosildenafil</p>
 <p>Chemical structure of 2-Hydroxypropylnortadalafil: A 1,2,3,4-tetrahydro-2H-1-benzopyrido[4,3-b]pyridin-6(1H)-one core substituted with a 2-hydroxypropyl group at position 2, a 2,3-dihydrobenzofuran-5-yl group at position 3, and a hydrogen atom at position 4.</p> <p>2-Hydroxypropylnortadalafil</p>	 <p>Chemical structure of Hydroxythiohomosildenafil: A 6-hydroxy-1,2,3,4-tetrahydro-1H-benzotriazin-4(1H)-one core substituted with a propyl group at position 4, a 4-(2-hydroxyethyl)piperazine-1-sulfonyl group at position 2, an ethoxy group at position 6, and a sulfur atom at position 5.</p> <p>Hydroxythiohomosildenafil</p>
 <p>Chemical structure of Hydroxythiovardenafil: A 6-hydroxy-1,2,3,4-tetrahydro-1H-benzotriazin-4(1H)-one core substituted with a propyl group at position 4, a 4-(2-hydroxyethyl)piperazine-1-sulfonyl group at position 2, an ethoxy group at position 6, and a sulfur atom at position 5.</p> <p>Hydroxythiovardenafil</p>	 <p>Chemical structure of Hydroxyvardenafil: A 6-hydroxy-1,2,3,4-tetrahydro-1H-benzotriazin-4(1H)-one core substituted with a propyl group at position 4, a 4-(2-hydroxyethyl)piperazine-1-sulfonyl group at position 2, an ethoxy group at position 6, and a carbonyl group at position 5.</p> <p>Hydroxyvardenafil</p>



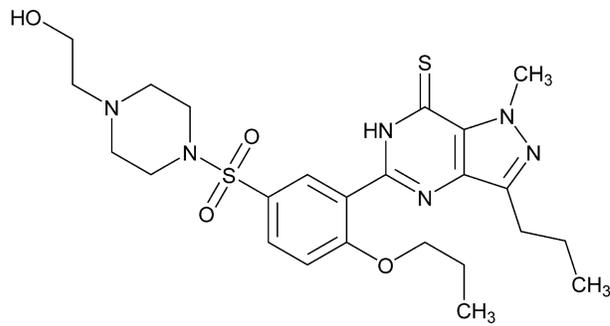
 <p>The structure shows a piperazine ring connected via a sulfonamide group to a benzene ring. This benzene ring is part of a larger fused system including a pyrazoloquinoline core. The pyrazoloquinoline core has a methyl group at the 7-position and a propyl group at the 5-position. An ethoxy group is attached to the benzene ring at the 4-position.</p> <p><i>N</i>-Desethylsildenafil</p>	 <p>The structure is similar to N-Desethylsildenafil, but the piperazine ring is substituted with a methyl group at the 4-position.</p> <p><i>N</i>-Desmethylsildenafil</p>
 <p>The structure features a pyrazoloquinoline core with a methyl group at the 7-position and a propyl group at the 5-position. A benzene ring is attached at the 4-position, which has a nitro group (NO₂) at the para position and a methoxy group (CH₃O) at the ortho position.</p> <p>Nitrodenafil</p>	 <p>The structure is similar to N-Desethylsildenafil, but the piperazine ring is substituted with a methyl group at the 4-position and a nitroso group (NO) at the 3-position.</p> <p>Nitroso-prodenafil</p>
 <p>The structure shows a piperazine ring with an octyl chain at the 4-position. It is connected via a sulfonamide group to a benzene ring, which is part of a complex fused system including a pyrazoloquinoline core and a benzofuran moiety.</p> <p><i>N</i>-Octylnortadalafil</p>	 <p>The structure is similar to N-Desethylsildenafil, but the piperazine ring is substituted with a methyl group at the 4-position and an acetamide group (-NHCOCH₃) at the 3-position.</p> <p>Noracetildenafil</p>



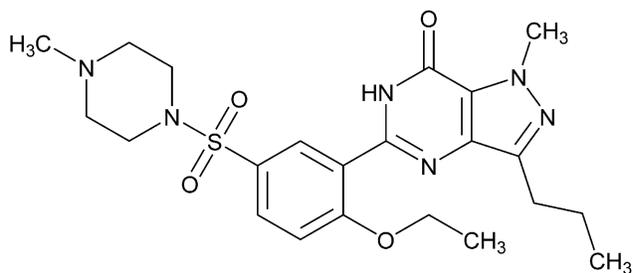




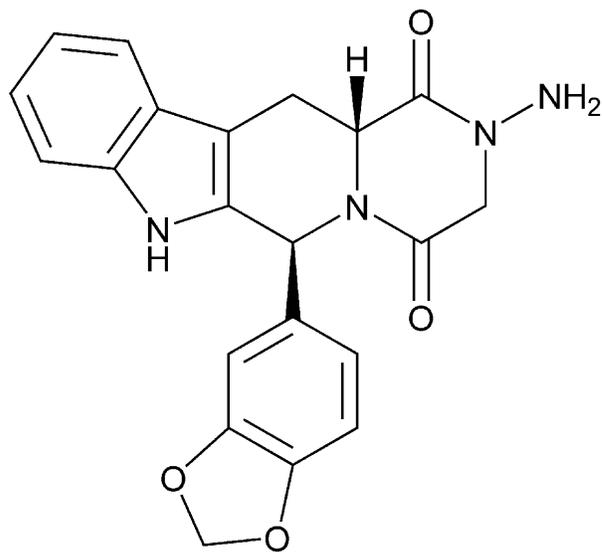
Propoxyphenyl thioaildenafil



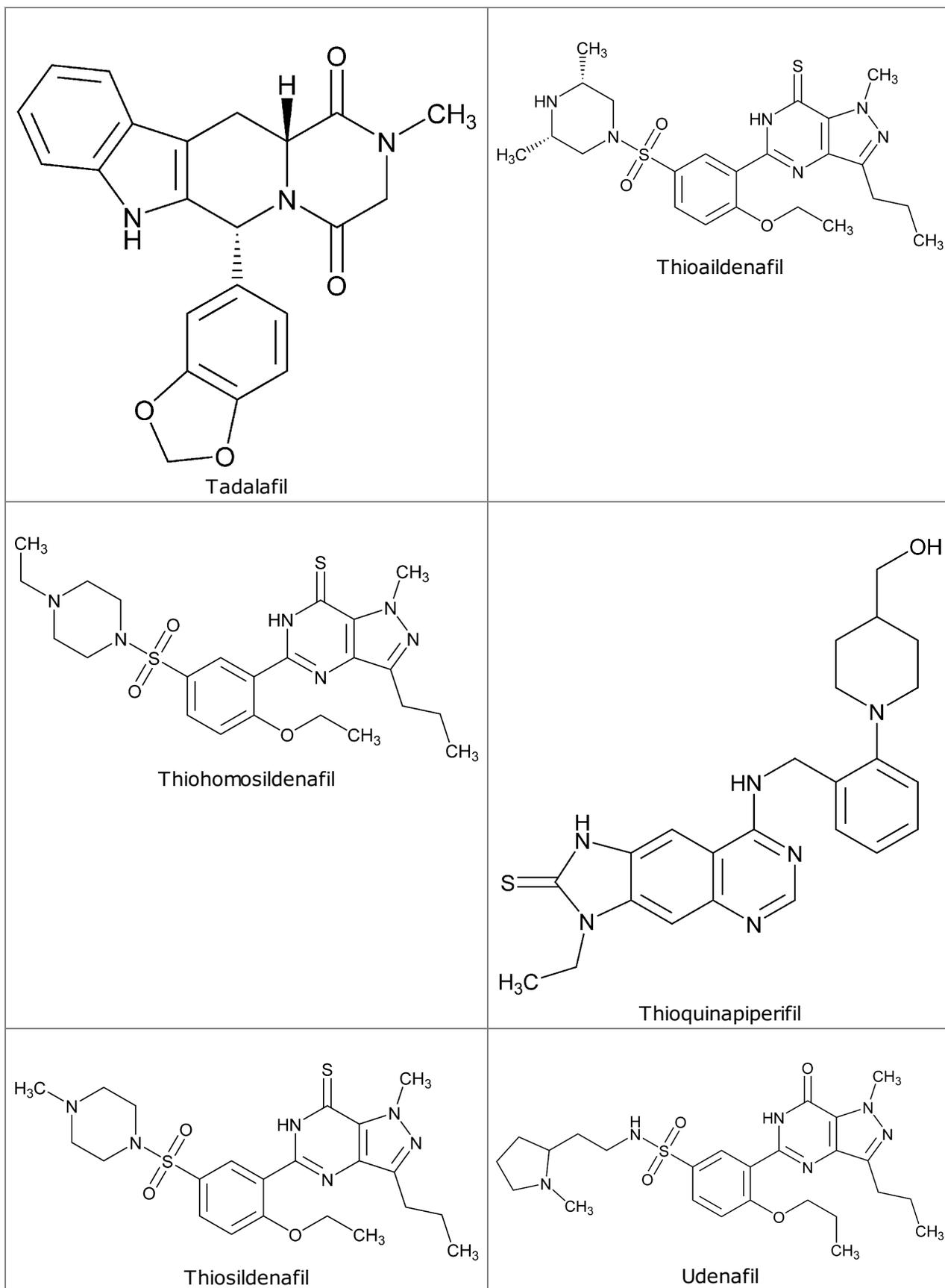
Propoxyphenyl thiohydroxyhomosildenafil

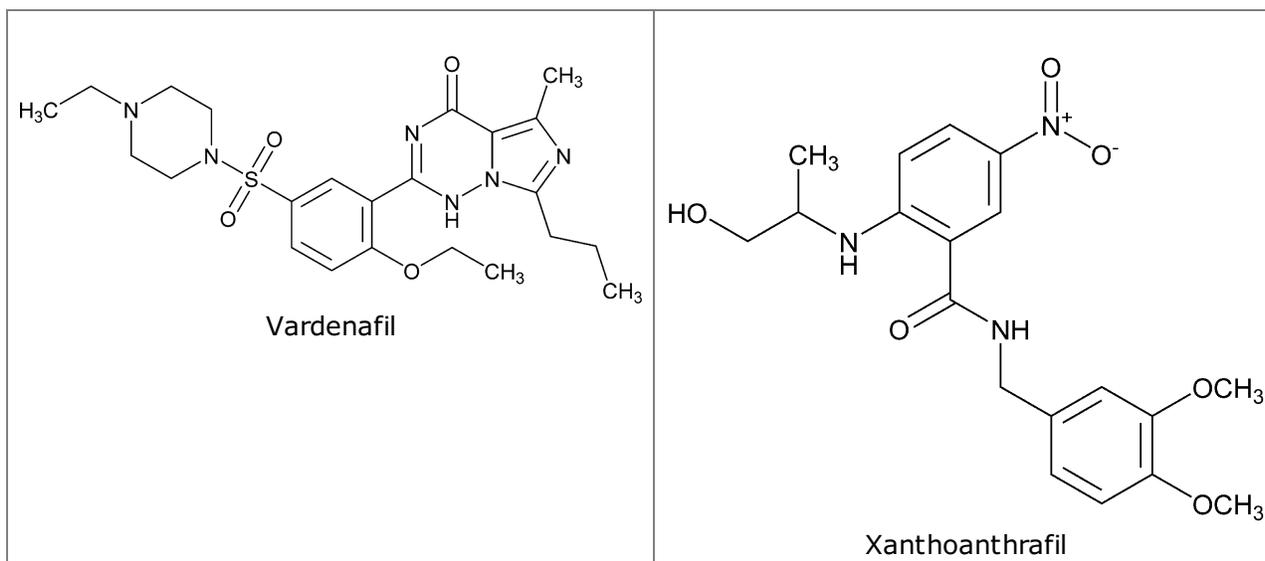


Sildenafil



(*S,R*)-Aminotadalafil





■ 1S (USP39)

¹ In the United States, dietary supplements are defined as substances that are ingested, in agreement with 21 U.S.Code §321(ff)(2)(A)(i). Definitions of dietary supplements, nutritional supplements, functional foods, and bioactive food additives may vary extensively, depending on local or national legislation. In the marketplace, there is a trend toward expanding the mode of delivery of the adulterating compounds to routes not covered by the regulatory definition for dietary supplements, i.e., topical oils, creams, lotions, e-cigarettes, chewing gums, sprays, and others. Such novel delivery systems present unique challenges, particularly from the standpoint of sample preparation, and are not considered for the purposes of this chapter to be dietary supplements. However, recognizing the emerging threat, USP chooses to highlight the existence of these products. In no way should mention of these products be interpreted as a comment on their legal status or be perceived as an expansion of the definition of DS.

² CacheSyn (<http://www.cachesyn.com/>); Santa Cruz Biotechnology, Inc. (<http://www.scbt.com>); TLC Pharmachem (<http://www.tlcpfarmachem.com/>); and Toronto Research Chemicals (<http://www.trc-canada.com/>) are some of the potential sources of rare and hard-to-find adulterant reference materials.

³ The procedure was developed on the Agilent Technologies Zorbax SB-C18 column.

⁴ The procedure was developed on the Agilent Technologies Zorbax SB-C18 column.

⁵ The settings were found appropriate for ThermoElectron LTQ XL Linear Ion Trap Mass Spectrometer. Users will need to optimize their respective instrumentation according to the manufacturer's recommendations.

⁶ 32% Ammonia solution is available from EMD Millipore.

⁷ Procedure was developed using Expression CMS mass spectrometer from Advion, equipped with a TLC-MS interface available from CAMAG. If other mass spectrometers are used, relevant settings will have to be optimized. The bands were directly eluted with a mixture of water and acetonitrile (80:20) containing 0.1% formic acid.

⁸ The procedures were developed using commercial Promega PDE-Glo™ Phosphodiesterase Assay Kit, Catalog # V1361. It includes the following reagents: PDE-Glo™ Reaction Buffer 5X (Catalog # V133A); PDE-Glo™ Detection Buffer 5X (Catalog # V134A); Protein Kinase A Solution (Catalog # V135A); PDE-Glo™ Termination Buffer 5X (Catalog # V136A); cGMP Stock Solution, 1 mM (Catalog # V641A); cAMP, 1 mM (Catalog # V642B); Kinase-Glo™ Substrate (Catalog # V672A); and Kinase-Glo™ Buffer (Catalog # V673A). Kits from alternative suppliers may also be used, e.g., BPS Science, Catalog # 60350, although re-optimization of test procedures will be required.

⁹ The procedures were developed using human phosphodiesterase 5A from BPS Bioscience Catalog # 60050. The enzyme is available from numerous suppliers, e.g., Sigma-Aldrich Catalog # E9034.

¹⁰ Conforming plates are available from Corning (Costar 3912 or Costar 3963), Thermo Scientific (Nunc™ Catalog # 236105), and other vendors.

¹¹ Data contributed by USP collaborators.

¹² Data compiled from published sources.

BRIEFING

2,6-Diaminopyridine. It is proposed to add this new reagent used in the *Organic Impurities* test in the monograph for *Phenazopyridine Hydrochloride*, previously published in PF 41(2) [Mar.–Apr. 2015].

(HDQ: M. Marques.)

Correspondence Number—C132945

Comment deadline: July 31, 2015

Add the following:

■ 2,6-Diaminopyridine, C₅H₇N₃—**109.13** [141-86-6]—Use a suitable grade with a content of NLT 98%.

[Note—A suitable grade is available as catalog number D24404 from www.sigma-aldrich.com.]

■ 1S (USP39)

BRIEFING

Benzethonium Chloride. It is proposed to add this new reagent for the preparation of 0.004 M benzethonium chloride VS used in the Assay for Sodium Lauryl Sulfate, which is posted as a Stage 6 harmonized monograph on www.usp.org.

(HDQ: M. Marques.)

Correspondence Number—C101945

Comment deadline: July 31, 2015

Add the following:

■ Benzethonium Chloride— Use *Benzethonium Chloride* (USP monograph). ■ 1S (USP39)

BRIEFING

Chelating Ion-Exchange Resin. It is proposed to add this new reagent used in the *Impurities, Gla-domainless eptacog alfa (Υ -carboxylation)* test in the monograph for *Eptacog Alfa* published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C149307

Comment deadline: July 31, 2015

Add the following:

■ Chelating Ion-Exchange Resin—Weak cation-exchange resin composed of styrene divinylbenzene copolymer containing paired iminodiacetate ions, which act as chelating groups in binding polyvalent metal ions. [Note—A suitable grade is available as Chelex® 100 Resin from www.bio-rad.com.] ■ 1S (USP39)

BRIEFING

Benzethonium Chloride, Two Hundred Fiftieth-Molar (0.004 M). This new volumetric solution is used in the *Assay for Sodium Lauryl Sulfate*, which is posted as a Stage 6 harmonized monograph on www.usp.org.

(HDQ: M. Marques.)

Correspondence Number—C101945

Comment deadline: July 31, 2015

Add the following:

■ **Benzethonium Chloride, Two Hundred Fiftieth-Molar (0.004 M)**

$C_{27}H_{42}ClNO_2$ **448.08**

Dissolve 1.792 g of benzethonium chloride, previously dried at 100° – 105° to constant weight, in water to make 1000 mL.

Calculate the molarity of the solution from the content of benzethonium chloride in the dried benzethonium chloride determined as follows. Dissolve 0.350 g of the dried benzethonium chloride in 30 mL of glacial acetic acid and add 6 mL of mercuric acetate TS. Titrate with 0.1 N perchloric acid VS, using 0.05 mL of crystal violet TS as an indicator. Carry out a blank titration. One mL of 0.1 N perchloric acid VS is equivalent to 44.81 mg of benzethonium chloride ($C_{27}H_{42}ClNO_2$). [Note—This solution is commercially available ready to be used. Use a suitable grade.]

■ 1S (USP39)

BRIEFING

L## (Eptacog Alfa, Dionex CarboPac PA100). It is proposed to add this new column packing used in the *Glycan Analysis* test in the monograph for *Eptacog Alfa*, published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C149307

Comment deadline: July 31, 2015

Add the following:

■ L## (Eptacog Alfa, Dionex CarboPac PA100)—Ethylvinylbenzene/divinylbenzene substrate (55% cross-linked) agglomerated with quaternary amine functionalized 275-nm latex microbeads (6% cross-linked), about 8.5 µm in diameter. ■ 1S (USP39)

BRIEFING

L## (Eptacog Alfa, Dionex DNAPac PA100). It is proposed to add this new column packing used in the test for *GLA-Domainless Eptacog Alpha (γ-Carboxylation)* in the monograph for *Eptacog Alfa*, published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C149307

Comment deadline: July 31, 2015

Add the following:

■ L## (Eptacog Alfa, Dionex DNAPac PA100)—Sulfonated ethylvinylbenzene/divinylbenzene substrate approximately 12–14 µm in diameter agglomerated with hydrophilic quaternary amine functionalized glycidyl-derivative methacrylate microbeads. ■ 1S (USP39)

BRIEFING

L## (Metoprolol Succinate, Halo Penta-HILIC). It is proposed to add this new column packing used in the test for *Content of Metoprolol Related Compound H and Metoprolol Related Compound I* in the revision to the monograph for *Metoprolol Succinate*, published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C142873

Comment deadline: July 31, 2015

Add the following:

■ L## (Metoprolol Succinate, Halo Penta-HILIC)—A highly polar alkyl ligand comprising five hydroxyl groups that are chemically bonded to totally porous or superficially porous silica or a monolithic silica rod. ■ 1S (USP39)

BRIEFING

L26, *USP 38* page 1902. It is proposed to expand the description of this column packing to include packings based on superficially porous particles.

Additionally, minor editorial changes have been made to update the packing to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C156426

Comment deadline: July 31, 2015

Change to read:

L26—Butyl silane chemically bonded to totally porous

■ or superficially porous ■ 1S (USP39)

silica particles, 1.5–10 µm in diameter.

CONTAINERS FOR DISPENSING CAPSULES AND TABLETS

BRIEFING

Container Specifications for Capsules and Tablets, page 7209 of the *First Supplement to USP 38*.

(HDQ.)

Correspondence Number—C118327; C120789; C127643; C133281

The following table is provided as a reminder for the pharmacist engaged in the typical dispensing situation who already is acquainted with the *Packaging and Storage* requirements set forth in the individual monographs. It lists the capsules and tablets that are official in the *United States Pharmacopeia* and indicates the relevant tight (T), well-closed (W), and light-resistant (LR) specifications applicable to containers in which the drug that is repackaged should be dispensed.

This table is not intended to replace, nor should it be interpreted as replacing, the definitive requirements stated in the individual monographs.

Container Specifications for Capsules and Tablets

Monograph Title	Container Specification
Add the following: ■ Abacavir and Lamivudine Tablets	T, LR ■ 1S (USP39)
Delete the following: ■ Oxtriphylline Delayed-Release Tablets	T ■ 1S (USP39)
Add the following: ■ Paricalcitol Capsules	T ■ 1S (USP39)
Add the following: ■ Sildenafil Tablets	W ■ 1S (USP39)

BRIEFING

Description and Relative Solubility of USP and NF Articles, page 7219 of the *First Supplement to USP 38*.

(HDQ.)

Correspondence Number—C145274; C152104

Add the following:

■ **Exemestane:** White to slightly yellow, crystalline powder. Freely soluble in *N,N*-dimethylformamide; soluble in methanol; insoluble in water. ■ 1S (USP39)

Change to read:

Nystatin: ~~Yellow to light tan powder, having an odor suggestive of cereals. Is hygroscopic, and is affected by long exposure to light, heat, and air. Freely soluble in dimethylformamide and in dimethyl sulfoxide; sparingly to slightly soluble in methanol, in *n*-propyl alcohol, and in *n*-butyl alcohol; practically insoluble in water and in alcohol; insoluble in chloroform and in ether.~~

■ Yellow to light tan powder. Is hygroscopic, and is affected by long exposure to light, heat, and air. Freely soluble in dimethylformamide and in dimethyl sulfoxide; very slightly soluble in methanol; practically insoluble or insoluble in water, in alcohol, in *n*-propyl alcohol, in *n*-butyl alcohol, in chloroform, and in ether. ■1S (USP39)

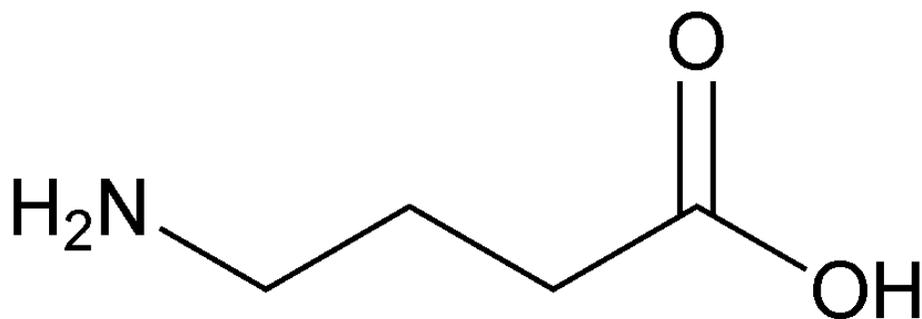
BRIEFING

Gamma-Aminobutyric Acid. Because there is no existing *USP* monograph for this dietary ingredient, a new monograph is being proposed. The liquid chromatographic procedure in the *Assay* and the test for *Related Compounds* is based on analyses performed with the Waters XBridge Amide brand of L68 column. Typical retention times for gamma-aminobutyric acid and monosodium glutamate are 7.2 and 8.7 min, respectively.

(DS: H. Dinh.)

Correspondence Number—C135182

Comment deadline: July 31, 2015

Add the following:■ **Gamma-Aminobutyric Acid**

C₄H₉NO₂ 103.12

4-Aminobutanoic acid [56-12-2].

DEFINITION

Gamma-Aminobutyric Acid contains NLT 98.0% and NMT 102.0% of gamma-aminobutyric acid (C₄H₉NO₂), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** { 197K }
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **Procedure**

Buffer solution: 20 mM monobasic sodium phosphate. Adjust with 1 N sodium hydroxide to a pH of 5.5.

Mobile phase: Acetonitrile and *Buffer solution* (78:22)

Diluent: Acetonitrile and water (60:40)

System suitability solution: 1 mg/mL of USP Gamma-Aminobutyric Acid RS and 0.1 mg/mL of monosodium glutamate in *Diluent*

Standard solution: 1 mg/mL of USP Gamma-Aminobutyric Acid RS in *Diluent*

Sample solution: 1 mg/mL of Gamma-Aminobutyric Acid in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing L68

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for gamma-aminobutyric acid and monosodium glutamate are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 2.0 between gamma-aminobutyric acid and monosodium glutamate, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of gamma-aminobutyric acid (C₄H₉NO₂) in the portion of Gamma-Aminobutyric Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Gamma-Aminobutyric Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Gamma-Aminobutyric Acid in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition** 〈 281 〉: NMT 0.1%

• **Chloride and Sulfate** 〈 221 〉, *Chloride*

Standard solution: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.73 g

Acceptance criteria: NMT 0.05%

- **Chloride and Sulfate** 〈 221 〉, *Sulfate*

Standard solution: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.33 g

Acceptance criteria: NMT 0.03%

- **Related Compounds**

Mobile phase, Diluent, System suitability solution, Chromatographic system, and

System suitability: Proceed as directed in the *Assay*.

Sample solution: 10 mg/mL of Gamma-Aminobutyric Acid in *Diluent*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Gamma-Aminobutyric Acid taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_T sum of the peak responses of all the peaks from the *Sample solution*

Acceptance criteria

Individual impurity: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **pH** 〈 791 〉

Sample solution: 20 mg/mL in water

Acceptance criteria: 6.5–7.5

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

- **USP Reference Standards** 〈 11 〉

USP Gamma-Aminobutyric Acid RS

■ 1S (USP39)

BRIEFING

Glutathione, *USP 38* page 6080. Based on comments received, it is proposed to add a procedure for preparing *Silver manganese paper* to the test for *Ammonium*.

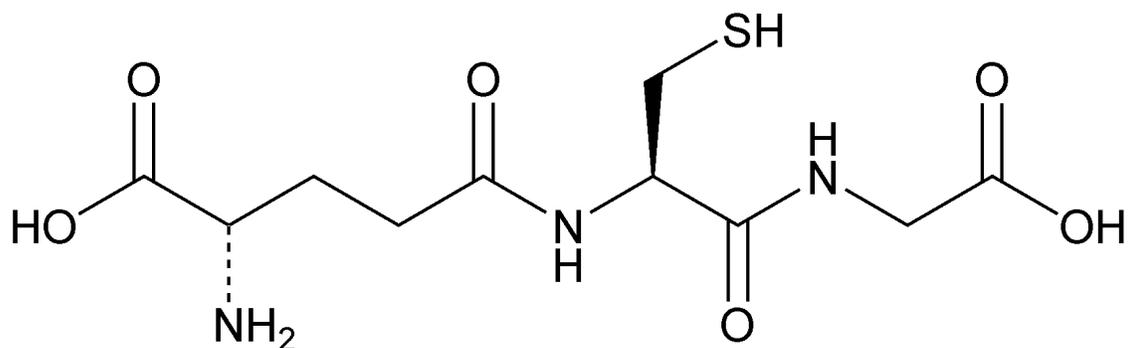
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(DS: H. Dinh.)

Correspondence Number—C157933

Comment deadline: July 31, 2015

Glutathione



$C_{10}H_{17}N_3O_6S$ 307.32

Pentanoic acid, 2-amino-5-[(*R*)-1-(carboxymethylamino)-3-mercapto-1-oxopropan-2-ylamino]-5-oxo, (*S*);

N-(*N*-L-γ-Glutamyl-L-cysteinyl)glycine [70-18-8].

DEFINITION

Glutathione contains NLT 98.0% and NMT 101.0% of glutathione ($C_{10}H_{17}N_3O_6S$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈 197K 〉
- **B. Optical Rotation, Specific Rotation** 〈 781S 〉
Sample solution: 40 mg/mL in water
Acceptance criteria: -15.5° to -17.5° , at 20°

ASSAY

• Procedure

Sample: 500 mg of Glutathione previously dried

Blank: 50 mL of metaphosphoric acid (1 in 50)

Titrimetric system

(See *Titrimetry* 〈 541 〉.)

Mode: Direct titration

Titrant: 0.1 N iodine VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 50 mL of metaphosphoric acid (1 in 50) and titrate with the *Titrant*.

Calculate the percentage of glutathione ($C_{10}H_{17}N_3O_6S$) in the portion of Glutathione taken:

$$\text{Result} = [(V_U - V_B) \times N \times F \times 100] / W$$

V_U titrant volume of the *Sample* (mL)

V_B = titrant volume of the *Blank* (mL)
 N = titrant normality (mEq/mL)
 F = equivalency factor, 307.32 mg/mEq
 W = weight of the *Sample* (mg)

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

Change to read:

• Ammonium

Standard solution: 10 µg of ammonium from a diluted ammonium chloride solution

Sample solution: 50 mg of Glutathione

■ **Silver manganese paper:** Immerse strips of slow filter paper (Whatman® filter paper, grade 5 or equivalent) into a solution containing 8.5 mg/mL of manganese sulfate and 8.5 mg/mL of silver nitrate. Maintain for a few minutes and allow to dry over phosphorus pentoxide protected from acid and alkaline vapors. ■1S (USP39)

Analysis: Transfer the *Sample solution* and the *Standard solution* to separate 25-mL jars fitted with caps, and dissolve in 1 mL of water. Add 0.30 g of magnesium oxide. Close immediately after placing a 5-mm square piece of *Silver manganese paper*, wetted with a few drops of water, under the caps. Swirl, avoiding projections of liquid, and allow to stand at 40° for 30 min.

Acceptance criteria: If the *Silver manganese paper* shows a gray color, it is not more intense than that of the Standard (NMT 200 ppm).

- **Arsenic** 〈 211 〉: NMT 2 ppm
- **Chloride and Sulfate, Chloride** 〈 221 〉: Dissolve 0.7 g in water to make 15 mL. The solution shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (NMT 200 ppm).
- **Chloride and Sulfate, Sulfate** 〈 221 〉: Dissolve 0.8 g in water to make 15 mL. The solution shows no more sulfate than corresponds to 0.25 mL of 0.020 N sulfuric acid (NMT 300 ppm).
- **Iron** 〈 241 〉: NMT 10 ppm
- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Delete the following:

• **Heavy Metals, Method I** 〈 231 〉: NMT 10 ppm • (Official 1-Dec-2015)

- **Residue on Ignition** 〈 281 〉: NMT 0.1%
- **Related Compounds**

Mobile phase: 6.8 g/L of potassium dihydrogen phosphate with 2.02 g/L of sodium 1-heptane sulfonate. Adjust with phosphoric acid to a pH of 3.0. Mix 970 mL of this solution with 30 mL of methanol.

System suitability solution: 0.1 mg/mL of USP L-Phenylalanine RS, 0.5 mg/mL of USP Glutathione RS, and 0.5 mg/mL of USP Ascorbic Acid RS in *Mobile phase*

Standard solution: 0.01 mg/mL of USP Glutathione RS in *Mobile phase*. [Note—This solution has a concentration equivalent to 2.0% of that of the *Sample solution*.]

Sample solution: 50 mg of Glutathione in 100 mL of *Mobile phase*. [Note—Allow the solution to stand for 5 min before use.]

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: Adjust so that the retention time of glutathione is about 5 min.

Injection volume: 10 μL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 5.0 between the ascorbic acid and glutathione peaks; and NLT 5.0 between the glutathione and l-phenylalanine peaks

Relative standard deviation: NMT 1.5% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any impurity in the portion of Glutathione taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any peak from the *Sample solution* other than glutathione

r_S peak response of the glutathione peak from the *Standard solution*

C_S concentration of USP Glutathione RS in the *Standard solution* (mg/mL)

C_U concentration of Glutathione in the *Sample solution* (mg/mL)

Acceptance criteria

Individual impurity: NMT 1.5% for the impurity with the relative retention time of about 4

Total impurities: NMT 2.0%

SPECIFIC TESTS

• Clarity and Color of Solution

Sample solution: 0.1 g/mL in water

Analysis: Using identical tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm, compare the liquid to be examined with water, the depth of the layer being 40 mm. Compare the colors in diffused daylight, viewing vertically against a white background.

Acceptance criteria: The solution is clear and colorless.

• Loss on Drying 〈 731 〉

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** { 11 }
 - USP Ascorbic Acid RS
 - USP Glutathione RS
 - USP L-Phenylalanine RS

BRIEFING

Krill Oil Capsules, *USP 38* page 6118. On the basis of comments received and to be consistent with the recent revision of the *Krill Oil* monograph published in *PF 41(2)* [Mar.–Apr. 2015], the following changes are proposed:

1. Add the content of myristic acid and palmitic acid and the ratio of palmitic acid to myristic acid content to *Table 1* of the *Fatty Acid Profile* in the *Identification* section.
2. Change the upper limits and lower limits for several fatty acids listed in *Table 1* to reflect new data recently received.
3. Change the range of total phospholipids content from 28%–52% to 30%–55% and the range of phosphatidylcholine from 60%–90% (w/w) to 60%–96% (w/w) of the total phospholipids content in the *Acceptance criteria* for the *Content of Total Phospholipids* test.
4. Change the range of phosphatidylcholine from 60%–90% (w/w) to 60%–96% (w/w) of the total phospholipids content in *Identification* test *B—Phospholipid Profile*.
5. Add a table of molecular weight values and approximate chemical shift values (in reference to triphenyl phosphate) for the phospholipids of importance to krill oil analysis in the test for *Total Phospholipids* in order to clarify the proper use of the NMR data obtained. The values proposed are based on data and comments received. Also, equations are included for the calculations required by this test.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(DS: N. Davydova.)

Correspondence Number—C157669

Comment deadline: July 31, 2015

Krill Oil Capsules**DEFINITION**

Krill Oil Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of Krill Oil where Krill Oil is the fixed oil extracted from Antarctic krill (*Euphausia superba* Dana) biomass by using a suitable extraction solvent.

IDENTIFICATION**Change to read:**

- **A. Fatty Acid Profile**

Antioxidant solution, System suitability solution 1, and Chromatographic system:

Proceed as directed in *Fats and Fixed Oils* { 401 }, *Omega-3 Fatty Acids Determination*

and Profile.

Standard solution: Proceed as directed for *Test Solution 1* in *Fats and Fixed Oils* (401), *Omega-3 Fatty Acids Determination and Profile*, except use 250 mg of USP Krill Oil RS.

Sample solution: Using the portion of oil from NLT 10 Capsules, proceed as directed for *Test Solution 1* in *Fats and Fixed Oils* (401), *Omega-3 Fatty Acids Determination and Profile*.

System suitability

Samples: *System suitability solution 1* and *Standard solution*

Suitability requirements

Chromatogram similarity: The chromatogram from the *Standard solution* is similar to the reference chromatogram provided with the lot of USP Krill Oil RS being used.

Resolution: NLT 1.0 between the methyl oleate and methyl *cis*-vaccinate peaks, *Standard solution*

Theoretical area percentages: Meet the requirements for *System suitability solution 1*

Analysis

Sample: *Sample solution*

Identify the retention times of the relevant fatty acid methyl esters in the *Sample solution* by comparing the chromatogram of the *Sample solution* with that of the *Standard solution* and the USP reference chromatogram.

Calculate the area percentage for each fatty acid as methyl esters in the portion of oil taken from the Capsules:

$$\text{Result} = (r_A/r_B) \times 100$$

r_A peak area of each individual fatty acid from the *Sample solution*

r_B total area of all peaks, except the solvent and butylated hydroxytoluene peaks, from the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Fatty Acid	Short-Hand Notation	Lower Limit (Area %)	Upper Limit (Area %)
■ Saturated fatty acids			
Myristic acid	14:0	6.4	13.0
Palmitic acid	16:0	17.0	24.6
Palmitic acid: myristic acid ratio	16:0/14:0	1.6	2.8 ■ 1S (USP39)
■ Monounsaturated fatty acids			
Palmitoleic acid	16:1 n-7	2.5	9.0
			7.0
<i>cis</i> -Vaccenic acid	18:1 n-7	4.7	■ 8.0 ■ 1S (USP39)
Oleic acid	18:1 n-9	7.0	14.5
			1.2
Eicosenic acid	20:1 n-9	0.1	■ 1.7 ■ 1S (USP39)

Fatty Acid	Short-Hand Notation	Lower Limit (Area %)	Upper Limit (Area %)
Erucic acid	22:1 n-9	0.0	0.9 ■ 1.3 ■ 1S (USP39)
Polyunsaturated fatty acids			
Linoleic acid	18:2 n-6	1.4	3.0
α-Linolenic acid	18:3 n-3	0.5	3.5 ■ 5.0 ■ 1S (USP39)
Morocctic acid	18:4 n-3	1.8	7.2 ■ 10.0 ■ 1S (USP39)
Eicosapentaenoic acid	20:5 n-3	14.0	22.1 ■ 24.3 ■ 1S (USP39)
Docosapentaenoic acid	22:5 n-3	0.0	0.7
Docosahexaenoic acid	22:6 n-3	7.5 ■ 7.1 ■ 1S (USP39)	13.2 ■ 15.7 ■ 1S (USP39)

Change to read:

- **B. Phospholipid Profile**

Solution A, Line shape standard (¹H), Sensitivity standard (¹H), Sensitivity standard (³¹P), Internal standard, Sample solution, Standard solution, Instrumental conditions, System suitability, and Analysis: Proceed as directed in the test for *Content of Total Phospholipids in Strength*.

Acceptance criteria: The *Sample solution* contains all of the following phospholipids: phosphatidylcholine (60%–90%

■ 96% ■ 1S (USP39)

(w/w) of the total phospholipids content), lysophosphatidylcholine, (~~as a mixture of 1-lysophosphatidylcholine and 2-lysophosphatidylcholine~~);

■ 1S (USP39)

and phosphatidylethanolamine.

STRENGTH

- **Content of Krill Oil**

Analysis: Weigh NLT 10 Capsules in a tared weighing bottle, and carefully open the Capsules, without loss of shell material. Transfer the combined Capsule contents to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of 2,2,4-trimethylpentane. Discard the washings, and allow the empty Capsules to dry in a current of dry air until the 2,2,4-trimethylpentane is completely evaporated. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average net weight per Capsule.

Acceptance criteria: 95.0%–105.0%

- **Content of EPA and DHA**

Standard solution 1a, Standard solution 1b, Standard solution 2a, Standard solution 2b, System suitability solution 1, and Chromatographic system: Proceed as directed

in *Fats and Fixed Oils* { 401 }, *Omega-3 Fatty Acids Determination and Profile*.

Test solution 1: Using the portion of 250 mg of oil from NLT 10 Capsules, proceed as directed for *Test solution 1* in *Fats and Fixed Oils* { 401 }, *Omega-3 Fatty Acids Determination and Profile*.

Test solution 2: Using the portion of 250 mg of oil from NLT 10 Capsules, proceed as directed for *Test solution 2* in *Fats and Fixed Oils* { 401 }, *Omega-3 Fatty Acids Determination and Profile*.

Analysis: Proceed as directed for *Analysis (for triglycerides)* in *Fats and Fixed Oils* { 401 }, *Omega-3 Fatty Acids Determination and Profile*.

Calculate the percentage of EPA and DHA in the portion of oil taken from the Capsules.

Acceptance criteria: NLT 10.0% (w/w) of EPA and NLT 5.0% (w/w) of DHA

Change to read:

• **Content of Total Phospholipids**

(See *Nuclear Magnetic Resonance Spectroscopy* { 761 }, *Qualitative and Quantitative NMR Analysis*.)

[Note—All deuterated solvents used in this method should be NLT 99.8 atom % D. Whenever water is used in this method, it should be of sufficient quality to ensure that no trace metals or other contaminants that may affect the analysis are present.]

Solution A: 0.2 M EDTA adjusted with a 1 M cesium carbonate solution to a pH of 7.2–7.5. Document the final pH and the amount of 1 M cesium carbonate solution necessary to attain the desired pH. [Note—Use cesium carbonate of a sufficient grade for trace metals analysis.]

Line shape standard (¹H): 1% Chloroform in acetone-d₆

Sensitivity standard (¹H): 0.1% Ethylbenzene in chloroform-d

Sensitivity standard (³¹P): 0.0485 M triphenyl phosphate in acetone-d₆

Internal standard: Use triphenyl phosphate NMR reference standard with NLT 99% purity.

Sample solution: [Note—NMR solvents containing tetramethylsilane (TMS) are readily available. If the solvents used do not contain TMS, it must be added to the *Sample solution* at an approximate concentration of 0.05% (v/v) for use as a chemical shift scale reference.] Transfer the portion of 300–350 mg of oil from NLT 10 Capsules to a 5-mL sealable glass vial. Add 25.0 mg of the *Internal standard* to the vial. Add 1 mL each of deuterated chloroform (chloroform-d) and deuterated methanol (methanol-d₄) of a grade suitable for NMR analysis to the vial to dissolve the sample. Once dissolution is complete, add 1 mL of *Solution A*, seal the vial, shake the solution for 10–20 min, then centrifuge the contents of the vial. Transfer the lower organic phase to an appropriate NMR tube. It is critical to collect the entire organic phase and transfer it to the NMR tube. It may be unavoidable to also transfer small amounts of the aqueous phase when collecting the organic phase in the NMR tube. This is an acceptable practice, so long as the aqueous phase remains completely separated and atop the organic phase in the NMR tube. The entire amount of aqueous phase must be above the probe's radio frequency (RF) coil (outside the analysis area of the tube). Should the organic phase contain undissolved materials, they must remain suspended at the aqueous-organic interface and be outside the analysis area of the tube as well. The organic phase must be free of bubbles and suspended materials that may interfere with NMR data acquisition.

Standard solution: Proceed as directed in the *Sample solution*, except use 300–350 mg of

USP Krill Oil RS.

Instrumental conditions

(See 〈 761 〉.)

Magnetic field strength: NLT 300 MHz for ^1H frequency

Probe: Direct observe probe capable of tuning to the resonance frequency of ^{31}P (dependent on the specific magnetic field strength used)

Instrument performance qualification

[Note—Testing for sensitivity and line shape should be performed on the interval specified by the manufacturer of the instrument used. Performing these tests on a minimum of a monthly basis is required for this method, but it may be done more often, as required. Resolution testing is to be performed during each analysis and documented as a part of the analytical results.]

^1H Line shape test: Using the *Line shape standard* (^1H) and the protocol recommended by the instrument manufacturer, the instrument must achieve the line shape specifications for the probe in use, as required by the instrument manufacturer. [Note—A different standard solution may be required or recommended by the manufacturer of the instrument; 1% chloroform in acetone- d_6 is most commonly used.]

^1H Sensitivity test: Using the *Sensitivity standard* (^1H) and the protocol recommended by the instrument manufacturer, the instrument must achieve the sensitivity specifications required by the instrument manufacturer. [Note—A different standard solution may be required or recommended by the manufacturer of the instrument; 0.1% ethylbenzene in chloroform- d is most commonly used.]

^{31}P Sensitivity test: Using the *Sensitivity standard* (^{31}P) and the protocol recommended by the instrument manufacturer, the instrument must achieve the sensitivity specifications required by the instrument manufacturer. [Note—A different standard solution may be required or recommended by the manufacturer of the instrument; 0.0485 M triphenyl phosphate in acetone- d_6 is most commonly used.]

^1H Resolution test: The resolution is demonstrated by the ability to detect both of the ^{29}Si satellite signals of TMS. The satellites must be resolved from the TMS signal in the spectrum with a line-broadening factor of NMT 0.5 ppm.

^{31}P Resolution test: The resolution is demonstrated using the phosphatidylcholine ether peak and the phosphatidylcholine peak. The separation of these peaks (with an applied line-broadening factor of 1.0) must be demonstrated as follows. Using the baseline as a reference, determine the total peak height of the phosphatidylcholine ether peak,

■ [Note—The PC ether signal appears just downfield from the PC signal.] ■ 1S (USP39)

and draw a line at 30% of that total peak height (intensity). The phosphatidylcholine ether peak and the neighboring phosphatidylcholine peak must be fully resolved at a point that is NMT 30% of the peak height of the phosphatidylcholine ether peak.

Data collection: Use the parameters specified in *Table 2*. Use 90 degree pulses, and calibrate pulses before use according to the recommendations supplied by the instrument manufacturer.

Table 2

Parameter	³¹ P NMR Quantitative Measurement	¹ H NMR Qualitative Measurement
Pulse program	¹ H-decoupled ³¹ P ■ (inverse gated) ■ 1S (USP39)	Single pulse ¹ H
Spectral width	50 ppm (25 to -25 ppm)	20 ppm (-3 to 17 ppm)
Transmitter offset	Center of spectral width, 0 ppm	Center of spectral width, 7 ppm
Relaxation delay	2-5 s ■ 5-15 s ■ 1S (USP39)	2-5 s
Acquisition time	1-6 s	1-6 s
Sum of relaxation delay and acquisition time	NLT 15 s	NLT 15 s ■ 1S (USP39)
Size of data set	NLT 64k (32k with zero-filling)	NLT 64k (32k with zero-filling)

[Note—The acquisition time is dependent upon the field strength and the time domain

■ the dwell time and the number of data points collected. ■ 1S (USP39)

The number of scans acquired using a 300-MHz instrument must be NLT 512.]

System suitability: Under the conditions outlined in *Data collection*, the ³¹P NMR signal of triphenyl phosphate should be observed at -17.80 ppm, and the ¹H NMR spectrum should be referenced to the ¹H signal of TMS (0 ppm) for all spectra acquired in the *Analysis*. For quantitative analysis, a sufficient number of scans should be acquired such that the signal-to-noise ratio for the phosphatidylcholine signal in the ³¹P spectrum of the *Sample solution* acquired in the *Analysis* is NLT 2000.

Analysis: Acquire the data outlined in *Data collection*. Minimally acquire the ¹H spectrum (fingerprint) of the *Sample solution* and the *Standard solution* as well as the quantitative ³¹P spectrum of the *Sample solution* and the *Standard solution*. Record the resulting spectra, and perform integration by hand or automated means on the quantitative ³¹P NMR spectrum of the *Sample solution*. The integration of the peaks in the spectrum of the *Sample solution* must be performed such that the complete set of phospholipid peaks (as identified by a comparison to the spectrum of the *Standard solution* and the *Standard solution* reference spectrum) is included in the integration. The integration region for each signal must extend ±0.05 ppm on either side of the ³¹P signal. Quantify the total phospholipids present, the phosphatidylcholine ether content, and the phosphatidylcholine content in the *Sample solution* by using a comparison to the concentration of the *Internal standard*.

Compare the ¹H spectrum of the *Sample solution* to that of the *Standard solution* to determine the similarity of fingerprints according to which phospholipids identified in the reference spectrum of the *Standard solution* are present in the spectrum of the *Sample solution*.

■ **Calculations:** Use the following equations and molecular weights listed in *Table 3* to determine the phospholipids content in the sample taken:

$$\text{mmol}_{IS} = (W_{IS} \times C_{IS}) / (MW_{IS} \times 100)$$

mmol_{IS}

= millimoles of the *Internal standard* in the *Sample solution* (mmol)

W_{IS}

= weight of the *Internal standard* added to the *Sample solution* (mg)

C_{IS}

= purity value of the *Internal standard*, based on quantitative ^{31}P NMR analysis (% by weight)

MW_{IS}

= molecular weight of the *Internal standard* (326.28 g/mol for triphenyl phosphate)

$$\text{mmol}_{PL} = (I_{PL} \times A_{IS} \times \text{mmol}_{IS}) / (I_{IS} \times A_{PL})$$

mmol_{PL} = millimoles of the phospholipid of interest in the *Sample solution* (mmol)

I_{PL} = integrated area under the phospholipid signal of interest obtained from the spectrum of the *Sample solution*

A_{IS} = number of phosphorus atoms per molecule expected from the *Internal standard* (1 for triphenyl phosphate)

mmol_{IS} = millimoles of the *Internal standard* in the *Sample solution*

I_{IS} = integrated area under the *Internal standard* obtained from the spectrum of the *Sample solution*

A_{PL} = number of phosphorus atoms per molecule expected from the phospholipid of interest (1 for any phospholipid listed in *Table 3*)

$$C_{PL} = (MW_{PL} \times \text{mmol}_{PL} \times 100) / W_S$$

C_{PL} = concentration of the phospholipid of interest in the *Sample solution* (% w/w)

MW_{PL} = molecular weight of the phospholipid of interest (g/mol, from *Table 3*) in the *Sample solution* (mg)

mmol_{PL} = millimoles of the phospholipid of interest in the *Sample solution* (mmol)

W_S = weight of sample present in the *Sample solution* (mg)

[Note—Use the molecular weight specified in *Table 3* for the calculations.]

Table 3

Component	Approximate Chemical Shift (ppm) in Reference to Triphenyl Phosphate	Molecular Weight (g/mol)
Triphenyl phosphate (<i>Internal standard</i>)	-17.8	—
Phosphatidylcholine (PC)	-0.89	791
1-Lysophosphatidylcholine (1-LPC) ^a	-0.48	534.5
2-Lysophosphatidylcholine (2-LPC) ^a	-0.4	534.5
Phosphatidylethanolamine (PE)	-0.24	770
<i>N</i> -Acylphosphatidylethanolamine (NAPE)	0	1032
Lysophosphatidylethanolamine (LPE)	0.25	492.5
Other	—	800

^a Ability to resolve the signals of 1-lysophosphatidylcholine and 2-lysophosphatidylcholine will depend upon the applied magnetic field strength of the NMR spectrometer used for the test procedure.

■ 1S (USP39)

Acceptance criteria

Total phospholipids: 28%–52%

■ 30%–55% ■ 1S (USP39)

Phosphatidylcholine: 60%–90%

■ 96% ■ 1S (USP39)

(w/w) of the total phospholipids content

● Content of Astaxanthin

[Note—Perform this analysis in subdued light using low-actinic glassware.]

Sample solution: 0.005 g/mL in chloroform using the portion of oil from NLT 10 Capsules.

[Note—If the solution is not clear, centrifuge it with an appropriate centrifuge to obtain a clear supernatant.]

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* ⟨ 851 ⟩ .)

Analytical wavelength: 486 nm

Cell path: 1 cm

Blank: Chloroform

Analysis

Sample: *Sample solution*

Calculate the percentage of astaxanthin in the portion of oil taken from the Capsules:

$$\text{Result} = A/(F \times C)$$

A = absorbance of the *Sample solution*

F = coefficient of extinction ($E_{1\%}^1$) of pure astaxanthin in chloroform ($100 \text{ mL} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$),
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 C = concentration of the *Sample solution* (g/mL)

Acceptance criteria: NLT 0.01%

PERFORMANCE TESTS

- **Disintegration and Dissolution** 〈 2040 〉, *Rupture Test for Soft Shell Capsules*: Meet the requirements
- **Weight Variation** 〈 2091 〉: Meet the requirements

CONTAMINANTS

• **Limit of Dioxins, Furans, and Polychlorinated Biphenyls**

Analysis: Determine the content of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by Method 1613, Revision B, of the Environmental Protection Agency. Determine the content of polychlorinated biphenyls (PCBs) by Method 1668, Revision A, of the Environmental Protection Agency.

Acceptance criteria: The sum of PCDDs and PCDFs is NMT 2.0 pg/g of World Health Organization (WHO) toxic equivalents. The sum of PCDDs, PCDFs, and dioxin-like PCBs (polychlorinated biphenyls; non-ortho IUPAC congeners PCB-77, PCB-81, PCB-126, and PCB-169; and mono-ortho IUPAC congeners PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, and PCB-189) is NMT 10.0 pg/g of WHO toxic equivalents.

- **Microbial Enumeration Tests** 〈 2021 〉: The total aerobic microbial count does not exceed 10^3 cfu/g, and the combined molds and yeasts count does not exceed 10^2 cfu/g.
- **Absence of Specified Microorganisms** 〈 2022 〉: Meet the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

• **Astaxanthin Esterification**

Standard solution A: 10 mg/mL of USP Astaxanthin Esters from *Haematococcus pluvialis* RS in acetone

Standard solution B: 10 mg/mL of USP Astaxanthin (Synthetic) RS in acetone

Sample solution: Using the portion of oil from NLT 10 Capsules, prepare a solution of 250 mg/mL in acetone.

Chromatographic system

(See *Chromatography* 〈 621 〉, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel. [Note—Dry silica gel at 110° for 1 h before use.]

Application volume: 5 μL

Developing solvent system: Hexane and acetone (70:30)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has

moved about 15 cm of the length of the plate. Remove the plate from the chamber, and allow to dry.

Acceptance criteria: The principal spot of *Standard solution B*, located in the bottom half of the plate, is free astaxanthin. The *Sample solution* may exhibit a light, minor spot in the same location. The principal spots of *Standard solution A* are from monoesters (primary spot, located slightly above the middle of the plate) and diesters (secondary spot, located in the top third of the plate). The principal spot of the *Sample solution* should correspond in color and R_F value to the diester spot of *Standard solution A*. The secondary spot of the *Sample solution* should correspond in color and approximately the same R_F value to the monoester spot of *Standard solution A*. [Note—Slight differences in R_F values within monoester spots and within diester spots may exist because of different intensities.]

- **Fats and Fixed Oils** 〈 401 〉, *Peroxide Value*: NMT 5.0 mEq peroxide/kg

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at room temperature. Protect from light.
- **Labeling:** The label states the amount of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), total phospholipids, and astaxanthin.
- **USP Reference Standards** 〈 11 〉
 - USP Astaxanthin Esters from *Haematococcus pluvialis* RS
 - USP Astaxanthin (Synthetic) RS
 - USP Docosahexaenoic Acid Ethyl Ester RS
 - USP Eicosapentaenoic Acid Ethyl Ester RS
 - USP Krill Oil RS
 - USP Methyl Tricosanoate RS

BRIEFING

Krill Oil Delayed-Release Capsules, *USP 38* page 6121. On the basis of comments received and to be consistent with the recent revision to the *Krill Oil* monograph published in *PF 41(2)* [Mar.–Apr. 2015], the following changes are proposed:

1. Add the content of myristic acid and palmitic acid and the ratio of palmitic acid to myristic acid content to *Table 1* of the *Fatty Acid Profile* in the *Identification* section.
2. Change the upper limits and lower limits for several fatty acids listed in *Table 1* to reflect new data recently received.
3. Change the range of total phospholipids content from 28%–52% to 30%–55% and the range of phosphatidylcholine from 60%–90% (w/w) to 60%–96% (w/w) of the total phospholipids content in the *Acceptance criteria* for the *Content of Total Phospholipids* test.
4. Change the range of phosphatidylcholine from 60%–90% (w/w) to 60%–96% (w/w) of the total phospholipids content in *Identification* test *B—Phospholipid Profile*.
5. Add a table of molecular weight values and approximate chemical shift values (in reference to triphenyl phosphate) for the phospholipids of importance to krill oil analysis in the test for *Total Phospholipids* in order to clarify the proper use of the NMR data obtained. The values proposed are based on data and comments received. Also, equations are included for the calculations required by this test.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(DS: N. Davydova.)

Correspondence Number—C157671

Comment deadline: July 31, 2015

Krill Oil Delayed-Release Capsules

DEFINITION

Krill Oil Delayed-Release Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of Krill Oil where Krill Oil is the fixed oil extracted from Antarctic krill (*Euphausia superba* Dana) biomass by using a suitable extraction solvent.

IDENTIFICATION

Change to read:

- **A. Fatty Acid Profile**

Antioxidant solution, System suitability solution 1, and Chromatographic system:

Proceed as directed in *Fats and Fixed Oils* 〈401〉, *Omega-3 Fatty Acids Determination and Profile*.

Standard solution: Proceed as directed for *Test Solution 1* in *Fats and Fixed Oils* 〈401〉, *Omega-3 Fatty Acids Determination and Profile*, except use 250 mg of USP Krill Oil RS.

Sample solution: Using the portion of oil from NLT 10 Capsules, proceed as directed for *Test Solution 1* in *Fats and Fixed Oils* 〈401〉, *Omega-3 Fatty Acids Determination and Profile*.

System suitability

Samples: *System suitability solution 1* and *Standard solution*

Suitability requirements

Chromatogram similarity: The chromatogram from the *Standard solution* is similar to the reference chromatogram provided with the lot of USP Krill Oil RS being used.

Resolution: NLT 1.0 between the methyl oleate and methyl *cis*-vaccinate peaks, *Standard solution*

Theoretical area percentages: Meets the requirements for *System suitability solution 1*

Analysis

Sample: *Sample solution*

Identify the retention times of the relevant fatty acid methyl esters in the *Sample solution* by comparing the chromatogram of the *Sample solution* with that of the *Standard solution* and the USP reference chromatogram.

Calculate the area percentage for each fatty acid as methyl esters in the portion of oil taken from the Capsules:

$$\text{Result} = (r_A/r_B) \times 100$$

r_A peak area of each individual fatty acid from the *Sample solution*

$r_{\bar{r}}$ total area of all peaks, except the solvent and butylated hydroxytoluene peaks, from the *Sample solution*

Acceptance criteria: See Table 1.

Table 1

Fatty Acid	Short-Hand Notation	Lower Limit (Area %)	Upper Limit (Area %)
■ Saturated fatty acids			
Myristic acid	14:0	6.4	13.0
Palmitic acid	16:0	17.0	24.6
Palmitic acid: myristic acid ratio	16:0/14:0	1.6	2.8 ■ 1S (USP39)
Monounsaturated fatty acids			
Palmitoleic acid	16:1 n-7	2.5	9.0
			7.0
<i>cis</i> -Vaccenic acid	18:1 n-7	4.7	■ 8.0 ■ 1S (USP39)
Oleic acid	18:1 n-9	7.0	14.5
			1.2
Eicosenic acid	20:1 n-9	0.1	■ 1.7 ■ 1S (USP39)
			0.9
Erucic acid	22:1 n-9	0.0	■ 1.3 ■ 1S (USP39)
Polyunsaturated fatty acids			
Linoleic acid	18:2 n-6	1.4	3.0
			3.5
α-Linolenic acid	18:3 n-3	0.5	■ 5.0 ■ 1S (USP39)
			7.2
Moroctic acid	18:4 n-3	1.8	■ 10.0 ■ 1S (USP39)
			22.1
Eicosapentaenoic acid	20:5 n-3	14.0	■ 24.3 ■ 1S (USP39)
Docosapentaenoic acid	22:5 n-3	0.0	0.7
			7.5
Docosahexaenoic acid	22:6 n-3	■ 7.1 ■ 1S (USP39)	■ 15.7 ■ 1S (USP39)

Change to read:

● **B. Phospholipid Profile**

Solution A, Line shape standard (¹H), Sensitivity standard (¹H), Sensitivity standard (³¹P), Internal standard, Sample solution, Standard solution, Instrumental conditions, System suitability, and Analysis: Proceed as directed in the test for *Content of Total Phospholipids in Strength*.

Acceptance criteria: The *Sample solution* contains all of the following phospholipids: phosphatidylcholine (60%–90%

■ 96% ■ 1S (USP39)

(w/w) of the total phospholipids content), lysophosphatidylcholine, ~~(as a mixture of 1-lysophosphatidylcholine and 2-lysophosphatidylcholine),~~

■ ■ 1S (USP39)

and phosphatidylethanolamine.

STRENGTH

- **Content of Krill Oil:** Weigh NLT 10 Capsules in a tared weighing bottle, and carefully open the Capsules, without loss of shell material. Transfer the combined Capsule contents to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of 2,2,4-trimethylpentane. Discard the washings, and allow the empty Capsules to dry in a current of dry air until the 2,2,4-trimethylpentane is completely evaporated. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average net weight per Capsule.

Acceptance criteria: 95.0%–105.0%

- **Content of EPA and DHA**

Standard solution 1a, Standard solution 1b, Standard solution 2a, Standard solution 2b, System suitability solution 1, and **Chromatographic system:** Proceed as directed in *Fats and Fixed Oils* 〈401〉, *Omega-3 Fatty Acids Determination and Profile*.

Test solution 1: Using the portion of 250 mg of oil from NLT 10 Capsules, proceed as directed for *Test Solution 1* in *Fats and Fixed Oils* 〈401〉, *Omega-3 Fatty Acids Determination and Profile*.

Test solution 2: Using the portion of 250 mg of oil from NLT 10 Capsules, proceed as directed for *Test Solution 2* in *Fats and Fixed Oils* 〈401〉, *Omega-3 Fatty Acids Determination and Profile*.

Analysis: Proceed as directed for *Analysis (for triglycerides)* in *Fats and Fixed Oils* 〈401〉, *Omega-3 Fatty Acids Determination and Profile*.

Calculate the percentage of EPA and DHA in the portion of oil taken from the Capsules.

Acceptance criteria: NLT 10.0% (w/w) of EPA and NLT 5.0% (w/w) of DHA

Change to read:

- **Content of Total Phospholipids**

(See *Nuclear Magnetic Resonance Spectroscopy* 〈761〉, *Qualitative and Quantitative NMR Analysis*).

[Note—All deuterated solvents used in this method should be NLT 99.8 atom % D. Whenever water is used in this method, it should be of sufficient quality to ensure that no trace metals or other contaminants that may affect the analysis are present.]

Solution A: 0.2 M EDTA adjusted with a 1 M cesium carbonate solution to a pH of 7.2–7.5. Document the final pH and the amount of 1 M cesium carbonate solution necessary to attain the desired pH. [Note—Use cesium carbonate of a sufficient grade for trace metals analysis.]

Line shape standard (¹H): 1% Chloroform in acetone-d₆

Sensitivity standard (¹H): 0.1% Ethylbenzene in chloroform-d

Sensitivity standard (³¹P): 0.0485 M triphenyl phosphate in acetone-d₆

Internal standard: Use triphenyl phosphate NMR reference standard with NLT 99% purity.

Sample solution: [Note—NMR solvents containing tetramethylsilane (TMS) are readily available. If the solvents used do not contain TMS, it must be added to the *Sample*

solution at an approximate concentration of 0.05% (v/v) for use as a chemical shift scale reference.]

Transfer the portion of 300–350 mg of oil from NLT 10 Capsules to a 5-mL sealable glass vial. Add 25.0 mg of the *Internal standard* to the vial. Add 1 mL each of deuterated chloroform (chloroform-d) and deuterated methanol (methanol-d₄) of a grade suitable for NMR analysis to the vial to dissolve the sample. Once dissolution is complete, add 1 mL of *Solution A*, seal the vial, and shake the solution for 10–20 min, then centrifuge the contents of the vial. Transfer the lower organic phase to an appropriate NMR tube. It is critical to collect the entire organic phase and transfer it to the NMR tube. It may be unavoidable to also transfer small amounts of the aqueous phase when collecting the organic phase in the NMR tube. This is an acceptable practice, so long as the aqueous phase remains completely separated and atop the organic phase in the NMR tube. The entire amount of aqueous phase must be above the probe's radio frequency (RF) coil (outside the analysis area of the tube). Should the organic phase contain undissolved materials, they must remain suspended at the aqueous-organic interface and be outside the analysis area of the tube as well. The organic phase must be free of bubbles and suspended materials that may interfere with NMR data acquisition.

Standard solution: Prepare as directed in the *Sample solution*, except use 300–350 mg of USP Krill Oil RS.

Instrumental conditions

(See 〈 761 〉.)

Magnetic field strength: NLT 300 MHz for ¹H frequency

Probe: Direct observe probe capable of tuning to the resonance frequency of ³¹P (dependent on the specific magnetic field strength used)

Instrument performance qualification

[Note—Testing for sensitivity and line shape should be performed on the interval specified by the manufacturer of the instrument used. Performing these tests on a minimum of a monthly basis is required for this method, but it may be done more often, as required. Resolution testing is to be performed during each analysis and documented as a part of the analytical results.]

¹H Line shape test: Using the *Line shape standard* (¹H) and the protocol recommended by the instrument manufacturer, the instrument must achieve the line shape specifications for the probe in use, as required by the instrument manufacturer. [Note—A different standard solution may be required or recommended by the manufacturer of the instrument; 1% chloroform in acetone-d₆ is most commonly used.]

¹H Sensitivity test: Using the *Sensitivity standard* (¹H) and the protocol recommended by the instrument manufacturer, the instrument must achieve the sensitivity specifications required by the instrument manufacturer. [Note—A different standard solution may be required or recommended by the manufacturer of the instrument; 0.1% ethylbenzene in chloroform-d is most commonly used.]

³¹P Sensitivity test: Using the *Sensitivity standard* (³¹P) and the protocol recommended by the instrument manufacturer, the instrument must achieve the sensitivity specifications required by the instrument manufacturer. [Note—A different standard solution may be required or recommended by the manufacturer of the instrument; 0.0485 M triphenyl phosphate in acetone-d₆ is most commonly used.]

¹H Resolution test: The resolution is demonstrated by the ability to detect both of the

^{29}Si satellite signals of TMS. The satellites must be resolved from the TMS signal in the spectrum with a line-broadening factor of NMT 0.5 ppm.

^{31}P Resolution test: The resolution is demonstrated using the phosphatidylcholine ether peak and the phosphatidylcholine peak. The separation of these peaks (with a line-broadening factor of 1.0) must be demonstrated as follows. Using the baseline as a reference, determine the total peak height of the phosphatidylcholine ether peak, ■ [Note—The PC ether signal appears just downfield from the PC signal.] ■ 1S (USP39)

and draw a line at 30% of that total peak height (intensity). The phosphatidylcholine ether peak and the neighboring phosphatidylcholine peak must be fully resolved at a point that is NMT 30% of the peak height of the phosphatidylcholine ether peak.

Data collection: Use the parameters specified in *Table 2*. Use 90 degree pulses, and calibrate pulses before use according to the recommendations supplied by the instrument manufacturer.

Table 2

Parameter	^{31}P NMR Quantitative Measurement	^1H NMR Qualitative Measurement
Pulse program	^1H -decoupled ^{31}P ■ (inverse gated) ■ 1S (USP39)	Single pulse ^1H
Spectral width	50 ppm (25 to -25 ppm)	20 ppm (-3 to 17 ppm)
Transmitter offset	Center of spectral width, 0 ppm	Center of spectral width, 7 ppm
Relaxation delay	2–5 s ■ 5–15 s ■ 1S (USP39)	2–5 s
Acquisition time	1–6 s	1–6 s
Sum of relaxation delay and acquisition time	NLT 15 s	NLT 15 s ■ 1S (USP39)
Size of data set	NLT 64k (32k with zero-filling)	NLT 64k (32k with zero-filling)

[Note—The acquisition time is dependent upon the field strength and the time domain ■ the dwell time and the number of data points collected. ■ 1S (USP39)

The number of scans acquired using a 300-MHz instrument must be NLT 512.]

System suitability: Under the conditions outlined in *Data collection*, the ^{31}P NMR signal of triphenyl phosphate should be observed at -17.80 ppm, and the ^1H NMR spectrum should be referenced to the ^1H signal of TMS (0 ppm) for all spectra acquired in the *Analysis*. For quantitative analysis, a sufficient number of scans should be acquired such that the signal-to-noise ratio for the phosphatidylcholine signal in the ^{31}P spectrum of the *Sample solution* acquired in the *Analysis* is NLT 2000.

Analysis: Acquire the data outlined in *Data collection*. Minimally acquire the ^1H spectrum (fingerprint) of the *Sample solution* and the *Standard solution* as well as the quantitative ^{31}P spectrum of the *Sample solution* and the *Standard solution*. Record the resulting spectra, and

perform integration by hand or automated means on the quantitative ^{31}P NMR spectrum of the *Sample solution*. The integration of the peaks in the spectrum of the *Sample solution* must be performed such that the complete set of phospholipid peaks (as identified by a comparison to the spectrum of the *Standard solution* and the *Standard solution* reference spectrum) is included in the integration. The integration region for each signal must extend ± 0.05 ppm on either side of the ^{31}P signal. Quantify the total phospholipids present, the phosphatidylcholine ether content, and the phosphatidylcholine content in the *Sample solution* by using a comparison to the concentration of the *Internal standard*.

Compare the ^1H spectrum of the *Sample solution* to that of the *Standard solution* to determine the similarity of fingerprints according to which phospholipids identified in the reference spectrum of the *Standard solution* are present in the spectrum of the *Sample solution*.

■ **Calculations:** Use the following equations and molecular weights listed in *Table 3* to determine the phospholipids content in the sample taken:

$$\text{mmol}_{IS} = (W_{IS} \times C_{IS}) / (MW_{IS} \times 100)$$

mmol_{IS}

= millimoles of the *Internal standard* in the *Sample solution* (mmol)

W_{IS}

= weight of the *Internal standard* added to the *Sample solution* (mg)

C_{IS}

= purity value of the *Internal standard*, based on quantitative ^{31}P NMR analysis (% by weight)

MW_{IS}

= molecular weight of the *Internal standard* (326.28 g/mol for triphenyl phosphate)

$$\text{mmol}_{PL} = (I_{PL} \times A_{IS} \times \text{mmol}_{IS}) / (I_{IS} \times A_{PL})$$

mmol_{PL} = millimoles of the phospholipid of interest in the *Sample solution* (mmol)

I_{PL} = integrated area under the phospholipid signal of interest obtained from the spectrum of the *Sample solution*

A_{IS} = number of phosphorus atoms per molecule expected from the *Internal standard* (1 for triphenyl phosphate)

mmol_{IS} = millimoles of the *Internal standard* in the *Sample solution*

I_{IS} = integrated area under the *Internal Standard* obtained from the spectrum of the *Sample solution*

A_{PL} = number of phosphorus atoms per molecule expected from the phospholipid of interest (1 for any phospholipid listed in *Table 3*)

$$C_{PL} = (MW_{PL} \times \text{mmol}_{PL} \times 100) / W_S$$

C_{PL} = concentration of the phospholipid of interest in the *Sample solution* (% w/w)

MW_{PL} = molecular weight of the phospholipid of interest (g/mol, from *Table 3*) in the *Sample solution* (mg)

$mmol_{PL}$ = millimoles of the phospholipid of interest in the *Sample solution* (mmol)

W_S = weight of sample present in the *Sample solution* (mg)

[Note—Use the molecular weight specified in *Table 3* for the calculations.]

Table 3

Component	Approximate Chemical Shift (ppm) in Reference to Triphenyl Phosphate	Molecular Weight (g/mol)
Triphenyl phosphate (<i>Internal standard</i>)	−17.8	—
Phosphatidylcholine (PC)	−0.89	791
1-Lysophosphatidylcholine (1-LPC) ^a	−0.48	534.5
2-Lysophosphatidylcholine (2-LPC) ^a	−0.4	534.5
Phosphatidylethanolamine (PE)	−0.24	770
<i>N</i> -Acylphosphatidylethanolamine (NAPE)	0	1032
Lysophosphatidylethanolamine (LPE)	0.25	492.5
Other	—	800

^a Ability to resolve the signals of 1-lysophosphatidylcholine and 2-lysophosphatidylcholine will depend upon the applied magnetic field strength of the NMR spectrometer used for the test procedure.

■ 1S (USP39)

Acceptance criteria

Total phospholipids: 28%–52%

■ 30%–55% ■ 1S (USP39)

Phosphatidylcholine: 60%–90%

■ 96% ■ 1S (USP39)

(w/w) of the total phospholipids content

● Content of Astaxanthin

[Note—Perform this analysis in subdued light using low-actinic glassware.]

Sample solution: 0.005 g/mL in chloroform using the portion of oil from NLT 10 Capsules.

[Note—If the solution is not clear, centrifuge it with an appropriate centrifuge to obtain a clear supernatant.]

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* 〈 851 〉.)

Analytical wavelength: 486 nm

Cell path: 1 cm

Blank: Chloroform

Analysis

Sample: *Sample solution*

Calculate the percentage of astaxanthin in the portion of oil taken from the Capsules:

$$\text{Result} = A/(F \times C)$$

A = absorbance of the *Sample solution*

F = coefficient of extinction ($E_{1\%}^{1\text{cm}}$) of pure astaxanthin in chloroform (100 mL·g₋₁·cm₋₁),
1692

C = concentration of the *Sample solution* (g/mL)

Acceptance criteria: NLT 0.01%

PERFORMANCE TESTS

- **Disintegration and Dissolution** 〈 2040 〉, *Disintegration, Delayed-release (enteric-coated) soft shell capsules*: Meet the requirements
- **Weight Variation** 〈 2091 〉: Meet the requirements

CONTAMINANTS

- **Limit of Dioxins, Furans, and Polychlorinated Biphenyls**

Analysis: Determine the content of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by Method 1613, Revision B, of the Environmental Protection Agency. Determine the content of polychlorinated biphenyls (PCBs) by Method 1668, Revision A, of the Environmental Protection Agency.

Acceptance criteria: The sum of PCDDs and PCDFs is NMT 2.0 pg/g of World Health Organization (WHO) toxic equivalents. The sum of PCDDs, PCDFs, and dioxin-like PCBs (polychlorinated biphenyls; non-ortho IUPAC congeners PCB-77, PCB-81, PCB-126, and PCB-169; and mono-ortho IUPAC congeners PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, and PCB-189) is NMT 10.0 pg/g of WHO toxic equivalents.

- **Microbial Enumeration Tests** 〈 2021 〉: The total aerobic microbial count does not exceed 10³ cfu/g, and the combined molds and yeasts count does not exceed 10² cfu/g.
- **Absence of Specified Microorganisms** 〈 2022 〉: Meet the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

- **Astaxanthin Esterification**

Standard solution A: 10 mg/mL of USP Astaxanthin Esters from *Haematococcus pluvialis* RS in acetone

Standard solution B: 10 mg/mL of USP Astaxanthin (Synthetic) RS in acetone

Sample solution: Using the portion of oil from NLT 10 Capsules, prepare a solution of 250 mg/mL in acetone.

Chromatographic system

(See *Chromatography* 〈 621 〉, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel. [Note—Dry silica gel at 110° for 1 h before use.]

Application volume: 5 µL

Developing solvent system: Hexane and acetone (70:30)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about 15 cm of the length of the plate. Remove the plate from the chamber, and allow to dry.

Acceptance criteria: The principal spot of *Standard solution B*, located in the bottom half of the plate, is free astaxanthin. The *Sample solution* may exhibit a light, minor spot in the same location. The principal spots of *Standard solution A* are from monoesters (primary spot, located slightly above the middle of the plate) and diesters (secondary spot, located in the top third of the plate). The principal spot of the *Sample solution* should correspond in color and R_F value to the diester spot of *Standard solution A*. The secondary spot of the *Sample solution* should correspond in color and approximately the same R_F value to the monoester spot of *Standard solution A*. [Note—Slight differences in R_F values within monoester spots and within diester spots may exist because of different intensities.]

- **Fats and Fixed Oils** 〈 401 〉, *Peroxide Value*: NMT 5.0 mEq peroxide/kg

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at room temperature. Protect from light.
- **Labeling:** The label states the amount of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), total phospholipids, and astaxanthin.
- **USP Reference Standards** 〈 11 〉
 - USP Astaxanthin Esters from *Haematococcus pluvialis* RS
 - USP Astaxanthin (Synthetic) RS
 - USP Docosahexaenoic Acid Ethyl Ester RS
 - USP Eicosapentaenoic Acid Ethyl Ester RS
 - USP Krill Oil RS
 - USP Methyl Tricosanoate RS

BRIEFING

Lecithin, *NF 33* page 6726. On the basis of comments and data received, it is proposed to make the following changes:

1. In the test for *Content of Phospholipids*, add the *Acceptance criteria* for *Content of phosphatidylcholine*. This proposed specification represents the quality of a lecithin product in commerce that is intended for use in the manufacture of injectable dosage forms.
2. In the test for *Content of Acetone-Insoluble Matter*, due to variability of the test

procedure, change the *Acceptance criteria* for Lecithin intended for use in the manufacture of injectable dosage forms from NLT 90.0% to NLT 80.0%.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC: H. Wang.)

Correspondence Number—C155497

Comment deadline: July 31, 2015

Lecithin

[8002-43-5].

DEFINITION

Lecithin is a complex mixture of acetone-insoluble phosphatides, which consist chiefly of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid, present in conjunction with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates, as separated from the crude vegetable oil source. The content of each of the phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid) is indicated on the certificate of analysis.

IDENTIFICATION

• **A. Identification of Phospholipids by Thin-Layer Chromatography**

Mobile phase: Chloroform, methanol, water (65:25:4, v/v/v)

Standard solution A: 10 mg/mL of USP Phosphatidic Acid (Soy) Monosodium RS and 10 mg/mL of USP Phosphatidylcholine (Soy) RS in *Mobile phase*

Standard solution B: 7 mg/mL of USP Phosphatidylethanolamine (Soy) RS and 7 mg/mL of USP Lysophosphatidylcholine (Soy) RS in *Mobile phase*

Sample solution: 20 mg/mL of Lecithin in *Mobile phase*

Chromatographic system

(See *Chromatography* { 621 }, *Thin-Layer Chromatography*.)

Mode: TLC

Plate: 20-cm × 20-cm, silica gel 60 on aluminum foil, 0.2-mm layer

Application volume: 20 µL

Spray reagent: Dilute 80 mL of phosphoric acid (85%) with 600 mL of water in a 1-L volumetric flask. [Note—Add water to the flask first.] While stirring, add 100 g of anhydrous cupric sulfate. After stirring for 10 min, most of the cupric sulfate is dissolved. Add water to volume and continue stirring until the solid completely dissolves.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Fill the chromatography chamber with the *Mobile phase* to a height of about 0.5 cm. Place a fat-free, U-shaped filter paper in the glass trough and press it against the wall. Sufficient saturation is reached once the *Mobile phase* has permeated to the upper rim of the filter paper. Apply the *Samples* in different bands to the previously marked starting point on a TLC plate. Place the TLC plate in the saturated chromatography chamber. When the *Mobile phase* front has reached the mark (12 cm above the starting point),

remove the TLC plate, and dry it using a dryer. Spray or immerse the TLC plate in the *Spray reagent*, and dry it again with a dryer (a current of hot air). Heat the plate to 170° for 10 min. Develop all lipids by charring as dark brown spots.

Acceptance criteria: The R_F values of the spots for phosphatidylcholine, phosphatidylethanolamine, phosphatidic acid, and lysophosphatidylcholine from the *Sample solution* correspond to those from *Standard solution A* and *Standard solution B*. [Note—Depending on the sample tested, if a phospholipid component presents in a low amount in the sample, the corresponding spot in the *Sample solution* on the TLC may not be visualized.]

ASSAY

Change to read:

• Content of Phospholipids

[Note—Perform the test for lysophosphatidylcholine for Lecithin intended for use in the manufacture of injectable dosage forms.]

Solution A: Mix 1342 g (2.0 L) of *n*-hexane, 334.1 g (425 mL) of isopropanol (2-propanol), 39.4 g (38 mL) of glacial acetic acid, and 2.0 mL of triethylamine.

Solution B: Mix 663.5 g (850 mL) of isopropanol, 15.8 g (15 mL) of glacial acetic acid, 140 g (140 mL) of water, and 0.8 mL of triethylamine.

Solvent: *n*-Hexane, isopropanol, and water (46:46:8, v/v/v). [Note—To avoid the formation of two phases, mix the isopropanol and water first, and then add the *n*-hexane.]

Mobile phase: See *Table 1*.

Table 1

Program Step	Time (min)	Flow Rate (mL/min)	Solution A (%)	Solution B (%)
1	0	1.0	95	5
2	5.0	1.0	80	20
3	8.5	1.0	60	40
4	14.0	1.0	55	45
5	15.0	1.0	0	100
6	17.5	1.0	0	100
7	17.6	1.0	95	5
8	21.0	1.0	95	5
9	22.0	2.0	95	5
10	27.0	2.0	95	5
11	29.0	1.0	95	5

Phospholipids standard stock solution (2X): 0.8 mg/mL of USP Phosphatidylcholine (Soy) RS, 0.4 mg/mL of USP Phosphatidylethanolamine (Soy) RS, 0.4 mg/mL of phosphatidylinositol prepared from USP Phosphatidylinositol (Soy) Sodium RS, and 0.2 mg/mL of phosphatidic acid prepared from USP Phosphatidic Acid (Soy) Monosodium RS in *Solvent*. [Note—Due to the highly hydroscopic nature of phospholipids, take special precaution in the Standard preparation.]

Phospholipids standard solutions: Prepare as directed in *Table 2*.

Table 2

Concentration	Phospholipids Standard Stock Solution (2X): Solvent (v/v)
0.6X	3:7
0.8X	4:6
1.0X	5:5
1.2X	6:4
1.4X	7:3

System suitability solution: *Phospholipids standard solution 1.0X*

Lysophosphatidylcholine standard stock solution (2X): 60 µg/mL of USP Lysophosphatidylcholine (Soy) RS in *Solvent*

Resolution solution: *Phospholipids standard stock solution (2X)* and *Lysophosphatidylcholine standard stock solution (2X)* (1:1)

Lysophosphatidylcholine standard solution: 30 µg/mL of USP Lysophosphatidylcholine (Soy) RS in *Solvent*

Sample solution: 1 mg/mL of Lecithin in *Solvent*. [Note—If necessary, adjust the concentration of the *Sample solution* to obtain the concentration of each of the phospholipids within the calibration range.]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Evaporative light-scattering

Column: 4-mm × 12.5-cm; 5-µm packing L20

Temperatures

Detector: 50°

Column: 55°

Flow rate: 1.0 mL/min with step gradient at 2.0 mL/min (see *Table 1*)

Injection volume: 20 µL

[Note—Depending on the different settings of the *Detector*, the *Detector* temperature and *Flow rate* can be adjusted as long as system suitability requirements are met.]

System suitability

Samples: *System suitability solution* and *Resolution solution*

[Note—The relative retention times for phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, and lysophosphatidylcholine are 0.4, 0.9, 1.0, 1.2, and 1.3, respectively, for the *Resolution solution*.]

Suitability requirements

Resolution: NLT 2.0, *System suitability solution*

Relative standard deviation: NMT 5.0%, *System suitability solution*

Analysis

Samples: *Phospholipids standard solutions*, *Lysophosphatidylcholine standard solution*, and *Sample solution*

Identify the peaks of the relevant phospholipids from the *Sample solution* by comparison with the *Phospholipids standard solutions*. Measure the areas of the phospholipid peaks. Plot the logarithms of the relevant responses versus the logarithms of the concentrations, in mg/mL, of each of the phospholipids from the *Standard solutions*, and determine the

linear regression line using a least-squares analysis. The correlation coefficient for the linear regression line is NLT 0.995.

From the graphs, determine the concentration (C), in mg/mL, of the relevant phospholipid in the *Sample solution*.

Calculate the percentage of each of the phospholipids (phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol) in the portion of Lecithin taken:

$$\text{Result} = (C_U/C_S) \times 100$$

C_U concentration of each of the phospholipids in the *Sample solution* (mg/mL)

C_S concentration of Lecithin (mg/mL)

Based on the *Lysophosphatidylcholine standard solution*, identify the peak of lysophosphatidylcholine. Compare the peak area of lysophosphatidylcholine from the *Lysophosphatidylcholine standard solution* and the *Sample solution*, respectively.

Acceptance criteria

Content of each of the phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid): Within the respective ranges stated on the label

For Lecithin intended for use in the manufacture of injectable dosage forms

Content of lysophosphatidylcholine: The peak area of lysophosphatidylcholine in the *Sample solution* is NMT the peak area of lysophosphatidylcholine in the *Lysophosphatidylcholine standard solution*, corresponding to NMT 3.0% of lysophosphatidylcholine in Lecithin.

■ **Content of phosphatidylcholine:** NLT 70.0% ■ 1S (NF34)

IMPURITIES

Delete the following:

●● **Heavy Metals, Method II** (231): NMT 20 ppm ● (Official 1-Dec-2015)

● **Lead** (251): NMT 10 ppm

● **Hexane-Insoluble Matter**

Sample: If the substance under test is plastic or semisolid, soften the Lecithin by warming it at a temperature not exceeding 60°, and then mix. Weigh 10.0 g into a 250-mL conical flask.

Analysis: To the *Sample* add 100 mL of hexane. Shake until solution is apparently complete or until no more residue seems to be dissolving. Pass through a coarse-porosity filtering funnel that previously has been heated at 105° for 1 h, cooled, and weighed. Wash the flask with two 25-mL portions of hexane, and pour both washings through the funnel. Dry the funnel at 105° for 1 h. [**Caution**—Hexane is flammable.] Cool to room temperature, and determine the gain in weight.

Acceptance criteria: NMT 0.3%

For Sunflower Lecithin: NMT 1.0%

SPECIFIC TESTS**Change to read:**

- **Content of Acetone-Insoluble Matter**

Sample: If the substance under test is plastic or semisolid, soften the Lecithin by warming it briefly at a temperature not exceeding 60°, and then mix. Transfer 2 g to a 40-mL centrifuge tube that previously has been tared along with a stirring rod, cool, and weigh.

Analysis: To the *Sample* add 15.0 mL of acetone, and warm carefully in a water bath to melt the test specimen without evaporating the acetone. Stir to help dissolve completely, and place in an ice-water bath for 5 min. De-oiled lecithin and fractions are suspended in acetone by stirring. Add acetone that has been previously chilled to 0°–5° to the 40-mL mark on the tube, stirring during the addition. Cool in an ice-water bath for 15 min, stir, remove the rod, clarify by centrifuging at about 2000 rpm for 5 min, and decant. Break up the residue with the stirring rod, and refill the centrifuge tube to the 40-mL mark with chilled acetone, while stirring. Cool in an ice-water bath for 15 min, stir, remove the rod, centrifuge, and decant. Break up the residue with the stirring rod. Place the tube in a horizontal position until most of the acetone has evaporated. Mix again, and heat the tube containing the acetone-insoluble residue and the stirring rod at 105° to constant weight. [Caution—Acetone is flammable.]

Determine the weight of the residue, and calculate the percentage of acetone-insoluble matter.

Acceptance criteria: NLT 50.0%

For Lecithin intended for use in the manufacture of injectable dosage forms: NLT 90.0%

■ NLT 80.0% ■ 1S (NF34)

- **Fats and Fixed Oils** 〈 401 〉, *Acid Value*

Sample: If the substance under test is plastic or semisolid, soften the Lecithin by warming it briefly at a temperature not exceeding 60°, and then mix. Transfer 2 g to a 250-mL conical flask.

Analysis: Dissolve the *Sample* in 50 mL of petroleum ether with 100°–120° boiling range. To this solution add 50 mL of alcohol, previously neutralized to phenolphthalein with 0.1 N sodium hydroxide, and mix. Add phenolphthalein TS. Titrate with 0.1 N sodium hydroxide VS to a pink endpoint that persists for 5 s.

Calculate the amount, in mg, of potassium hydroxide required to neutralize the free acids in 1.0 g of Lecithin:

$$\text{Result} = (M_r \times N \times V) / W$$

M_r = molecular weight of potassium hydroxide, 56.11

N = normality of the sodium hydroxide VS

V = volume of the sodium hydroxide VS consumed in the titration (mL)

W = weight of Lecithin taken (g)

Acceptance criteria: NMT 36

- **Peroxide Value**

Sample: 5 g of Lecithin

Analysis: Transfer the *Sample* into a 250-mL Erlenmeyer flask with a ground-glass stopper, add 35 mL of a mixture of chloroform and glacial acetic acid (2:1), and mix. Completely dissolve the test specimen while shaking gently. The solution becomes transparent. Completely replace the air in the flask with nitrogen. While purging with nitrogen, add 1 mL of potassium iodide solution (165 mg/mL of potassium iodide), then stop the flow of the nitrogen, and immediately place a stopper in the flask. Shake for 1 min, and allow to stand in a dark place for 5 min. Add 75 mL of water, replace the stopper again, and shake vigorously. Titrate with 0.01 N sodium thiosulfate VS, adding starch TS as the endpoint is approached, and continue the titration until the blue starch color has just disappeared.

Perform a blank determination (see *Titrimetry* 〈 541 〉), and make any necessary correction.

Calculate the peroxide value, as mEq of peroxide per 1000 g of Lecithin:

$$\text{Result} = (S \times N/W) \times 1000$$

S = net volume of sodium thiosulfate solution required for titration (mL)

N = normality of the sodium thiosulfate solution

W = weight of Lecithin taken (g)

Acceptance criteria: NMT 10

For Lecithin intended for use in the manufacture of injectable dosage forms: NMT 3

- **Microbial Enumeration Tests** 〈 61 〉 **and Tests for Specified Microorganisms** 〈 62 〉: The total aerobic microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g. It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.
- **Water Determination** 〈 921 〉, *Method I*: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers. Store at the temperature indicated on the label. Protect from excess heat and moisture.
- **Labeling:** Label to indicate the content of each of the phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid). The labeling also indicates the natural source of lecithin. Where Lecithin is intended for use in the manufacture of injectable dosage forms, it is so labeled. Label it to indicate the storage conditions.
- **USP Reference Standards** 〈 11 〉
 - USP Lysophosphatidylcholine (Soy) RS
 - USP Phosphatidic Acid (Soy) Monosodium RS
 - USP Phosphatidylcholine (Soy) RS
 - USP Phosphatidylethanolamine (Soy) RS
 - USP Phosphatidylinositol (Soy) Sodium RS

BRIEFING

Abacavir and Lamivudine Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph is proposed based on validated methods of analysis. The liquid chromatographic procedures used in the *Assay* and the test for *Organic Impurities* are based on analyses performed using the Waters Xterra MS C18 brand of L1 column. The

typical retention times for abacavir and lamivudine are about 7 and 11 min, respectively.

The calculations in the test for *Dissolution* are performed using multi-component analysis software typically used for analysis of multi-component mixtures.

(SM1: S. Shivaprasad.)

Correspondence Number—C120789

Comment deadline: July 31, 2015

Add the following:

■ Abacavir and Lamivudine Tablets

DEFINITION

Abacavir and Lamivudine Tablets contain an amount of abacavir sulfate and lamivudine equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of abacavir ($C_{14}H_{18}N_6O$) and NLT 90.0% and NMT 110.0% of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$), respectively.

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Diluent: 0.1 N hydrochloric acid

Solution A: Water and trifluoroacetic acid (2000:1)

Solution B: Acetonitrile, methanol, and trifluoroacetic acid (1000:1000:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
12	70	30
12.1	40	60
13.1	40	60
13.2	100	0

System suitability solution: Dissolve the contents of 1 vial of USP Lamivudine Resolution Mixture C RS in 2.5 mL of *Diluent*. [Note—1 vial of USP Lamivudine Resolution Mixture C RS contains 0.8 mg of USP Lamivudine Resolution Mixture C RS.]

Standard solution: 0.35 mg/mL of USP Abacavir Sulfate RS and 0.15 mg/mL of USP Lamivudine RS in *Diluent*. Sonicate to dissolve prior to final dilution.

Sample stock solution: Nominally 3 mg/mL of abacavir and 1.5 mg/mL of lamivudine in *Diluent* prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask. Add *Diluent* to about 50% of the final volume and shake for NMT 30 min to disperse the Tablets. Dilute with *Diluent* to volume. Pass through a suitable filter.

Sample solution: Nominally 0.3 mg/mL of abacavir and 0.15 mg/mL of lamivudine in *Diluent*

from *Sample stock solution*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV, 270 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for lamivudine-*S*-oxide and lamivudine-*R*-oxide, in relation to the lamivudine peak, are 0.31 and 0.36, respectively; the relative retention times for lamivudine-diastereomer and lamivudine are 0.95 and 1.0, respectively; *System suitability solution*.]

Suitability requirements

Resolution: NLT 1.0 between lamivudine-*S*-oxide and lamivudine-*R*-oxide; NLT 1.0 between lamivudine diastereomer and lamivudine, *System suitability solution*

Relative standard deviation: NMT 1.5% each for abacavir and lamivudine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of abacavir (C₁₄H₁₈N₆O) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of abacavir from the *Sample solution*

r_S = peak response of abacavir from the *Standard solution*

C_S = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of abacavir in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of abacavir multiplied by 2, 572.66

M_{r2} = molecular weight of abacavir sulfate, 670.74

Acceptance criteria: 90.0%–110.0% of the labeled amount of abacavir

Calculate the percentage of the labeled amount of lamivudine (C₈H₁₁N₃O₃S) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lamivudine from the *Sample solution*

r_S = peak response of lamivudine from the *Standard solution*

C_S = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lamivudine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of lamivudine

PERFORMANCE TESTS

- **Dissolution** 〈 711 〉

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 75 rpm

Time: 30 min

Standard solution 1: 0.79 mg/mL of USP Abacavir Sulfate RS in *Medium*. Sonicate to dissolve prior to final dilution.

Standard solution 2: 0.33 mg/mL of USP Lamivudine RS in *Medium*. Sonicate to dissolve prior to final dilution.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

Mode: UV

Wavelength: 240–320 nm

Cell length: 0.2 mm

Blank: *Medium*

Analysis: The calculations of the percentages dissolved are performed using multi-component analysis software.

Tolerances: NLT 80% (Q) of the labeled amount of abacavir and lamivudine is dissolved.

- **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

IMPURITIES

- **Organic Impurities**

Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample stock solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual abacavir related impurity and each unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_{\bar{U}}$ peak response of each abacavir related impurity or unspecified impurity

r_T sum of the peak responses of abacavir, all abacavir related impurities, and all unspecified impurities

Calculate the percentage of each lamivudine related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_{\bar{U}}$ peak response of each lamivudine related impurity

r_T sum of the peak responses of lamivudine and all lamivudine related impurities

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cytosine ^a	0.12	0.2
Lamivudine- <i>S</i> -sulfoxide ^b	0.19	0.2
Lamivudine- <i>R</i> -sulfoxide ^c	0.21	0.2
Lamivudine-carboxylic acid ^d	0.49	— ^e
Lamivudine diastereomer (Lamivudine- <i>trans</i>) ^f	0.52	— ^e
Lamivudine	0.60	—
Lamivudine-uracil derivative ^g	0.78	0.2
Cyclopropyldiaminopurine abacavir ^h	0.80	0.2
Descyclopropyl abacavir ⁱ	0.85	0.2
3-Hydroxyabacavir ^j	0.89	— ^e
Abacavir	1.0	— ^e
Any individual unspecified impurity	—	0.2
Total lamivudine related impurities ^k	—	0.6
Total abacavir related impurities ^l	—	1.0
^a 4-Aminopyrimidin-2(1 <i>H</i>)-one (Lamivudine related impurity).		

^b 1-[(2*R*,3*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.

^c 1-[(2*R*,3*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.

^d (2*RS*,5*SR*)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.

^e Process impurity monitored in the drug substance.

^f 1-[(2*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.

^g 1-[(2*RS*,5*SR*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]uracil.

^h *N*⁶-Cyclopropyl-9*H*-purine-2,6-diamine.

ⁱ [(1*S*,4*R*)-4-(2,6-Diamino-9*H*-purin-9-yl)cyclopent-2-enyl]methanol.

^j (1*R*,2*R*,4*S*)-2-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-4-(hydroxymethyl)cyclopentan-1-ol.

^k Includes all lamivudine related impurities.

^l Includes all abacavir related and all individual unspecified impurities.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

- **USP Reference Standards** 〈 11 〉

USP Abacavir Sulfate RS

USP Lamivudine RS

USP Lamivudine Resolution Mixture C RS

This is a mixture of Lamivudine and the following impurities (other impurities may also be present).

Uracil, Pyrimidine-2,4(1*H*,3*H*)-dione.

C₄H₄N₂O₂ 112.09

Lamivudine-uracil derivative, 1-[(2*RS*,5*SR*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]uracil.

C₈H₁₀N₂O₄S 230.24

Cytosine, 4-Aminopyrimidin-2(1*H*)-one.

C₄H₅N₃O 111.10

Lamivudine-*S*-sulfoxide, 1-[(2*R*,3*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.

C₈H₁₁N₃O₄S 245.26

Lamivudine-*R*-sulfoxide, 1-[(2*R*,3*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.

C₈H₁₁N₃O₄S 245.26

Lamivudine carboxylic acid, (2*RS*,5*SR*)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.

C₈H₉N₃O₄S 243.24

Lamivudine diastereomer, 1-[(2*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.

C₈H₁₁N₃O₃S 229.26

Salicylic acid, 2-Hydroxybenzoic acid.

C₇H₆O₃ 138.12

■ 1S (USP39)

BRIEFING

Acetazolamide, USP 38 page 2041. As part of the USP monograph modernization effort, it is proposed to make the following changes:

1. The nonspecific wet chemistry *Identification* test *B* is replaced with a retention time agreement based on the *Assay*.
2. The IR *Assay* is replaced with an HPLC *Assay* based on the current USP monograph for *Acetazolamide Tablets*. The liquid chromatography procedure for the *Assay* is based on analysis performed with the Phenomenex Nucleosil C18 100 **A** brand of L1 column. The typical retention time for acetazolamide is about 8 min.
3. The nonspecific tests for *Silver-Reducing Substances* and *Ordinary Impurities* are replaced with an HPLC test for *Organic Impurities* based on the current monograph for *Acetazolamide* in the *European Pharmacopoeia*. The liquid chromatography procedure in the test for *Organic Impurities* is based on analysis performed with the Phenomenex Synergi Polar RP brand of L11 column. The typical retention time for

acetazolamide is about 8 min.

- The new Reference Standards, USP Acetazolamide Related Compound D RS and USP Acetazolamide Related Compound E RS, are added in the *USP Reference Standards* section to support the proposed revision to the test for *Organic Impurities*.

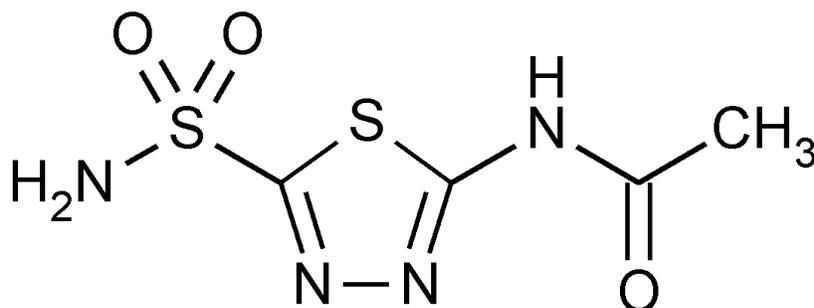
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: F. Mao.)

Correspondence Number—C111437

Comment deadline: July 31, 2015

Acetazolamide



$C_4H_6N_4O_3S_2$ 222.25

Acetamide, *N*-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]-;
N-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide [59-66-5].

DEFINITION

Acetazolamide contains NLT 98.0% and NMT 102.0% of acetazolamide ($C_4H_6N_4O_3S_2$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)

Delete the following:

• **B. Procedure**

Sample solution: 20 mg/mL in 1 N sodium hydroxide

Analysis: To 5 mL of *Sample solution* add 5 mL of a solution made by dissolving 100 mg of hydroxylamine hydrochloride and 80 mg of cupric sulfate in 10 mL of water. Heat the resulting pale yellow solution on a steam bath for 5 min.

Acceptance criteria: A clear, bright yellow solution is produced. No heavy precipitate or dark brown color results after the mixing or heating. ■ 1S (USP39)

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

ASSAY**Change to read:**● **Procedure**~~**Standard solution:** 20 mg/mL of USP Acetazolamide RS in pyridine~~~~**Sample solution:** 20 mg/mL of Acetazolamide in pyridine~~~~**Blank:** Pyridine~~~~**Instrumental conditions**~~~~(See *Spectroscopy and Light Scattering* (851),)~~~~**Mode:** IR~~~~**Analytical wavelength:** 7.38 μm (1350 cm^{-1})~~~~**Cell:** 0.1 mm~~~~**Analysis**~~~~**Samples:** *Standard solution* and *Sample solution*~~~~Calculate the quantity, as a percentage, of acetazolamide ($\text{C}_4\text{H}_6\text{N}_4\text{O}_3\text{S}_2$) in the portion of Acetazolamide taken:~~

~~$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$~~

 ~~A_S = absorbance of the *Sample solution*~~ ~~A_U = absorbance of the *Standard solution*~~ ~~C_S = concentration of USP Acetazolamide RS in the *Standard solution* (mg/mL)~~ ~~C_U = concentration of acetazolamide in the *Sample solution* (mg/mL)~~~~**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis~~

- **Mobile phase:** Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water, add 20 mL of methanol and 30 mL of acetonitrile, and mix. Adjust with glacial acetic acid to a pH of 4.0.

Standard solution: 0.1 mg/mL of USP Acetazolamide RS prepared as follows. Transfer USP Acetazolamide RS into a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the final volume, and dilute with water to volume.

Sample solution: 0.1 mg/mL of Acetazolamide prepared as follows. Transfer Acetazolamide into a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the final volume, and dilute with water to volume.

Chromatographic system~~(See *Chromatography* (621), *System Suitability*.)~~~~**Mode:** LC~~~~**Detector:** UV 254 nm~~~~**Column:** 4.6-mm \times 25-cm; packing L1~~~~**Flow rate:** 2 mL/min~~~~**Injection volume:** 20 μL~~ **System suitability**~~**Sample:** *Standard solution*~~

Suitability requirements**Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 0.73%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acetazolamide ($C_4H_6N_4O_3S_2$) in the portion of Acetazolamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of acetazolamide from the *Sample solution* r_S = peak response of acetazolamide from the *Standard solution* C_S = concentration of USP Acetazolamide RS in the *Standard solution* (mg/mL) C_U = concentration of Acetazolamide in the *Sample solution* (mg/mL)

■ 1S (USP39)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis**IMPURITIES**

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

- **Chloride and Sulfate** 〈 221 〉, *Chloride*

Sample solution: Digest 1.5 g with 75 mL of water at about 70° for 5 min. Cool to room temperature, and filter.

Acceptance criteria: A 25-mL portion of the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).

- **Chloride and Sulfate** 〈 221 〉, *Sulfate*

Sample solution: A 25-mL portion of the filtrate prepared in the test for *Chloride*

Acceptance criteria: It shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.04%).

- **Selenium** 〈 291 〉

Sample: 200 mg

Acceptance criteria: NMT 30 ppm

Delete the following:

- **Heavy Metals, Method II** 〈 231 〉: NMT 20 ppm •(Official 1-Dec-2015)

Delete the following:

- **Silver-Reducing Substances**

Sample: 5-g

Analysis: Thoroughly wet the *Sample* with alcohol. Add 125 mL of water, 10 mL of nitric acid, and 5.0 mL of 0.1 N silver nitrate VS. Stir with a mechanical stirrer for 30 min. Filter, add 5 mL of ferric ammonium sulfate TS to the filtrate, and titrate with 0.1 N ammonium thiocyanate VS to a reddish-brown endpoint.

Acceptance criteria: NLT 4.8 mL of 0.1 N ammonium thiocyanate is required. ■ 1S (USP39)

Change to read:

• Organic Impurities

• ~~Procedure: Ordinary Impurities (466)~~

~~**Standard solution:** Acetone and methanol (1:1)~~

~~**Test solution:** Acetone and methanol (1:1)~~

~~**Eluant:** *n*-Propyl alcohol and 1 N ammonium hydroxide (88:12)~~

~~**Visualization:** 1~~

■ **Solution A:** 6.8 g/L of monobasic potassium phosphate, in water

Mobile phase: Acetonitrile and *Solution A* (10:90)

System suitability solution: 0.16 µg/mL each of USP Acetazolamide Related Compound D RS and USP Acetazolamide Related Compound E RS in *Mobile phase*

Standard solution: 0.4 µg/mL of USP Acetazolamide RS in *Mobile phase*

Sample solution: 0.4 mg/mL of Acetazolamide in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 15-cm; 4-µm packing L11

Flow rate: 1.0 mL/min

Injection volume: 25 µL

Run time: 3.5 times the retention time of acetazolamide

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between acetazolamide related compound E and acetazolamide related compound D

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Acetazolamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U

= peak area of each impurity from the *Sample solution*

r_S

= peak area of acetazolamide from the *Standard solution*

C_S = concentration of USP Acetazolamide RS in the *Standard solution* (mg/mL) C_U = concentration of Acetazolamide in the *Sample solution* (mg/mL) F = relative response factor for each individual impurity (see *Table 1*)**Acceptance criteria:** See *Table 1*. Disregard any impurity peak less than 0.05%.**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Acetazolamide related compound E	0.3	1.0	0.15
Acetazolamide related compound D	0.4	0.63	0.15
Acetamidothiadiazole ^a	0.6	0.43	0.15
Acetazolamide	1.0	—	—
Mercaptothiadiazole analog ^b	1.4	0.38	0.15
Chlorothiadiazole analog ^c	2.1	1.0	0.15
Acetazolamide dimer ^d	2.6	1.0	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.6

a *N*-(1,3,4-Thiadiazol-2-yl)acetamide.

b *N*-(5-Mercapto-1,3,4-thiadiazol-2-yl)acetamide.c *N*-(5-Chloro-1,3,4-thiadiazol-2-yl)acetamide.d *N,N*-{5,5'-[(Hydrosulfonylamino)sulfonyl]bis(1,3,4-thiadiazole-5,2-diyl)}diacetamide.

■ 1S (USP39)

SPECIFIC TESTS

- **Water Determination** { 921 }, *Method I*: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at room temperature.

Change to read:

- **USP Reference Standards** { 11 }

USP Acetazolamide RS

- USP Acetazolamide Related Compound D RS

5-Amino-1,3,4-thiadiazole-2-sulfonamide.

$C_2H_4N_4O_2S_2$ 180.21

USP Acetazolamide Related Compound E RS

5-Acetamido-1,3,4-thiadiazole-2-sulfonic acid.

$C_4H_5N_3O_4S_2$ 223.23

■ 1S (USP39)

BRIEFING

Acetazolamide for Injection, USP 38 page 2041. On the basis of comments received, the following revisions to the monograph are proposed:

1. The nonspecific wet chemistry *Identification* test A is replaced with a retention time agreement based on the *Assay*.
2. The nonselective UV method in the *Assay* is replaced with an HPLC procedure based on the current USP monograph for *Acetazolamide Tablets*. The liquid chromatography procedure in the *Assay* is based on analysis performed with the Phenomenex Nucleosil C18 100 Å brand of L1 column. The typical retention time for acetazolamide is about 8 min.
3. The redundant test *Completeness of Solution* is deleted because the monograph already contains the test for *Constituted Solutions* from *Injections* (1).

Additionally, minor editorial changes have been made to update the monograph to current USP style.

This proposal highlights the need to add a procedure in the test for *Organic Impurities*. Interested parties are encouraged to submit comments and proposals to the Small Molecules-3 Expert Committee.

(SM3: F. Mao.)

Correspondence Number—C139063

Comment deadline: July 31, 2015

Acetazolamide for Injection

DEFINITION

Acetazolamide for Injection is prepared from Acetazolamide with the aid of sodium hydroxide. It is suitable for parenteral use. The contents of each container, when constituted as directed in the labeling, yield a solution containing NLT 95.0% and NMT 110.0% of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$).

IDENTIFICATION

Delete the following:

■ • **A. Procedure**

Sample: Dissolve 500 mg in 5 mL of water, add 2 drops of hydrochloric acid, and allow the mixture to stand for about 15 min. Filter through a fine sintered glass funnel, wash with several small portions of water, and dry in vacuum over silica gel for 3 h: the crystals

~~meet the requirements of the following tests.~~

Analysis 1: ~~Infrared Absorption (197K)~~

Analysis 2

Sample solution: ~~20 mg/mL of the Sample in 1N sodium hydroxide~~

~~To 5 mL of the Sample solution, add 5 mL of a solution made by dissolving 100 mg of hydroxylamine hydrochloride and 80 mg of cupric sulfate in 10 mL of water. Mix, and heat the resulting pale yellow solution on a steam bath for 5 min.~~

Acceptance criteria: ~~A clear, bright yellow solution is produced. No heavy precipitate or dark brown color results after the mixing or heating.~~

■ 1S (USP39)

Add the following:

■ ● **A. Infrared Absorption (197K)**

Sample: Dissolve 500 mg in 5 mL of water, add 2 drops of hydrochloric acid, and allow the mixture to stand for about 15 min. Filter through a fine sintered-glass funnel, wash with several small portions of water, and dry under vacuum over silica gel for 3 h.

Acceptance criteria: Meets the requirements ■ 1S (USP39)

Add the following:

- ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

Change to read:

- **B-**

■ **C.** ■ 1S (USP39)

Identification Tests—General (191), *Sodium*: Meets the requirements

ASSAY

Change to read:

- **Procedure**

Standard stock solution: ~~100 µg/mL of USP Acetazolamide RS in sodium hydroxide solution (1 in 100)~~

Standard solution: ~~10 µg/mL of USP Acetazolamide RS prepared as follows. Dilute 10.0 mL of Standard stock solution with 0.1 N hydrochloric acid to 100 mL.~~

Sample stock solution: ~~Nominally equivalent to 500 µg/mL of acetazolamide prepared as follows. Dissolve the contents of 1 container of Acetazolamide for Injection in a measured volume of water corresponding to the volume of solvent specified in the labeling. Dilute a portion of this solution quantitatively and stepwise with water.~~

Sample solution: ~~Nominally equivalent to 10 µg/mL of Acetazolamide prepared as follows. Transfer 5 mL of the Sample stock solution into a 250 mL volumetric flask, add 25 mL of 1 N hydrochloric acid, then add water to volume.~~

Blank: ~~0.1 N hydrochloric acid~~

Instrumental conditions

(See *Spectroscopy and Light Scattering* ~~(851)~~.)

Mode: UV

Analytical wavelength: 265 nm

Analysis

Samples: *Standard solution and Sample Solution*

Calculate the percentage of acetazolamide ($C_4H_6N_4O_3S_2$) in the portion of Acetazolamide for Injection taken:

$$250C(A_U/A_S)$$

~~A_U~~ absorbance of the *Sample solution*

~~A_S~~ absorbance of the *Standard solution*

~~C_S~~ concentration of USP Acetazolamide RS in the *Standard solution* ($\mu\text{g/mL}$)

~~C_U~~ nominal concentration of acetazolamide in the *Sample solution* ($\mu\text{g/mL}$)

■ Mobile phase:

Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water, add 20 mL of methanol and 30 mL of acetonitrile, and mix. Adjust with glacial acetic acid to a pH of 4.0.

Standard solution: 0.1 mg/mL of USP Acetazolamide RS prepared as follows. Transfer USP Acetazolamide RS into a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the final volume, and dilute with water to volume.

Sample solution: Nominally equivalent to 0.1 mg/mL of acetazolamide prepared as follows. Dissolve the contents of 1 container of Acetazolamide for Injection in a measured volume of water corresponding to the volume of solvent specified in the labeling. Dilute a portion of this solution quantitatively and stepwise with water.

Chromatographic system

(See *Chromatography* ~~(621)~~, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of acetazolamide from the *Sample solution*

r_s

= peak response of acetazolamide from the *Standard solution*

C_s

= concentration of USP Acetazolamide RS in the *Standard solution* (mg/mL)

C_u

= nominal concentration of acetazolamide in the *Sample solution* (mg/mL)

■ 1S (USP39)

Acceptance criteria: 95.0%–110.0%

PERFORMANCE TESTS

- **Uniformity of Dosage Units** 〈 905 〉: Meets the requirements

SPECIFIC TESTS

- **pH** 〈 791 〉: 9.0–10.0, in a freshly prepared solution (1 in 10)
- **Bacterial Endotoxins Test** 〈 85 〉: It contains NMT 0.5 USP Endotoxin Units/mg of acetazolamide.
- **Injections** 〈 1 〉, *Labeling*: Meets the requirements
- **Injections** 〈 1 〉, *Constituted Solutions*: Meets the requirements at the time of use
- **Sterility Tests** 〈 71 〉: Meets the requirements

Delete the following:

- ~~● **Completeness of Solution** 〈 641 〉: 100 mg/mL in carbon dioxide-free water dissolves to yield a clear solution. ■ 1S (USP39)~~

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described in *Injections* 〈 1 〉, *Containers for Sterile Solids*, preferably of Type III glass, and store at room temperature.
- **USP Reference Standards** 〈 11 〉
USP Acetazolamide RS
USP Endotoxin RS

BRIEFING

Acetazolamide Tablets, USP 38 page 2042. On the basis of comments received, the following revisions are proposed:

1. The nonspecific wet chemistry *Identification* test *B* is replaced with a retention time agreement based on the *Assay*.
2. The internal standard in the *Assay* is removed, and as a result the system suitability requirement is revised.

3. An equation in the *Dissolution* test is added.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

This proposal highlights the need to add a procedure in the test for *Organic Impurities*. Interested parties are encouraged to submit comments and proposals to the Small Molecules-3 Expert Committee.

(SM3: F. Mao.)

Correspondence Number—C156061

Comment deadline: July 31, 2015

Acetazolamide Tablets

DEFINITION

Acetazolamide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$).

IDENTIFICATION

- **A. Infrared Absorption** (197K)

Sample: Extract a quantity of finely powdered Tablets, equivalent to about 500 mg of acetazolamide, with 50 mL of acetone. Filter, and add sufficient solvent hexane to the filtrate to cause formation of a heavy, white precipitate. Collect the precipitate on a medium-porosity, sintered-glass funnel, and dry with suction.

Acceptance criteria: Meet the requirements

Delete the following:

- **B. Procedure**

Sample solution: 20 mg/mL of ~~Sample~~ from *Identification A* in 1 N sodium hydroxide

Analysis: ~~To 5 mL of Sample solution add 5 mL of a solution made by dissolving 100 mg of hydroxylamine hydrochloride and 80 mg of cupric sulfate in 10 mL of water. Mix and heat the resulting pale yellow solution on a steam bath for 5 min.~~

Acceptance criteria: ~~A clear, bright yellow solution is produced. No heavy precipitate or dark brown color results after the mixing or heating.~~ ■1S (USP39)

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

ASSAY

Change to read:

- **Procedure**

Mobile phase: Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water, add 20 mL of methanol and 30 mL of acetonitrile, and mix. Adjust with glacial acetic acid to a pH of 4.0.

~~**Internal standard solution:** Transfer 100 mg of sulfadiazine to a 100-mL volumetric flask, add 10 mL of 0.5 N sodium hydroxide, and mix to dissolve. Dilute with water to volume.~~

~~**Standard stock solution:** Dissolve 25 mg of USP Acetazolamide RS in 2.5 mL of 0.5 N sodium hydroxide. Dilute with water to 25 mL.~~

~~**Standard solution:** Transfer 10.0 mL of *Standard stock solution*, 10.0 mL of *Internal standard solution* and 10 mL of 0.5 N sodium hydroxide to a 100-mL volumetric flask. Dilute with water to volume.~~

~~**Sample stock solution:** Transfer a portion of the powder, from NLT 20 Tablets, equivalent to 100 mg acetazolamide into a 100-mL volumetric flask. Add 10 mL of 0.5 N sodium hydroxide, sonicate for 5 min, cool to room temperature, and dilute with water to volume. Filter a portion of this solution, discarding the first 20 mL of the filtrate.~~

~~**Sample solution:** Transfer 10.0 mL of *Sample stock solution*, 10.0 mL of *Internal standard solution*, and 10 mL of 0.5 N sodium hydroxide to a 100-mL volumetric flask, and dilute with water to volume.~~

■ **Standard solution:** 0.1 mg/mL of USP Acetazolamide RS prepared as follows. Transfer USP Acetazolamide RS into a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the final volume, and dilute with water to volume.

Sample stock solution: Nominally equivalent to 1.0 mg/mL of acetazolamide prepared as follows. Transfer a portion of the powder, from NLT 20 Tablets, equivalent to 100 mg acetazolamide into a 100-mL volumetric flask. Add 10 mL of 0.5 N sodium hydroxide, sonicate for 5 min, cool to room temperature, and dilute with water to volume. Filter a portion of this solution, discarding the first 20 mL of the filtrate.

Sample solution: Nominally equivalent to 0.1 mg/mL of acetazolamide prepared as follows. Transfer 10.0 mL of *Sample stock solution* and 10 mL of 0.5 N sodium hydroxide to a 100-mL volumetric flask, and dilute with water to volume. ■ 1S (USP39)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[~~Note—The relative retention times for acetazolamide and sulfadiazine are about 0.7 and 1.0, respectively.~~]

~~Suitability requirements~~

~~**Resolution:** NLT 2.0 between the acetazolamide and sulfadiazine peaks~~

~~**Relative standard deviation:** NMT 1.0% for the ratios of the acetazolamide peak response to the sulfadiazine peak response~~

■ Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

■ 1S (USP39)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

~~R_U~~ peak response ratio of acetazolamide peak to sulfadiazine peak from the *Sample solution*

~~R_S~~ peak response ratio of acetazolamide peak to sulfadiazine peak from the *Standard solution*

~~C_S~~ concentration of USP Acetazolamide RS in *Standard solution* (mg/mL)

~~C_U~~ nominal concentration of acetazolamide in the *Sample solution* (mg/mL)

■

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= acetazolamide peak response from the *Sample solution*

r_S

= acetazolamide peak response from the *Standard solution*

C_S

= concentration of USP Acetazolamide RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of acetazolamide in the *Sample solution* (mg/mL)

■ 1S (USP39)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

Change to read:

● **Dissolution** 〈 711 〉

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 60 min

Standard solution: USP Acetazolamide RS in *Medium*

Sample solution: Dilute with *Medium* if necessary.

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* 〈 851 〉.)

Mode: UV

Analytical wavelength: 265 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) dissolved:
by comparing the *Sample solution* with the *Standard solution*

■

$$(A_U/A_S) \times C_S \times D \times (V/L) \times 100$$

$A_U =$

absorbance of the *Sample solution*

$A_S =$

absorbance of the *Standard solution*

$C_S =$

concentration of the *Standard solution* (mg/mL)

$D =$

dilution factor of the *Sample solution*, if needed

$V =$

volume of *Medium*, 900 mL

$L =$

label claim (mg/Tablet)

■ 1S (USP39)

Tolerances: NLT 75% (Q) of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) is dissolved.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
- **USP Reference Standards** { 11 }
USP Acetazolamide RS

BRIEFING

Aminophylline, *USP 38* page 2186. As a part of USP monograph modernization efforts, the following revisions are proposed:

1. Replace the nonspecific melting temperature test in *Identification* test A with an infrared spectra agreement between the *Sample* and USP Theophylline RS.
2. Replace the wet chemistry-based *Identification* test B with a retention time agreement from the proposed chromatographic procedures in the *Assay*.
3. Replace the HPLC procedure in the *Assay* with a stability-indicating UHPLC procedure. The liquid chromatographic procedure is performed using the Acquity BEH C18 brand of L1 column. The typical retention time for theophylline is about 4 min.
4. Add a stability-indicating UHPLC procedure in the test for *Organic Impurities* using the same chromatographic system as the *Assay*.
5. Add the new Reference Standards used in the *Assay* and the test for *Organic Impurities* to the *USP Reference Standards* section.

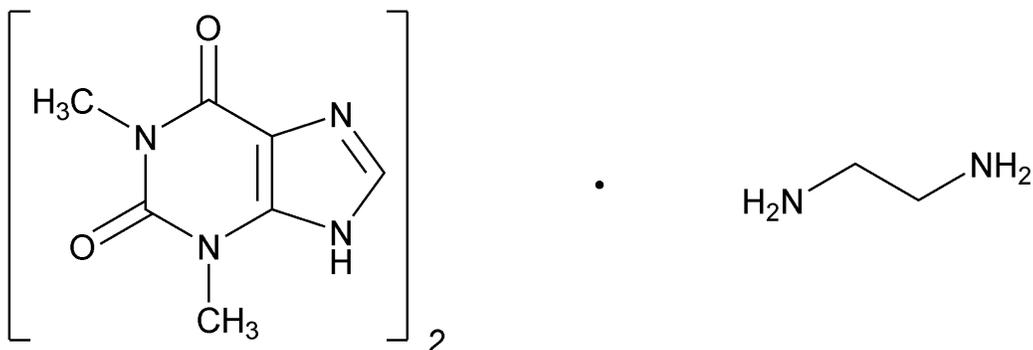
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: R.-H. Yeh.)

Correspondence Number—C131509

Comment deadline: July 31, 2015

Aminophylline



$C_{16}H_{24}N_{10}O_4$ 420.43

$C_{16}H_{24}N_{10}O_4 \cdot 2H_2O$ 456.46

1*H*-Purine-2,6-dione, 3,7-dihydro-1,3-dimethyl-, compd. with 1,2-ethanediamine (2:1);
Theophylline compound with ethylenediamine (2:1).

Anhydrous [317-34-0].

Dihydrate [5897-66-5].

DEFINITION

Aminophylline is anhydrous or contains NMT two molecules of water of hydration. It contains NLT 84.0% and NMT 87.4% of anhydrous theophylline ($C_7H_8N_4O_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• A.

■ **Infrared Absorption** < 197K > ■_{1S} (USP39)

Sample: 500 mg of Aminophylline

Analysis: Dissolve the *Sample* in 20 mL of water, add, with constant stirring, 1 mL of 3 N hydrochloric acid, filter (retain the filtrate), wash the precipitate with small portions of cold water, and dry at 105° for 1 h.

Acceptance criteria: ~~The precipitate of theophylline so obtained melts at 270°–274°.~~

■ The IR spectrum of the precipitate of theophylline so obtained corresponds to that of USP Theophylline RS. ■_{1S} (USP39)

Change to read:

- **B.**

Sample: 10 mg of the precipitate from *Identification* test A

Analysis: Transfer the *Sample* to a porcelain dish, add 1 mL of hydrochloric acid and 100 mg of potassium chlorate, evaporate on a steam bath to dryness, and invert the dish over a vessel containing a few drops of 6 N ammonium hydroxide.

Acceptance criteria: The residue acquires a purple color, which is destroyed by solutions of fixed alkalis.

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

- **C.**

Sample: The filtrate obtained in *Identification* test A

Analysis: To the *Sample* add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline. Shake by mechanical means for 10 min, add 5 mL of 3 N hydrochloric acid to acidify, chill, collect the precipitated disulfonamide of ethylenediamine, wash with water, recrystallize from water, and dry at 105° for 1 h.

Acceptance criteria: The dried precipitate melts at 164°–171°.

ASSAY

Change to read:

- **Procedure**

Mobile phase: 200 mL of methanol, 960 mg of sodium 1-pentanesulfonate, and sufficient water to make 1 L. Adjust with glacial acetic acid to a pH of 2.9 ± 0.1.

Diluent: Methanol and water (1:4)

Standard solution: 0.08 mg/mL of USP Theophylline RS in *Diluent*

System suitability stock solution: 80 µg/mL each of USP Theophylline RS and theobromine, prepared by dissolving a suitable quantity of theobromine with *Standard solution*

System suitability solution: 64 µg/mL each of USP Theophylline RS and theobromine, from *System suitability stock solution* in *Diluent*

Sample solution: 0.096 mg/mL of Aminophylline in *Diluent*

Chromatographic system

(See *Chromatography* ~~621~~, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9 mm × 15 cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for theobromine and theophylline are 0.65 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between theobromine and theophylline, *System suitability solution*

Tailing factor: NMT 2.0 for theophylline, *System suitability solution*

Relative standard deviation: ~~NMT 2.0%, Standard solution~~
Analysis:

Samples: ~~Standard solution and Sample solution~~

Calculate the percentage of theophylline ($C_7H_8N_4O_2$) in the portion of Aminophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

~~r_U~~ peak response from the ~~Sample solution~~

~~r_S~~ peak response from the ~~Standard solution~~

~~C_S~~ concentration of USP Theophylline RS in the ~~Standard solution~~ (mg/mL)

~~C_U~~ concentration of Aminophylline in the ~~Sample solution~~ (mg/mL)

■ Solution A:

10 mM ammonium acetate prepared as follows. Transfer 770.8 mg of ammonium acetate to a 1-L volumetric flask, and dissolve in water to 80% of the flask volume. Adjust with glacial acetic acid to a pH of 5.5, and dilute with water to volume. Pass through a suitable filter of 0.2- μ m pore size.

Solution B: Methanol

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

Impurity stock solution: 25 μ g/mL of USP Theophylline Related Compound F RS in water

System suitability solution: 0.8 mg/mL of USP Theophylline RS and 1 μ g/mL of USP

Theophylline Related Compound F RS in water prepared as follows. Transfer 21 mg of USP Theophylline RS to a 25-mL volumetric flask and add 15 mL of water. Sonicate to dissolve, add 1 mL of *Impurity stock solution*, and dilute with water to volume.

Standard solution: 0.17 mg/mL of USP Theophylline RS in water. Sonicate to dissolve as needed.

Sample solution: 0.2 mg/mL of Aminophylline in water

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 2.1-mm \times 10-cm; 1.7- μ m packing L1

Column temperature: 40 $^{\circ}$

Flow rate: 0.4 mL/min

Injection volume: 1 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between theophylline and theophylline related compound F,
System suitability solution

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of theophylline ($C_7H_8N_4O_2$) in the portion of Aminophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of theophylline from the *Sample solution*

r_S

= peak response of theophylline from the *Standard solution*

C_S

= concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U

= concentration of Aminophylline in the *Sample solution* (mg/mL)

■ 1S (USP39)

Acceptance criteria: 84.0%–87.4% of theophylline on the anhydrous basis

OTHER COMPONENTS

Change to read:

● **Ethylenediamine Content**

■ **Content of Ethylenediamine** ■ 1S (USP39)

Sample: 500 mg of Aminophylline

Diluent: Water

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N hydrochloric acid VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 30 mL of *Diluent*, add methyl orange TS, and titrate. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine ($C_2H_8N_2$).

Acceptance criteria: 157–175 mg of ethylenediamine ($C_2H_8N_2$) per g of theophylline ($C_7H_8N_4O_2$) found in the *Assay*

IMPURITIES

● **Residue on Ignition** 〈 281 〉: NMT 0.15%

Add the following:

■ • Organic Impurities

Solution A, Solution B, Mobile phase, System suitability solution, and

Chromatographic system: Proceed as directed in the Assay.

Standard stock solution: 25.0 µg/mL each of USP Caffeine RS, USP Theophylline RS, USP Theophylline Related Compound B RS, USP Theophylline Related Compound C RS, USP Theophylline Related Compound D RS, and USP Theophylline Related Compound F RS in water

Standard solution: 1.0 µg/mL each of USP Caffeine RS, USP Theophylline RS, USP Theophylline Related Compound B RS, USP Theophylline Related Compound C RS, USP Theophylline Related Compound D RS, and USP Theophylline Related Compound F RS in water, from *Standard stock solution*

Sample solution: 1.0 mg/mL of Aminophylline in water

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

Relative standard deviation: NMT 3.0% for each peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of caffeine, theophylline related compound B, theophylline related compound C, theophylline related compound D, and theophylline related compound F in the portion of Aminophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_{\bar{U}}$ peak response of caffeine, theophylline related compound B, theophylline related compound C, theophylline related compound D, or theophylline related compound F from the *Sample solution*

$r_{\bar{S}}$ peak response of the corresponding Reference Standard from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Caffeine RS, USP Theophylline Related Compound B RS, USP Theophylline Related Compound C RS, USP Theophylline Related Compound D RS, or USP Theophylline Related Compound F RS in the *Standard solution* (mg/mL)

$C_{\bar{U}}$ concentration of Aminophylline in the *Sample solution* (mg/mL)

Calculate the percentage of dimethyl uric acid, theobromine, and any other individual unspecified impurity in the portion of Aminophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{\bar{U}}$ peak response of dimethyl uric acid, theobromine, or any other individual unspecified impurity from the *Sample solution*

$r_{\bar{S}}$ peak response of theophylline from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

$C_{\bar{U}}$ concentration of Aminophylline in the *Sample solution* (mg/mL)

F = relative response factor

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Theophylline related compound C	0.36	—	0.10
Theophylline related compound B	0.63	—	0.10
Theophylline related compound D	0.69	—	0.10
Dimethyl uric acid ^a	0.76	0.55	0.10
Theobromine ^b	0.82	1.0	0.10
Theophylline	1.0	—	—
Theophylline related compound F	1.09	—	0.10
Caffeine	1.20	—	0.10
Any other individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5
a 1,3-Dimethyl-7,9-dihydro-1 <i>H</i> -purine-2,6,8(3 <i>H</i>)-trione.			
b 3,7-Dihydro-3,7-dimethylpurine-2,6(1 <i>H</i>)-dione.			

■ 1S (USP39)

SPECIFIC TESTS

- **Water Determination** 〈 921 〉, Method I

Sample: 1.5 g of Aminophylline

Solvent: 50 mL of chloroform and methanol (50:50) in place of methanol

Acceptance criteria

Anhydrous: NMT 0.75%

Hydrous: NMT 7.9%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label it to indicate whether it is anhydrous or hydrous, and also to state the content of anhydrous theophylline.

Change to read:

- **USP Reference Standards** 〈 11 〉

■ USP Caffeine RS

■ 1S (USP39)

USP Theophylline RS

■ USP Theophylline Related Compound B RS

3-Methyl-1*H*-purine-2,6-dione.

C₆H₆N₄O₂ 166.14

USP Theophylline Related Compound C RS

N-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide.

C₇H₁₀N₄O₃ 198.18

USP Theophylline Related Compound D RS

N-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide.

C₆H₁₀N₄O 154.17

USP Theophylline Related Compound F RS

7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

C₉H₁₂N₄O₃ 224.22

■ 1S (USP39)

BRIEFING

Aminophylline Injection, USP 38 page 2187. As a part of USP monograph modernization efforts, the following revisions are proposed:

1. Replace the nonspecific melting temperature test in *Identification* test *B* with an infrared spectra agreement between the *Sample* and USP Theophylline RS.
2. Replace the wet chemistry-based *Identification* test *C* with a retention time agreement from the proposed chromatographic procedures in the *Assay*.
3. Replace the HPLC procedure in the *Assay* with a stability-indicating UHPLC procedure. The liquid chromatographic procedure is performed using the Acquity BEH C18 brand of L1 column. The typical retention time for theophylline is about 4 min.
4. Add a UHPLC procedure in the test for *Organic Impurities* using the same chromatographic system as the *Assay*.
5. Revise the *Packaging and Storage* requirements to be consistent with the product label.
6. Add the new Reference Standards used in the *Assay* and the test for *Organic Impurities* to the *USP Reference Standards* section.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: R.-H. Yeh.)

Correspondence Number—C132400

Comment deadline: July 31, 2015

Aminophylline Injection

DEFINITION

Aminophylline Injection is a sterile solution of Aminophylline in Water for Injection, or is a sterile solution of Theophylline in Water for Injection prepared with the aid of Ethylenediamine. It contains, in each mL, an amount of aminophylline (C₁₆H₂₄N₁₀O₄) equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of anhydrous theophylline (C₇H₈N₄O₂).

Aminophylline Injection may contain an excess of Ethylenediamine, but no other substance may be added for the purpose of pH adjustment.

[Note—Do not use the Injection if crystals have separated.]

IDENTIFICATION

• A.

Analysis: Dilute a volume of Injection equivalent to 500 mg of aminophylline with water to 20 mL, and add, with constant stirring, 1 mL of 3 N hydrochloric acid or enough to

completely precipitate the theophylline, and filter. To the filtrate add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline. Shake by mechanical means for 10 min, add 5 mL of 3 N hydrochloric acid to acidify, chill, collect the precipitated disulfonamide of ethylenediamine, wash with water, recrystallize from water, and dry at 105° for 1 h.

Acceptance criteria: The precipitate melts at 164°–171°.

Change to read:

• B.

■ **Infrared Absorption** 〈 197K〉 ■ 1S (USP39)

Analysis: Wash the precipitated theophylline from *Identification* test A with small portions of cold water, and dry at 105° for 1 h.

Acceptance criteria: The theophylline precipitate melts at 270°–274°.

■ The IR spectrum of the precipitate of theophylline so obtained corresponds to that of USP Theophylline RS. ■ 1S (USP39)

Change to read:

• C.

Analysis: Transfer 10 mg of the precipitated theophylline from *Identification* test A to a porcelain dish, add 1 mL of hydrochloric acid and 100 mg of potassium chlorate, evaporate on a steam bath to dryness, and invert the dish over a vessel containing a few drops of 6 N ammonium hydroxide.

Acceptance criteria: The residue acquires a purple color, which is destroyed by solutions of fixed alkalis.

■ The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

ASSAY

Change to read:

• Procedure

Mobile phase: 200 mL of methanol, 960 mg of sodium 1-pentanesulfonate, and sufficient water to make 1 L. Adjust with glacial acetic acid to a pH of 2.9 ± 0.1.

Diluent: Methanol and water (1:4)

Standard solution: 0.08 mg/mL of USP Theophylline RS in *Diluent*

System suitability stock solution: 80 µg/mL each of USP Theophylline RS and theobromine, prepared by dissolving a suitable quantity of theobromine with *Standard solution*

System suitability solution: 64 µg/mL each of USP Theophylline RS and theobromine, from *System suitability stock solution* in *Diluent*

Sample stock solution: Equivalent to 1 mg/mL of theophylline, prepared as follows. Transfer a volume of Injection equivalent to 100 mg of theophylline to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Sample solution: Equivalent to 0.08 mg/mL of theophylline in *Diluent*, from *Sample stock*

*solution***Chromatographic system***(See Chromatography 621, System Suitability.)***Mode:** LC**Detector:** UV 254 nm**Column:** 3.9 mm × 15 cm; packing L1**Flow rate:** 1 mL/min**Injection volume:** 10 µL**System suitability****Samples:** *Standard solution and System suitability solution*

[Note—The relative retention times for theobromine and theophylline are about 0.65 and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 3.0 between theobromine and theophylline, *System suitability solution***Tailing factor:** NMT 2.0 for theophylline, *System suitability solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution and Sample solution*Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL) C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)**■ Solution A:**

10 mM ammonium acetate prepared as follows. Transfer 770.8 mg of ammonium acetate to a 1-L volumetric flask, and dissolve in water to 80% of the flask volume. Adjust with glacial acetic acid to a pH of 5.5 and dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.

Solution B: Methanol**Mobile phase:** See Table 1.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

Impurity stock solution: 25 µg/mL of USP Theophylline Related Compound F RS in water

System suitability solution: 0.8 mg/mL of USP Theophylline RS and 1 µg/mL of USP Theophylline Related Compound F RS in water prepared as follows. Transfer 21 mg of USP Theophylline RS to a 25-mL volumetric flask, and add 15 mL of water. Sonicate to dissolve, add 1 mL of *Impurity stock solution*, and dilute with water to volume.

Standard solution: 0.17 mg/mL of USP Theophylline RS in water. Sonicate to dissolve as needed.

Sample solution: Nominally 0.17 mg/mL of anhydrous theophylline in water prepared as follows. Transfer 8.5 mg of anhydrous theophylline from a volume of Injection to a 50-mL volumetric flask. Dissolve and dilute with water to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 2.1-mm × 10-cm; 1.7-µm packing L1

Column temperature: 40°

Flow rate: 0.4 mL/min

Injection volume: 1 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline (C₇H₈N₄O₂) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of theophylline from the *Sample solution*

r_S

= peak response of theophylline from the *Standard solution*

C_S

= concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of theophylline in the *Sample solution* (mg/mL)

■ 1S (USP39)

Acceptance criteria: 93.0%–107.0%

OTHER COMPONENTS

- **Content of Ethylenediamine**

Sample: A volume of Injection equivalent to 500 mg of aminophylline

Diluent: Water

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N hydrochloric acid VS

Endpoint detection: Visual

Analysis: If necessary, dilute the *Sample* with *Diluent* to 30 mL, add methyl orange TS, and titrate with *Titrant*. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine (C₂H₈N₂).

Acceptance criteria: 166–192 mg of ethylenediamine (C₂H₈N₂) per g of theophylline (C₇H₈N₄O₂) found in the Assay

IMPURITIES**Add the following:**

- ● **Organic Impurities**

Solution A, Solution B, Mobile phase, Impurity stock solution, System suitability solution, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 2.0 µg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS in water

Sample solution: Nominally 1.0 mg/mL of anhydrous aminophylline in water prepared as follows. Transfer 25 mg of anhydrous aminophylline from a volume of Injection to a 25-mL volumetric flask. Dissolve and dilute with water to volume.

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

Relative standard deviation: NMT 3.0% for each peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of theophylline related compound D in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline related compound D from the *Sample solution*

r_S = peak response of theophylline related compound D from the *Standard solution*

C_S = concentration of USP Theophylline Related Compound D RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of aminophylline in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any other individual unspecified degradation product from the *Sample solution*

r_S peak response of theophylline from the *Standard solution*

C_S concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U nominal concentration of aminophylline in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound C ^{a,b}	0.36	—
Theophylline related compound B ^{a,c}	0.63	—
Theophylline related compound D	0.69	0.2
Dimethyl uric acid ^{a,d}	0.76	—
Theobromine ^{a,e}	0.82	—
Theophylline	1.0	—
Theophylline related compound F ^a	1.09	—
Caffeine ^a	1.20	—
Any other individual unspecified degradation product	—	0.2
Total impurities	—	0.5

^a Process impurity included for identification only and not to be included in the calculation of total degradation products.

^b *N*-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide.

^c 3-Methyl-1*H*-purine-2,6-dione.

^d 1,3-Dimethyl-7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione.

^e 3,7-Dihydro-3,7-dimethylpurine-2,6(1*H*)-dione.

■ 1S (USP39)

SPECIFIC TESTS

- **pH** 〈 791 〉: 8.6–9.0
- **Particulate Matter in Injections** 〈 788 〉: Meets the requirements for small-volume injections
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* 〈 1 〉
- **Bacterial Endotoxins Test** 〈 85 〉: It contains NMT 1.0 USP Endotoxin Unit/mg of

aminophylline.

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in single-dose containers from which carbon dioxide has been excluded, preferably of Type I glass, protected from light.

■ Store at controlled room temperature. ■ 1S (USP39)

- **Labeling:** Label the Injection to state the content of anhydrous theophylline.

Change to read:

- **USP Reference Standards** { 11 }

USP Endotoxin RS

USP Theophylline RS

■ USP Theophylline Related Compound D RS

N-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide.

C₆H₁₀N₄O 154.17

USP Theophylline Related Compound F RS

7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

C₉H₁₂N₄O₃ 224.22

■ 1S (USP39)

BRIEFING

Aminophylline Oral Solution, USP 38 page 2188. As a part of USP monograph modernization efforts, the following revisions are proposed:

1. Replace the nonspecific melting temperature in *Identification* test A with an infrared spectra agreement between the sample and the USP Theophylline Reference Standard.
2. Replace the HPLC procedure in the *Assay* with the same UHPLC procedure that is proposed in the test for *Organic Impurities*. The liquid chromatographic procedure is performed using the Acquity BEH C18 brand of L1 column. The typical retention time for theophylline is about 4 min.
3. Add a stability-indicating UHPLC procedure in the test for *Organic Impurities* using the same chromatographic system as the *Assay*.
4. Add the new Reference Standards used in the *Assay* and the test for *Organic Impurities* to the *USP Reference Standards* section.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: D.A. Porter.)

Correspondence Number—C132401

Comment deadline: July 31, 2015

Aminophylline Oral Solution

DEFINITION

Aminophylline Oral Solution is an aqueous solution of Aminophylline, prepared with the aid of Ethylenediamine. It contains an amount of aminophylline ($C_{16}H_{24}N_{10}O_4$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$). Aminophylline Oral Solution may contain an excess of ethylenediamine, but no other substance may be added for the purpose of pH adjustment.

IDENTIFICATION**Change to read:**• **A.**

■ **Infrared Absorption** $\langle 197K \rangle$ ■ 1S (USP39)

Analysis: Transfer a volume of Oral Solution equivalent to 500 mg of aminophylline to a suitable container and add, with constant stirring, 1 mL of 3 N hydrochloric acid or enough to completely precipitate the theophylline. Filter (retain the filtrate), wash the precipitate with small portions of cold water until free from chloride, and dry at 105° for 1 h.

Acceptance criteria: ~~The dried precipitate melts at 270° – 274° .~~

■ The IR spectrum of the precipitate of theophylline so obtained matches that of USP Theophylline RS. ■ 1S (USP39)

• **B.**

Analysis: To the filtrate obtained in *Identification* test A add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline. Shake by mechanical means for 10 min, add 5 mL of 3 N hydrochloric acid to acidify, chill, collect the precipitated disulfonamide of ethylenediamine, wash with water, recrystallize from water, and dry at 105° for 1 h.

Acceptance criteria: The dried precipitate melts at 164° – 171° .

ASSAY**Change to read:**• **Procedure**

~~**Mobile phase:** 200 mL of methanol, 960 mg of sodium 1-pentanesulfonate, and sufficient water to make 1 L.~~

~~Adjust with glacial acetic acid to a pH of 2.9 ± 0.1 .~~

~~**Diluent:** Methanol and water (1:4)~~

~~**Standard solution:** 0.08 mg/mL of USP Theophylline RS in *Diluent*~~

~~**System suitability stock solution:** 80 μ g/mL each of USP Theophylline RS and theobromine, prepared by dissolving a suitable quantity of theobromine with *Standard solution*~~

~~**System suitability solution:** 64 μ g/mL each of USP Theophylline RS and theobromine, from *System suitability stock solution* in *Diluent*~~

~~**Sample solution:** Equivalent to 0.072 mg/mL of anhydrous theophylline, from Oral Solution~~

in Diluent

Chromatographic system

(See *Chromatography* < 621 >, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9 mm x 15 cm, packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for theobromine and theophylline are about 0.65 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between theobromine and theophylline, *System suitability solution*

Tailing factor: NMT 2.0 for theophylline, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

■ **Solution A:** 10 mM ammonium acetate prepared as follows. Transfer an appropriate amount of ammonium acetate to a volumetric flask and dissolve in water (about 80% of the flask volume). Adjust with glacial acetic acid to a pH of 5.5 and dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

Impurity stock solution: 25 µg/mL of USP Theophylline Related Compound F RS in water

System suitability solution: 0.8 mg/mL of USP Theophylline RS and 2 µg/mL of USP Theophylline Related Compound F RS in water prepared as follows. Transfer 1 mg of USP Theophylline RS to a 25-mL volumetric flask and add 15 mL of water. Sonicate to dissolve, add 2 mL of *Impurity stock solution*, and dilute with water to volume.

Standard solution: 0.17 mg/mL of USP Theophylline RS in water. Sonicate to dissolve, as needed.

Sample solution: Nominally 0.17 mg/mL of anhydrous theophylline in water prepared as follows. Transfer an appropriate amount of anhydrous theophylline from a volume of Oral Solution to a suitable volumetric flask. Dissolve and dilute with water to volume.

Chromatographic system

(See *Chromatography* < 621 >, *System Suitability*.)

Mode: LC**Detector:** UV 270 nm**Column:** 2.1-mm × 10-cm; 1.7-μm packing L1**Column temperature:** 40°**Flow rate:** 0.4 mL/min**Injection volume:** 1 μL**System suitability****Samples:** *System suitability solution and Standard solution***Suitability requirements****Resolution:** NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution***Relative standard deviation:** NMT 1.0%, *Standard solution*

■ 1S (USP39)

Analysis**Samples:** *Standard solution and Sample solution*

Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak response from the *Sample solution* r_S peak response from the *Standard solution* C_S concentration of USP Theophylline RS in the *Standard solution* (mg/mL) C_U nominal concentration of

■ anhydrous ■ 1S (USP39)

theophylline in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**OTHER COMPONENTS**● **Content of Ethylenediamine****Sample:** A volume of Oral solution equivalent to 500 mg of aminophylline**Diluent:** Water**Titrimetric system****Mode:** Direct titration**Titrant:** 0.1 N hydrochloric acid VS**Endpoint detection:** Visual**Analysis:** If necessary, dilute the *Sample* with *Diluent* to 30 mL, add methyl orange TS, and titrate. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine ($C_2H_8N_2$).**Acceptance criteria:** 176–283 mg of ethylenediamine ($C_2H_8N_2$) per g of theophylline ($C_7H_8N_4O_2$) found in the Assay**IMPURITIES****Add the following:**■ ● **Organic Impurities**

Solution A, Solution B, Mobile phase, Impurity stock solution, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 2.0 µg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS in water

Sample solution: Nominally 1.0 mg/mL of anhydrous aminophylline in water prepared as follows. Transfer an appropriate amount of anhydrous aminophylline from a volume of Oral Solution to a suitable volumetric flask. Dissolve and dilute with water to volume.

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

Relative standard deviation: NMT 3.0% for each peak present in the *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of theophylline related compound D in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of theophylline related compound D from the *Sample solution*

r_S peak response of theophylline related compound D from the *Standard solution*

C_S concentration of USP Theophylline Related Compound D RS in the *Standard solution* (mg/mL)

C_U nominal concentration of anhydrous theophylline in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any other individual unspecified degradation product from the *Sample solution*

r_S peak response of theophylline from the *Standard solution*

C_S concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U nominal concentration of anhydrous theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.086%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound Ca,b	0.36	—
Theophylline related compound Ba,c	0.63	—
Theophylline related compound D	0.69	0.2
Dimethyl uric acid ^{a,d}	0.76	—

Theobrominea,e	0.82	—
Theophylline	1.0	—
Theophylline related compound Fa	1.09	—
Caffeinea	1.20	—
Any other individual unspecified degradation product	—	0.2
Total impurities	—	0.5

a Process impurity included for identification only and not to be included in the calculation of total degradation products.

b *N*-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide.

c 3-Methyl-1*H*-purine-2,6-dione.

d 1,3-Dimethyl-7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione.

e 3,7-Dihydro-3,7-dimethylpurine-2,6(1*H*)-dione.

■ 1S (USP39)

SPECIFIC TESTS

- pH 〈 791 〉: 8.5–9.7

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label the Oral Solution to state the content of anhydrous theophylline.

Change to read:

- **USP Reference Standards 〈 11 〉**

USP Theophylline RS

■ USP Theophylline Related Compound D RS

N-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide.

C₆H₁₀N₄O 154.17

USP Theophylline Related Compound F RS

7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

C₉H₁₂N₄O₃ 224.22

■ 1S (USP39)

BRIEFING

Aminophylline Tablets, USP 38 page 2190. As a part of USP monograph modernization efforts, the following revisions are proposed:

1. Replace the wet chemistry-based *Identification* test A with an infrared spectra agreement between the *Sample* and USP Theophylline RS. The precipitation procedure of theophylline is retained in this test.
2. Replace the nonspecific melting temperature test in *Identification* test B with the

retention time agreement from the proposed chromatographic procedure in the *Assay*.

3. Replace the titration procedure in the *Assay* with a stability-indicating UHPLC procedure. The liquid chromatographic procedure is performed using the Waters Acquity BEH C18 brand of L1 column. The typical retention time for theophylline is about 4 min.
4. Add a stability-indicating UHPLC procedure in the test for *Organic Impurities* that is consistent with the chromatographic system used in the *Assay*.
5. Revise the *Packaging and Storage* requirements to be consistent with the product label.
6. Add the new Reference Standards used in the *Assay* and the test for *Organic Impurities* to the *USP Reference Standards* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R.-H. Yeh.)

Correspondence Number—C132403

Comment deadline: July 31, 2015

Aminophylline Tablets

DEFINITION

Aminophylline Tablets contain an amount of aminophylline equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

[Note—The ammoniacal odor present in the vapor space above the Tablets is often quite strong, especially when bottles having suitably tight closures are newly opened. This is due to ethylenediamine vapor pressure build-up, a natural condition in the case of aminophylline.]

IDENTIFICATION

Change to read:

- **A.**

■ **Infrared Absorption** 〈 197K〉 ■ 1S (USP39)

Analysis: Macerate a quantity of Tablets, equivalent to 500 mg of aminophylline, with 25 mL of water, and filter. The filtrate is alkaline to litmus. To the filtrate add 1 mL of 3 N hydrochloric acid, stir, and if necessary, chill to precipitate the theophylline. Filter, and retain the filtrate, free from washings.

■ Use the filtrate in *Identification* test C. ■ 1S (USP39)

Wash the theophylline crystals so obtained with small quantities of ice-cold water, and dry at 105° for 1 h. ~~Transfer 10 mg of the dried theophylline crystals to a porcelain dish, add 1 mL of hydrochloric acid and 100 mg of potassium chlorate, evaporate on a steam bath to dryness, and invert the dish over a vessel containing a few drops of 6 N ammonium hydroxide.~~

■ ■ 1S (USP39)

Acceptance criteria: ~~The residue acquires a purple color, which is destroyed by solutions of~~

~~fixed alkalis.~~

- The IR spectrum of the theophylline so obtained corresponds to that of USP Theophylline RS.
 - 1S (USP39)

Change to read:

- B.

~~**Analysis:** Recrystallize the dried theophylline crystals from *Identification test A* from water, and dry at 105° for 1 h.~~

~~**Acceptance criteria:** The recrystallized theophylline melts at 270°–274°.~~

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

- C.

Sample: The filtrate obtained in *Identification test A*

Analysis: To the *Sample* add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline, shake by mechanical means for 10 min, and add 5 mL of 3 N hydrochloric acid to acidify. Chill, collect the precipitated disulfonamide of ethylenediamine, and wash with water. Recrystallize the washed precipitate from water, and dry at 105° for 1 h.

Acceptance criteria: The dried precipitate melts at 164°–171°.

ASSAY

Change to read:

- Procedure

~~**Sample solution:** Transfer the equivalent to 2 g of aminophylline, from finely powdered Tablets (NLT 20), to a 200-mL volumetric flask with the aid of a mixture of 50 mL of water and 15 mL of 6 N ammonium hydroxide. Allow to stand for 30 min with frequent shaking, warming to 50° if necessary to dissolve the aminophylline. Cool the mixture to room temperature if it has been warmed, and add water to volume. Centrifuge 50 mL of the mixture, and pipet a portion of the clear supernatant, equivalent to 250 mg of aminophylline, into a 250-mL conical flask, and dilute with water if necessary to make 40 mL. Add 8 mL of 6 N ammonium hydroxide and 20.0 mL of 0.1 N silver nitrate VS, mix, heat to boiling, and continue boiling for 15 min. Cool to between 5° and 10° for 20 min; filter, preferably through a filtering crucible under reduced pressure; and wash the precipitate with three 10-mL portions of water. Acidify the combined filtrate and washings with nitric acid, and add an additional 3 mL of the acid. Cool, and add 2 mL of ferric ammonium sulfate TS.~~

~~**Titrimetric system**~~

~~**Mode:** Residual titration~~

~~**Titrant:** 0.1 N ammonium thiocyanate VS~~

~~**Endpoint detection:** Visual~~

~~**Analysis:** Titrate the excess silver nitrate with *Titrant*. Each mL of 0.1 N silver nitrate is equivalent to 18.02 mg of theophylline (C₇H₈N₄O₂).~~

■ **Solution A:** 10 mM ammonium acetate prepared as follows. Transfer 770.8 mg of

ammonium acetate to a 1-L volumetric flask, and dissolve in water to 80% of the flask volume. Adjust with glacial acetic acid to a pH of 5.5 and dilute with water to volume. Pass through a suitable filter of 0.2- μ m pore size.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

Impurity stock solution: 25 μ g/mL of USP Theophylline Related Compound F RS in water

System suitability solution: 0.8 mg/mL of USP Theophylline RS and 1 μ g/mL of USP Theophylline Related Compound F RS in water prepared as follows. Transfer 21 mg of USP Theophylline RS to a 25-mL volumetric flask and add 15 mL of water. Sonicate to dissolve, add 1 mL of *Impurity stock solution*, and dilute with water to volume.

Standard solution: 0.17 mg/mL of USP Theophylline RS in water. Sonicate to dissolve as needed.

Sample solution: Nominally 0.17 mg/mL of anhydrous theophylline from NLT 20 finely powdered Tablets in water prepared as follows. Transfer 34 mg of anhydrous theophylline from a portion of the powder to a 200-mL volumetric flask. Add 20 mL of water and mix for 1 min. Add an additional 140 mL of water and sonicate for 30 min. Dilute with water to volume. Pass through a suitable filter of 0.22- μ m pore size, discarding the first 2–3 mL.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 2.1-mm \times 10-cm; 1.7- μ m packing L1

Column temperature: 40 $^{\circ}$

Flow rate: 0.4 mL/min

Injection volume: 1 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline (C₇H₈N₄O₂) in the portion

of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of theophylline from the *Sample solution*

r_S

= peak response of theophylline from the *Standard solution*

C_S

= concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of theophylline in the *Sample solution* (mg/mL)

■ 1S (USP39)

Acceptance criteria: 93.0%–107.0%

OTHER COMPONENTS

• Content of Ethylenediamine

Sample solution: Transfer a portion of the powdered Tablets, equivalent to 350 mg of aminophylline, prepared in the *Assay*, into a 100-mL conical flask. Add 20 mL of water, and digest at 50°, with frequent shaking, for 30 min. Cool, filter into a 250-mL conical flask, and wash with water until the last washing is neutral to litmus. Use the combined filtrate and washings.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N hydrochloric acid VS

Endpoint detection: Visual

Analysis: Add methyl orange TS to the *Sample solution*, and titrate. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine (C₂H₈N₂).

Acceptance criteria: 140–190 mg of ethylenediamine (C₂H₈N₂) per g of theophylline (C₇H₈N₄O₂) found in the *Assay*

PERFORMANCE TESTS

• Dissolution 〈 711 〉

For uncoated or plain coated tablets

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: USP Theophylline RS in *Medium*

Sample solution: Proceed as directed in the chapter for sample. Dilute with water to a concentration that is similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: UV about 269 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved.

Tolerances: NLT 75% (*Q*) of the labeled amount of theophylline ($C_7H_8N_4O_2$) is dissolved.

• **Uniformity of Dosage Units** 〈 905 〉

Procedure for content uniformity

Standard solution: 10 $\mu\text{g/mL}$ of USP Theophylline RS

Sample solution: Place 1 Tablet in a 250-mL volumetric flask, add 200 mL of water, and shake by mechanical means until disintegration is complete. Add water to volume. Filter a portion of the mixture, discarding the first 20 mL of the filtrate.

Instrumental conditions

Mode: UV

Analytical wavelength: About 269 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_{\bar{U}}$ absorbance of the *Sample solution*

$A_{\bar{S}}$ absorbance of the *Standard solution*

$C_{\bar{S}}$ concentration of USP Theophylline RS in the *Standard solution* ($\mu\text{g/mL}$)

$C_{\bar{U}}$ nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

Add the following:

■ • **Organic Impurities**

Solution A, Solution B, Mobile phase, Impurity stock solution, System suitability solution, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 2.0 $\mu\text{g/mL}$ each of USP Theophylline RS and USP Theophylline Related Compound D RS in water

Sample solution: Nominally 1.0 mg/mL of anhydrous aminophylline from NLT 20 finely powdered Tablets in water prepared as follows. Transfer 10 mg of anhydrous aminophylline from a portion of the powder to a 10-mL volumetric flask. Add 5 mL of water and sonicate for 30 min. Dilute with water to volume. Pass through a suitable filter of 0.22- μm pore size, discarding the first 2-3 mL.

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See Table 2 for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

Relative standard deviation: NMT 3.0% for each peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of theophylline related compound D in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of theophylline related compound D from the *Sample solution*

r_S peak response of theophylline related compound D from the *Standard solution*

C_S concentration of USP Theophylline Related Compound D RS in the *Standard solution* (mg/mL)

C_U nominal concentration of aminophylline in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any other individual unspecified degradation product from the *Sample solution*

r_S peak response of theophylline from the *Standard solution*

C_S concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U nominal concentration of aminophylline in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound Ca,b	0.36	—
Theophylline related compound Ba,c	0.63	—
Theophylline related compound D	0.69	0.2
Dimethyl uric acida,d	0.76	—
Theobrominea,e	0.82	—
Theophylline	1.0	—
Theophylline related compound Fa	1.09	—
Caffeinea	1.20	—
Any other individual unspecified degradation product	—	0.2
Total impurities	—	0.5

^a Process impurity included for identification only and not to be included in the calculation of total degradation products.

^b *N*-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide.

c 3-Methyl-1*H*-purine-2,6-dione.

d 1,3-Dimethyl-7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione.

e 3,7-Dihydro-3,7-dimethylpurine-2,6(1*H*)-dione.

■ 1S (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers.

■ Store at controlled room temperature. ■ 1S (USP39)

- **Labeling:** Label the Tablets to state the content of anhydrous theophylline.

Change to read:

- **USP Reference Standards** { 11 }

USP Theophylline RS

■ USP Theophylline Related Compound D RS

N-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide.

C₆H₁₀N₄O 154.17

USP Theophylline Related Compound F RS

7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

C₉H₁₂N₄O₃ 224.22

■ 1S (USP39)

BRIEFING

Argatroban, PF 39(3) [May–June 2013]. On the basis of comments received, the previously published monograph proposal for this drug substance in PF 39(3) is canceled and replaced with the following new proposal. The liquid chromatographic procedures in the *Assay* and the tests for *Organic Impurities*, *Content of Argatroban Related Compound C*, and *Content of Stereoisomers* are proposed based on analysis performed with the Inertsil ODS-3 brand of L1 column. The typical retention times for (*R*)-argatroban and (*S*)-argatroban are about 31 and 32 min based on conditions specified for the *Assay* and test for *Organic Impurities*. The typical retention times for (*R*)-argatroban and (*S*)-argatroban are about 66 and 71 min based on conditions specified for *Content of Argatroban Related Compound C*. The typical retention times for (*R*)-argatroban and (*S*)-argatroban are about 49 and 52 min based on conditions specified for *Content of Stereoisomers*.

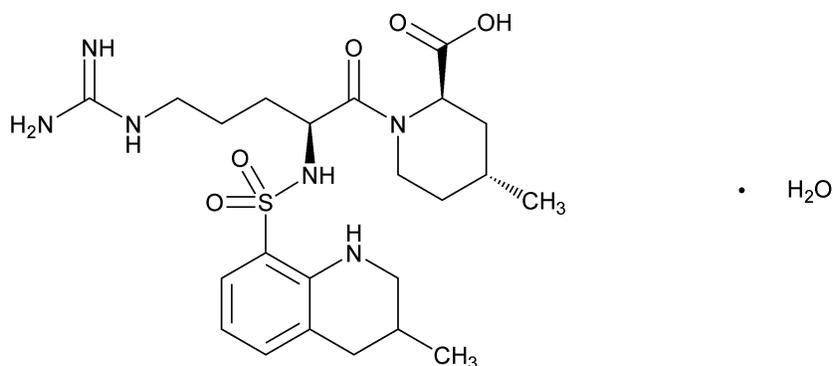
(SM2: S. Ramakrishna.)

Correspondence Number—C137076; C134779

Comment deadline: July 31, 2015

Add the following:

■ Argatroban



C₂₃H₃₆N₆O₅·H₂O 526.65

2-Piperidinecarboxylic acid, 1-[(S)-5-[(aminoiminomethyl)amino]-1-oxo-2-[(1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]amino}pentyl]-4-methyl-, (2R,4R)-monohydrate; (2R,4R)-4-Methyl-1-{N²-[(1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]-l-arginyl}piperidinecarboxylic acid, monohydrate [141396-28-3].

DEFINITION

Argatroban contains NLT 98.0% and NMT 102.0% of argatroban (C₂₃H₃₆N₆O₅S), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. Infrared Absorption** < 197K >
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

[Note—It is recommended to keep all solutions containing argatroban at about 4°.]

Solution A: 10 mM ammonium acetate and 5 mM sodium 1-heptanesulfonate

Solution B: Acetonitrile and methanol (500:300)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
20	60	40
35	50	50
50	20	80
60	20	80
60.1	60	40
72.1	60	40

Standard solution: 4 mg/mL of USP Argatroban RS in methanol

Sample solution: 4 mg/mL of Argatroban in methanol

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 259 nm

Column: 4.6-mm × 25-cm; 3-μm packing L1

Temperatures

Column: 50°

Autosampler: 4°

Flow rate: 0.6 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for both peaks

Relative standard deviation: NMT 1.0% for the sum of the peak responses of (R)-argatroban and (S)-argatroban

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of argatroban (C₂₃H₃₆N₆O₅S) in the portion of Argatroban taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak responses of (R)-argatroban and (S)-argatroban from the *Sample solution*

r_S = sum of the peak responses of (R)-argatroban and (S)-argatroban from the *Standard solution*

C_S = concentration of USP Argatroban RS in the *Standard solution* (mg/mL)

C_U = concentration of Argatroban in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% of argatroban (C₂₃H₃₆N₆O₅S) on the anhydrous and solvent-free basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%
- **Organic Impurities**

[Note—It is recommended to keep all solutions containing argatroban at about 4°.]

Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Sensitivity solution: 2 μg/mL of USP Argatroban RS in methanol

Standard solution: 4 μg/mL each of USP Argatroban RS, USP Argatroban Related Compound A RS, and USP Argatroban Related Compound B RS in methanol

System suitability

Samples: *Sensitivity solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between (*R*)-argatroban and (*S*)-argatroban, *Standard solution*

Signal-to-noise ratio: NLT 10 for (*R*)-argatroban, *Sensitivity solution*

Relative standard deviation: NMT 5% for all peaks. For argatroban, use the sum of the peak responses of (*R*)-argatroban and (*S*)-argatroban, *Standard solution*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of argatroban related compound A and argatroban related compound B in the portion of Argatroban taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of argatroban related compound A or argatroban related compound B from the *Sample solution*

r_S peak response of argatroban related compound A or argatroban related compound B from the *Standard solution*

C_S concentration of USP Argatroban Related Compound A RS or USP Argatroban Related Compound B RS in the *Standard solution* (mg/mL)

C_U concentration of Argatroban in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Argatroban taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any unspecified impurity from the *Sample solution*

r_S sum of the peak responses of (*R*)-argatroban and (*S*)-argatroban from the *Standard solution*

C_S concentration of USP Argatroban RS in the *Standard solution* (mg/mL)

C_U concentration of Argatroban in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Argatroban related compound A ^a	0.23	0.15
Argatroban related compound B ^b	0.39	0.15
(<i>R</i>)-Argatroban ^c	1.00	—
(<i>S</i>)-Argatroban ^d	1.03	—
Any unspecified impurity	—	0.10
Total impurities ^e	—	0.5

- a (2*R*,4*R*)-1-[*N*⁸-Nitro-*N*²-(3-methylquinoline-8-sulfonyl)-l-arginyl]-4-methylpiperidine-2-carboxylic acid.
- b Ethyl (2*R*,4*R*)-1-[*N*⁸-nitro-l-arginyl]-4-methylpiperidine-2-carboxylate hydrochloride.
- c (2*R*,4*R*)-4-Methyl-1-{*N*²-[*(R)*-1,2,3,4-tetrahydro-3-methyl-8-quinolyl]sulfonyl}-l-arginyl}pipercolic acid.
- d (2*R*,4*R*)-4-Methyl-1-{*N*²-[*(S)*-1,2,3,4-tetrahydro-3-methyl-8-quinolyl]sulfonyl}-l-arginyl}pipercolic acid.
- e Total impurities include specified and unspecified impurities and argatroban related compound C from the test for *Content of Argatroban Related Compound C*.

● **Content of Argatroban Related Compound C**

[Note—It is recommended to keep all solutions containing argatroban at about 4°.]

Buffer: 10 mM ammonium acetate and 5 mM sodium 1-heptanesulfonate

Solution A: Acetonitrile, dehydrated alcohol, and *Buffer* (80:240:680)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Acetonitrile (%)
0	100	0
70	100	0
71	30	70
91	30	70
92	100	0
102	100	0

Sensitivity solution: 4 µg/mL of USP Argatroban Related Compound C RS in methanol

System suitability solution: 10 mg/mL of USP Argatroban RS and 0.1 mg/mL of USP Argatroban Related Compound C RS in methanol

Sample solution: 10 mg/mL of Argatroban in methanol

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Samples: *Sensitivity solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 1.4 between argatroban related compound C and (*R*)-argatroban, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Relative standard deviation: NMT 2.5% for argatroban related compound C, *System suitability solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of argatroban related compound C in the portion of Argatroban taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_{\bar{C}}$ peak response of argatroban related compound C from the *Sample solution*

$r_{\bar{T}}$ total of all peak responses from the *Sample solution*

Acceptance criteria: See Table 4.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Argatroban related compound C ^a	0.94	0.15
(R)-Argatroban	1.00	—
(S)-Argatroban	1.07	—

^a (2R,4R)-1-[N⁸-Amino-N²-(3-methyl-1,2,3,4-tetrahydroquinoline-8-sulfonyl)-l-arginyl]-4-methylpiperidine-2-carboxylic acid.

• **Content of Stereoisomers**

[Note—It is recommended to keep all solutions containing argatroban at about 4 °.]

Mobile phase: Methanol and water (520:480)

Standard solution: 0.16 mg/mL of USP Argatroban RS in methanol

Sample solution: 0.16 mg/mL of Argatroban in methanol

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 259 nm

Column: 4.6-mm × 25-cm; 3-μm packing L1

Column temperature: 50 °

Flow rate: 0.6 mL/min

Injection volume: 10 μL

Run time: NLT 1.4 times the retention time of (R)-argatroban

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between (R)-argatroban and (S)-argatroban

Relative standard deviation: NMT 2.0% for (R)-argatroban and (S)-argatroban

Analysis

Sample: *Sample solution*

Calculate the percentage of (R)-argatroban and (S)-argatroban in the portion of Argatroban taken:

$$\text{Result} = [(r_U \text{ or } r_S)/(r_U + r_S)] \times 100$$

$r_{\bar{R}}$ peak response of (R)-argatroban from the *Sample solution*

$r_{\bar{S}}$ peak response of (S)-argatroban from the *Sample solution*

Acceptance criteria: See Table 5.

Table 5

Name	Relative Retention Time	Acceptance Criteria, (%)
(R)-Argatroban ^a	1.00	63–67
(S)-Argatroban ^b	1.06	33–37
^a (2R,4R)-4-Methyl-1- $\{N^2-[(R)-1,2,3,4\text{-tetrahydro-}3\text{-methyl-}8\text{-quinoly}]sulfonyl\}$ -l-arginyl}pipecolic acid.		

^b (2R,4R)-4-Methyl-1- $\{N^2-[(S)-1,2,3,4\text{-tetrahydro-}3\text{-methyl-}8\text{-quinoly}]sulfonyl\}$ -l-arginyl}pipecolic acid.

SPECIFIC TESTS

- **Water Determination** $\langle 921 \rangle$, *Method Ia*: 3.0%–6.0%
- **Bacterial Endotoxins Test** $\langle 85 \rangle$: NMT 2.0 Endotoxin Units/mg of argatroban
- **Microbial Enumeration Tests** $\langle 61 \rangle$ and **Tests for Specified Microorganisms** $\langle 62 \rangle$: The total aerobic microbial count is NMT 10^2 cfu/g, and the total combined molds and yeasts count is NMT 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage**: Preserve in tight, light resistant containers, and store at controlled room temperature.
- **USP Reference Standards** $\langle 11 \rangle$
 - USP Argatroban RS
 - USP Argatroban Related Compound A RS
(2R,4R)-1- $[N^8\text{-Nitro-}N^2\text{-}(3\text{-methylquinoline-}8\text{-sulfonyl})\text{-l-arginyl}]\text{-}4\text{-methylpiperidine-}2\text{-carboxylic acid}$.
 $C_{23}H_{31}N_7O_7S$ 549.60
 - USP Argatroban Related Compound B RS
Ethyl (2R,4R)-1- $[N^8\text{-nitro-l-arginyl}]\text{-}4\text{-methylpiperidine-}2\text{-carboxylate hydrochloride}$.
 $C_{15}H_{28}N_6O_5 \cdot HCl$ 408.88
 - USP Argatroban Related Compound C RS
(2R,4R)-1- $[N^8\text{-Amino-}N^2\text{-}(3\text{-methyl-}1,2,3,4\text{-tetrahydroquinoline-}8\text{-sulfonyl})\text{-l-arginyl}]\text{-}4\text{-methylpiperidine-}2\text{-carboxylic acid}$.
 $C_{23}H_{37}N_7O_5S$ 523.65
 - USP Endotoxin RS

■ 1S (USP39)

BRIEFING

Barium Hydroxide Lime, *USP 38* page 2369. In preparation for the omission of the flame tests from *Identification Tests—General* $\langle 191 \rangle$, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to revise *Identification* test *B* to include only the *Chemical Identification Tests* as described in the proposal. The optional test for the identification of potassium using the flame test is proposed for deletion for consistency with the *Soda Lime* monograph as both of

these articles are used only to remove carbon dioxide from anesthetic gases.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R. Ravichandran.)

Correspondence Number—C157837

Comment deadline: July 31, 2015

Barium Hydroxide Lime

DEFINITION

Barium Hydroxide Lime is a mixture of barium hydroxide octahydrate and Calcium Hydroxide. It may also contain Potassium Hydroxide and may contain an indicator that is inert toward anesthetic gases such as Ether, Cyclopropane, and Nitrous Oxide and that changes color when the Barium Hydroxide Lime no longer can absorb carbon dioxide.

[**Caution**—Because Barium Hydroxide Lime contains a soluble form of barium, it is toxic if swallowed.]

IDENTIFICATION

- **A.**

Analysis: Place a granule of it on a piece of moistened red litmus paper.

Acceptance criteria: The paper turns blue immediately.

Delete the following:

- ~~• **B. Identification Tests—General, Barium, Calcium, and Potassium** < 191 >~~

~~**Sample solution:** 100 mg/mL in 6 N acetic acid~~

~~**Acceptance criteria:** Meets the requirements for *Barium* and for *Calcium*, and it may meet the requirement of the flame test for *Potassium*. ■ 1S (USP39)~~

Add the following:

- ~~• **B. Identification Tests—General** < 191 >, *Barium*~~

~~**Sample solution:** 100 mg/mL in 6 N acetic acid~~

~~**Acceptance criteria:** Meets the requirements ■ 1S (USP39)~~

Add the following:

- ~~• **C. Identification Tests—General** < 191 >, *Calcium*~~

~~**Sample solution:** 100 mg/mL in 6 N acetic acid~~

~~**Acceptance criteria:** Meets the requirements ■ 1S (USP39)~~

SPECIFIC TESTS

- **Particle Size Distribution Estimation by Analytical Sieving** < 786 >

Sample: 100 g

Analysis: Screen the *Sample* for 5 min as directed in the chapter, using a mechanical shaker.

Acceptance criteria: It passes completely through a No. 2 standard-mesh sieve, and NMT 2.0% passes through a No. 40 standard-mesh sieve. NMT 7.0% is retained on the coarse-mesh sieve, and NMT 15.0% passes through the fine-mesh sieve designated on the label.

- **Loss on Drying** 〈 731 〉

Sample: 10 g

Analysis: Weigh the *Sample* in a tared weighing bottle, and dry at 105° for 2 h.

Acceptance criteria: 11.0%–16.0%

- **Hardness**

Sample: 200 g

Analysis: Screen the *Sample* on a mechanical sieve shaker (see 〈 786 〉) with a frequency of oscillation of 285 ± 3 cycles/min, for 3 min, to remove granules coarser than 4-mesh and finer than 8-mesh. Weigh 50 g of the granules retained on the screen, and place them in a hardness pan that has a diameter of 200 mm and a concave brass bottom. The bottom of the pan is 7.9 mm thick at the circumference and 3.2 mm thick at the center and has an inside spherical radius of curvature of 109 cm. Add 15 steel balls of 7.9-mm diameter, and shake on a mechanical sieve shaker for 30 min. Remove the steel balls, brush the contents of the hardness pan onto a sieve of the fine-mesh size designated on the label, shake for 3 min on the mechanical sieve shaker, and weigh.

Acceptance criteria: The percentage of Barium Hydroxide Lime retained on the screen is NLT 75.0% and represents the hardness.

- **Carbon Dioxide Absorbency**

Analysis: Fill the lower transverse section of a U-shaped drying tube of about 15-mm internal diameter and 15-cm height with loosely packed glass wool. Place in one arm of the tube about 5 g of anhydrous calcium chloride, and weigh the tube and the contents. Into the other arm of the tube, place 9.5–10.5 g of Barium Hydroxide Lime, and again weigh. Insert stoppers in the open arms of the U-tube, and connect the side tube of the arm filled with Barium Hydroxide Lime to a calcium chloride drying tube, which in turn is connected to a suitable supply source of carbon dioxide. Pass the carbon dioxide through the U-tube at a rate of 75 mL/min for 20 min, timed. Disconnect the U-tube, cool to room temperature, remove the stoppers, and weigh.

Acceptance criteria: The increase in weight is NLT 19.0% of the weight of Barium Hydroxide Lime used for the test.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** If an indicator has been added, the name and color change of such indicator are stated on the container label. The container label indicates also the mesh size in terms of standard-mesh sieve sizes (see *Powder Fineness* 〈 811 〉).

BRIEFING

Biological Indicator for Dry-Heat Sterilization, Paper Carrier, *USP 38* page 2435. This monograph is being proposed for deletion, and appropriate content from this monograph has been moved into *USP* general information chapter *Biological Indicators for Sterilization* 〈 1229.5 〉, which was proposed in *PF 41(2)* [Mar.–Apr. 2015]. No monograph or chapter is impacted by

this omission.

(GCM: R. Tirumalai.) Correspondence Number—C157029

Comment deadline: July 31, 2015

Delete the following:

■ Biological Indicator for Dry Heat Sterilization, Paper Carrier

» ~~Biological Indicator for Dry Heat Sterilization, Paper Carrier, is a defined preparation of viable spores made from a culture derived from a specified strain of *Bacillus subtilis* subspecies *niger*, on a suitable grade of paper carrier, individually packaged in a container readily penetrable by dry heat, and characterized for predictable resistance to dry heat sterilization. The packaged Biological Indicator for Dry Heat Sterilization, Paper Carrier, has a particular labeled spore count per carrier of not less than 10^4 and not more than 10^9 spores. When labeled for and subjected to dry heat sterilization conditions at a particular temperature, it has a survival time and kill time appropriate to the labeled spore count and to the decimal reduction value (*D value*, in min) of the preparation, specified by:~~

~~*Survival time* (in minutes) = not less than (labeled *D value*) × (log labeled spore count per carrier − 2); and~~

~~*Kill time* (in minutes) = not more than (labeled *D value*) × (log labeled spore count per carrier + 4).~~

~~**Packaging and storage**—Preserve in the original package under the conditions recommended on the label, and protect from light, toxic substances, excessive heat, and moisture. The packaging and container materials do not adversely affect the performance of the article used as directed in the labeling.~~

~~**Expiration date**—The expiration date is determined on the basis of stability studies and is not less than 18 months from the date of manufacture, the date of manufacture being the date on which the first determination of the total viable spore count was made.~~

~~**Labeling**—Label it to state that it is a Biological Indicator for Dry Heat Sterilization, Paper Carrier; to indicate its *D value* and the method used to determine such *D value*, i.e., by spore count or fraction negative procedure after graded exposures to the sterilization conditions; the survival time and kill time under the specified sterilization conditions stated on the label; its particular total viable spore count, with a statement that such count has been determined after preliminary heat treatment; and its recommended storage conditions. State in the labeling the size of the paper carrier, the strain and ATCC number from which the spores were derived, and instructions for spore recovery and for safe disposal of the indicator. Indicate in the labeling that the stated *D value* is reproducible only under the exact conditions under which it was determined, that the user would not necessarily obtain the same result, and that the user should determine the suitability of the biological indicator for the particular use.~~

Identification—The biological indicator organism complies substantially with the morphological, cultural, and biochemical characteristics of the strain of *Bacillus subtilis*, ATCC No. 9372, designated subspecies *niger*, detailed for that biological indicator organism under *Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier*.

Resistance performance tests—

D value— Proceed as directed for the relevant procedure for *D value* under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the determined *D value* is within 20% of the labeled *D value* for the selected sterilizing temperature, and if the confidence limits of the estimate are within 10% of the determined *D value*.

Survival time and kill time— Proceed as directed for *Survival Time and Kill Time* in the section *Dry Heat Sterilization, Paper Carrier*, under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if all of the specimens subjected to dry-heat sterilization for the survival time show evidence of growth, while none of the specimens subjected to dry-heat sterilization for the kill time show growth. If for either the survival time test or the kill time test not more than 1 specimen out of both groups fails the survival requirement or the kill requirement (whichever is applicable), continue the corresponding test with 4 additional groups, each consisting of 20 specimens, according to the procedure described. If all of the additional specimens subjected to dry-heat sterilization either meet the survival requirement for the survival time test or meet the kill requirement for the kill time test, whichever is applicable, the requirements of the test are met.

Total viable spore count— Proceed as directed for *Total Viable Spore Count* under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the log average number of viable spores per carrier is not less than 0.3 log of the labeled spore count per carrier and does not exceed the log labeled spore count per carrier by 0.48.

Purity—

Presence of contamination by other microorganisms— By examination of the spores on a suitable plate culture medium, there is no evidence of contamination with other microorganisms.

Disposal—Prior to destruction or discard, sterilize it by steam at 121° for not less than 30 minutes, or by not less than an equivalent method recommended by the manufacturer. This includes a test strip employed in any test procedures for the strips themselves. ■1S (USP39)

BRIEFING

Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier, USP 38 page 2435. This monograph is being proposed for deletion, and appropriate content from this monograph has been moved into USP general information chapter *Biological Indicators for Sterilization* (1229.5), which was proposed in PF 41(2) [Mar.–Apr. 2015]. No monograph or chapter is impacted by this omission.

(GCM: R. Tirumalai.) Correspondence Number—C157030

Comment deadline: July 31, 2015

Delete the following:**■ Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier**

» ~~Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier, is a defined preparation of viable spores made from a culture derived from a specified strain of *Bacillus subtilis* subspecies *niger* on a suitable grade of paper carrier, individually packaged in a suitable container readily penetrable by ethylene oxide sterilizing gas mixture, and characterized for predictable resistance to sterilization with such gas mixture. The packaged Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier, has a particular labeled spore count per carrier of not less than 10^4 and not more than 10^9 spores. Where labeled for and subjected to particular ethylene oxide sterilization conditions of a stated gaseous mixture, temperature, and relative humidity, it has a survival time and kill time appropriate to the labeled spore count and to the decimal reduction value (*D value*, in minutes) of the preparation, specified by:~~

~~*Survival time* (in minutes) = not less than (labeled *D value*) × (log labeled spore count per carrier — 2), and~~

~~*Kill time* (in minutes) = not more than (labeled *D value*) × (log labeled spore count per carrier + 4).~~

~~**Packaging and storage**—Preserve in the original package under the conditions recommended on the label, and protect it from light, toxic substances, excessive heat, and moisture. The packaging and container material shall be such that it does not adversely affect the performance of the article used as directed in the labeling.~~

~~**Expiration date**—The expiration date is determined on the basis of stability studies and is not less than 18 months from the date of manufacture, the date of manufacture being the date on which the first determination of the total viable spore count was made.~~

~~**Labeling**—Label it to state that it is a Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier; to indicate its *D value*, the method used to determine such *D value*, i.e., by spore count or fraction negative procedure after graded exposures to the sterilization conditions; the survival time and kill time under specified sterilization conditions stated on the label; its particular total viable spore count, with a statement that such count has been determined after preliminary heat treatment; and its recommended storage conditions. State in the labeling the size of the paper carrier, the strain and ATCC number from which the spores were derived, and instructions for spore recovery and for safe disposal of the indicator. Indicate in the labeling that the stated *D value* is reproducible only under the exact conditions under which it was determined, that the user would not necessarily obtain the same result, and that the user should determine the suitability of the biological indicator for the particular use.~~

~~**Identification**—The biological indicator organism complies substantially with the morphological, cultural, and biochemical characteristics of the strain of *Bacillus subtilis*, ATCC No. 9372,~~

designated subspecies *niger*: under microscopic examination it consists of Gram-positive rods of width 0.7 to 0.8 μm , and length 2 to 3 μm ; the endospores are oval and central and the cells are not swollen; when incubated aerobically in appropriate media at 30° to 35°, growth occurs within 24 hours, and similar inoculated media incubated concomitantly at 55° to 60° show no evidence of growth in the same period; agar colonies have a dull appearance and may be cream or brown colored; when incubated in nutrient broth it develops a pellicle, and shows little or no turbidity; when examined under conventional biochemical tests for microbial characterization, it develops a black pigment with tyrosine, it liquefies gelatin, utilizes citrate but not propionate or hippurate, reduces nitrate, and hydrolyzes both starch and glucose with no gas production; it shows a positive catalase reaction and gives a positive result with the Voges-Proskauer test.

Resistance performance tests—

D value— Proceed as directed for the relevant procedure for *D value* under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the determined *D value* is within 20% of the labeled *D value* for the selected sterilizing temperature and if the confidence limits of the estimate are within 10% of the determined *D value*.

Survival time and kill time— Proceed as directed for *Survival Time and Kill Time* in the section *Ethylene Oxide Sterilization, Paper Carrier*, under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if all of the specimens subjected to the ethylene oxide sterilization conditions for the survival time show evidence of growth, while none of the specimens subjected to the ethylene oxide sterilization conditions for the kill time shows evidence of growth. If for either the survival time test or the kill time test, not more than 1 specimen out of both groups fails the survival requirement or the kill requirement (whichever is applicable), continue the corresponding test with 4 additional groups, each consisting of 20 specimens, according to the procedure described. If all of the additional specimens subjected to ethylene oxide sterilization meet either the survival requirement for the survival time test or the kill requirement for the kill time test, whichever is applicable, the requirements of the test are met.

Total viable spore count— Follow the procedure for *Total Viable Spore Count* in the section *Ethylene Oxide Sterilization, Paper Carrier*, under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the log average number of viable spores per carrier is not less than 0.3 log of the labeled spore count per carrier and does not exceed the log labeled spore count per carrier by 0.48.

Purity—

Presence of contamination by other microorganisms— By examination of the spores on a suitable plate culture medium, there is no evidence of contamination with other microorganisms.

Disposal— Prior to destruction or discard, sterilize it by steam at 121° for not less than 30 minutes, or by not less than an equivalent method recommended by the manufacturer. This includes a strip used in test procedures for strips themselves. ■ 1S (USP39)

BRIEFING

Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization,

Liquid Spore Suspensions, *USP 38* page 2436. This monograph is being proposed for deletion, and appropriate content from this monograph has been moved into *USP* general information chapter *Biological Indicators for Sterilization* { 1229.5 }, which was proposed in *PF 41(2)* [Mar.–Apr. 2015]. No monograph or chapter is impacted by this omission.

(GCM: R. Tirumalai.) Correspondence Number—C157031

Comment deadline: July 31, 2015

Delete the following:

■ Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Liquid Spore Suspensions

» ~~Liquid spore suspensions may be used to prepare biological indicators for moist heat, dry heat, and gaseous modes of sterilization. On the basis of the intended sterilization use, the suspension is prepared inoculated from a culture of viable spores derived from one of several sterilization resistant microorganisms. Cultures used for liquid spore suspensions include, among others, the following: *Clostridium sporogenes*, *Geobacillus stearothermophilus* (formerly *B. stearothermophilus*), *Bacillus atrophaeus* (formerly *B. subtilis*), *Bacillus subtilis*, or *Bacillus coagulans*. Each tube or container containing the spore suspension is individually packaged for use. The packaged biological indicator spore suspension has a particular labeled spore count of not less than 10^3 , and not more than 10^9 , spores per mL of suspension. The suspending medium or vehicle is identified according to chemical composition. It has a survival time and kill time appropriate to the labeled spore count, and to the decimal reduction value (the *D value*, in minutes), specified by the following:~~

~~*Survival time* (in minutes) = not less than (labeled *D value*) × (log of labeled spore count per mL from 1:100 dilution of original suspension – 2);~~
and

~~*Kill time* (in minutes) = not more than (labeled *D value*) × (log of labeled spore count per mL from 1 : 100 dilution of original suspension + 4).~~

Packaging and storage—~~Preserve in the original tube or container under the conditions recommended on the label, and protect the contents of the tube or container from light, toxic substances, and excessive heat. The materials of composition of the tube or container must not adversely affect the performance of the spore suspension.~~

Expiration date—~~The expiration date is determined on the basis of stability studies. The date of manufacture is the date on which the first determination of the total viable count was made.~~

Labeling—~~Label the spore suspension tube or container or package insert to state that it is a biological indicator spore suspension for use in label specified applications for moist heat, dry~~

heat, and/or gaseous sterilization. State the biological indicator *D value* obtained under defined exposures to stated sterilization conditions using the Survival Curve Method of *D value* analysis. State the *Survival time and kill time* for the biological indicator suspension under specified conditions on the label. The total viable spore count per mL of the suspension following heat shock treatment must also appear on the label. State in the labeling the strain and ATCC number of the microorganisms used in the spore suspension and instructions for spore recovery and for safe disposal of the suspension. Indicate in the labeling that the stated *D value* is reproducible only under the exact conditions determined by the manufacturer and that the user would not necessarily obtain the same results if different exposure conditions were used. State that the user should determine the suitability of the biological indicator spore suspension for the user's particular purpose and exposure conditions.

Identification—Identification for the biological indicator is of lesser importance than the more relevant concerns of population and resistance to the sterilization processes. The manufacturer should identify the species used.

D value—If the biological indicators are being used in moist heat or dry heat sterilization, proceed as directed for the relevant procedure in the section *D Value Determination under Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the determined *D value* is within 20% of the labeled *D value* for the selected sterilizing conditions, and if the confidence limits of the estimate are within 10% of the determined *D value*. The *D value* determination method used should be that identified by the biological indicator manufacturer.

Survival time and kill time—Follow the procedure under *Survival Time and Kill Time* in the section *D Value Determination under Biological Indicators—Resistance Performance Tests* (55). The test is conducted using 1:100 dilution aliquots of the original suspension to inoculate carrier substrates that are most likely to be used by the purchaser of the spore suspensions for a given mode of sterilization. Following a total viable count analysis, the inoculated substrates are subjected to sterilization exposure conditions intended to indicate survival. The inoculated carriers must show evidence of growth among the exposed carriers. A second study is conducted to demonstrate the conditions necessary to result in total kill of the carriers. None of the carriers subjected to conditions designed to induce total kill should show growth. If for either the survival time test or the kill time test, not more than one carrier out of both groups fails the survival or kill requirements, continue the corresponding test with four additional groups, each consisting of 10 carriers, according to the procedure described. For biological indicators for use with moist heat or dry heat sterilization, if all of the additional specimens subjected to the specific sterilization process either meet the survival requirements for the survival time test or meet the kill requirement for the kill time test, whichever is applicable, the requirements are met.

Total viable spore count—Proceed as directed for *Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Liquid Spore Suspensions* in the section *Total Viable Spore Count* under *Biological Indicators—Resistance Performance Tests* (55). The requirements for this test are met if the total viable spore count within the suspension is within ± 1 log of the value stipulated by the manufacturer.

Purity—There is no evidence of contamination with other microorganisms following examination of spores recovered from the metal carriers using suitable plate culture medium.

~~**Shipment**—Spore suspensions must be shipped following EPA requirements for the shipment of biological and/or etiological agents.~~

~~**Disposal**—Spore suspensions that a user or manufacturer wishes to dispose of are first sterilized by moist heat by a process that achieves temperatures of approximately 121 °C for not less than 30 minutes. Alternative sterilization methods yielding equivalent or greater levels of lethality may be used. ■1S (USP39)~~

BRIEFING

Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Nonpaper Carriers, *USP 38* page 2437. This monograph is being proposed for deletion, and appropriate content from this monograph has been moved into *USP* general information chapter *Biological Indicators for Sterilization* { 1229.5 }, which was proposed in *PF 41(2)* [Mar.–Apr. 2015]. No monograph or chapter is impacted by this omission.

(GCM: R. Tirumalai.) Correspondence Number—C157160

Comment deadline: July 31, 2015

Delete the following:

■ Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Nonpaper Carriers

~~» Biological indicators for moist heat, dry heat, and gaseous modes of sterilization may be nonpaper carriers inoculated with a culture of viable spores derived from one of several sterilization resistant microorganisms, based on the intended sterilization use. Cultures used for inoculation of carriers include, among others, *Clostridium sporogenes*, *Geobacillus stearothermophilus* (formerly *B. stearothermophilus*), *Bacillus atrophaeus* (formerly *B. subtilis*), or *Bacillus coagulans*. The carriers should be individually packaged for use either within the package or for use upon removal from the package as an unpackaged biological indicator. The packaged biological indicator on the carrier has a particular labeled spore count of not less than 10^3 and not more than 10^9 spores per carrier. It has a survival time and kill time appropriate to the labeled spore count and to the decimal reduction value (*D value*, in minutes), specified by:~~

~~*Survival time* (in minutes) = not less than (labeled *D value*) × (log of labeled spore count per carrier – 2), and~~

~~*Kill time* (in minutes) = not more than (labeled *D value*) × (log of labeled spore count per carrier + 4).~~

~~**Packaging and storage**—Preserve in the original package under the conditions recommended on the label, and protect the package from light, toxic substances, excessive heat, and high~~

relative humidity or moisture. The packaging or container materials do not adversely affect the performance of the article used as directed in the labeling.

Expiration date—The expiration date is determined on the basis of stability studies and is not more than 18 months from the date of manufacture. The date of manufacture is the date on which the first determination of the total viable count was made.

Labeling—Label the package or package insert to state that it is a biological indicator prepared on a carrier for use in label specified applications for moist heat, dry heat, and/or gaseous sterilization. State the biological indicator *D value* obtained under defined exposures to stated sterilization conditions using the Survival Curve method, Spearman-Kärber method, or Stumbo-Murphy-Cochran method of *D value* analysis. State the survival time and kill time for the biological indicator carrier under specified conditions on the label. The total viable spore count per carrier following heat shock treatment must also appear on the label or package insert. State in the labeling the strain and ATCC number of the spore suspension used to inoculate the carriers and instructions for spore recovery and for safe disposal of the carriers. Indicate in the labeling that the stated *D value* is reproducible only under the exact conditions determined by the manufacturer and that the user would not necessarily obtain the same results if different exposure conditions were used. State that the user should determine the suitability of the carrier biological indicator for the user's particular purpose and exposure conditions.

Identification—Identification for the biological indicator is of less importance than the more relevant concerns of population and resistance to the sterilization processes. The manufacturer should identify the species used.

D value—Proceed as directed in the relevant procedure for *D Value Determination under Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the determined *D value* is within 20% of the labeled *D value* for the selected sterilizing conditions, and if the confidence limits of the estimate are within 10% of the determined *D value*. The *D value* determination method used should be that identified by the biological indicator manufacturer.

Survival time and kill time—Follow the procedure in the subsection *Survival Time and Kill Time* in the section *D Value Determination* under the chapter *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if all of the carriers subjected to sterilization exposure conditions intended to indicate survival show evidence of growth among the exposed carriers, while none of the carriers subjected to conditions designed to induce total kill show growth. If for either the survival test or the kill time test, not more than one carrier out of both groups fails the survival or kill requirements, continue the corresponding test with four additional groups, each consisting of 10 carriers, according to the procedure described. If all of the additional specimens subjected to the specific sterilization process either meet the survival requirements for the survival test time or meet the kill requirement for the kill test, whichever is applicable, the requirements are met.

Total viable spore count—Proceed as directed in the subsection *Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Nonpaper Carriers* in the section *Total Viable Spore Count* under the chapter *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the average number of viable spores per carrier are within $-50%$ and $+300%$ of the labeled count per carrier or within a lesser range that may be

~~stated by the manufacturer.~~

~~**Purity**—There is no evidence of contamination with other microorganisms following examination of spores recovered from the carriers using a suitable plate culture medium.~~

~~**Disposal**—Prior to destruction or discarding the carriers, sterilize by moist heat sterilization to ensure that the carrier surface is exposed to 121 °C for not less than 30 minutes, or by an equivalent method recommended by the manufacturer. ■1S (USP39)~~

BRIEFING

Biological Indicator for Steam Sterilization, Paper Carrier, *USP 38* page 2438. This monograph is being proposed for deletion, and appropriate content from this monograph has been moved into *USP* general information chapter *Biological Indicators for Sterilization* (1229.5), which was proposed in *PF* 41(2) [Mar.–Apr. 2015]. No monograph or chapter is impacted by this omission.

(GCM: R. Tirumalai.) Correspondence Number—C157032

Comment deadline: July 31, 2015

Delete the following:

■ **Biological Indicator for Steam Sterilization, Paper Carrier**

~~» Biological Indicator for Steam Sterilization, Paper Carrier, is a defined preparation of viable spores made from a culture derived from a specified strain of *Bacillus stearothermophilus*, on a suitable grade of paper carrier, individually packaged in a suitable container readily penetrable by steam, and characterized for predictable resistance to steam sterilization. The packaged Biological Indicator for Steam Sterilization, Paper Carrier, has a particular labeled spore count per carrier of not less than 10^4 and not more than 10^9 spores. When labeled for and subjected to steam sterilization conditions at a particular temperature, it has a survival time and kill time appropriate to the labeled spore count and to the decimal reduction value (*D value*, in minutes) of the preparation, specified by:~~

~~*Survival time* (in minutes) = not less than (labeled *D value*) × (log labeled spore count per carrier − 2); and~~

~~*Kill time* (in minutes) = not more than (labeled *D value*) × (log labeled spore count per carrier + 4).~~

~~**Packaging and storage**—Preserve in the original package under the conditions recommended on the label, and protect it from light, toxic substances, excessive heat, and moisture. The packaging and container materials do not adversely affect the performance of the article used as directed in the labeling.~~

Expiration date—The expiration date is determined on the basis of stability studies and is not less than 18 months from the date of manufacture, the date of manufacture being the date on which the first determination of the total viable spore count was made.

Labeling—Label it to state that it is a Biological Indicator for Steam Sterilization, Paper Carrier; to indicate its *D value*, the method used to determine such *D value*, i.e., by spore count or fraction negative procedure after graded exposures to the sterilization conditions; the survival time and kill time under specified sterilization conditions stated on the label; its particular total viable spore count with a statement that such count has been determined after preliminary heat treatment; and its recommended storage conditions. State in the labeling the size of the paper carrier, the strain and ATCC number from which the spores were derived, and instructions for spore recovery and for safe disposal of the indicator. Indicate in the labeling that the stated *D value* is reproducible only under the exact conditions under which it was determined; that the user would not necessarily obtain the same result and that the user should determine the suitability of the biological indicator for the particular use.

Identification—The biological indicator organism complies substantially with the morphological, cultural, and biochemical characteristics of the strain of *Bacillus stearothermophilus*, ATCC No. 7953 or 12980, whichever is stated in the labeling: under microscopic examination it consists of Gram positive rods with oval endospores in subterminally swollen cells; when incubated in nutrient broth for 17 hours and used to inoculate appropriate solid media, growth occurs when the inoculated media are incubated aerobically for 24 hours at 55 ° to 60 °, and similar inoculated media incubated concomitantly at 30 ° to 35 ° show no evidence of growth in the same period. When examined under conventional biochemical tests for microbial characterization, it shows a delayed weak positive catalase reaction, it does not utilize citrate, propionate or hippurate, it reduces nitrate, but it does not liquefy gelatin, and it gives a negative result with the Voges-Proskauer test. Organisms derived from ATCC strain No. 7953 show negative egg yolk and starch hydrolysis reactions, while those derived from ATCC strain No. 12980 show positive reactions in both tests.

Resistance performance tests—

D value— Proceed as directed for the relevant procedure for *D Value* under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the determined *D value* is within 20% of the labeled *D value* for the selected sterilizing temperature, and if the confidence limits of the estimate are within 10% of the determined *D value*.

Survival time and kill time— Follow the procedure for *Survival Time and Kill Time* in the section *Steam Sterilization, Paper Carrier, under Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if all of the specimens subjected to the steam sterilization for the survival time show evidence of growth, while none of the specimens subjected to the steam sterilization for the kill time shows growth. If for either the survival time test or the kill time test, not more than 1 specimen out of both groups fails the survival requirement or the kill requirement (whichever is applicable), continue the corresponding test with 4 additional groups, each consisting of 20 specimens, according to the procedure described. If all of the additional specimens subjected to steam sterilization either meet the survival requirement for the survival time test or meet the kill requirement for the kill time test, whichever is applicable, the requirements of the test are met.

~~Total viable spore count— Proceed as directed for *Total Viable Spore Count* in the section *Steam Sterilization, Paper Carrier*, under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the tests are met if the log average number of viable spores per carrier is not less than 0.3 log of the labeled spore count per carrier and does not exceed the log labeled spore count per carrier by 0.48.~~

~~**Purity—**~~

~~*Presence of contamination by other microorganisms—* By examination of the spores on a suitable plate culture medium, there is no evidence of contamination with other microorganisms.~~

~~**Disposal—** Prior to destruction or discard, sterilize it by steam at 121° for not less than 30 minutes, or by not less than an equivalent method recommended by the manufacturer. This includes a test strip employed in any test procedures for the strips themselves. ■ 1S (USP39)~~

BRIEFING

Biological Indicator for Steam Sterilization, Self-Contained, USP 38 page 2439. This monograph is being proposed for deletion, and appropriate content from this monograph has been moved into USP general information chapter *Biological Indicators for Sterilization* (1229.5), which was proposed in PF 41(2) [Mar.–Apr. 2015]. No monograph or chapter is impacted by this omission.

(GCM: R. Tirumalai.) Correspondence Number—C157033

Comment deadline: July 31, 2015

Delete the following:

■ **Biological Indicator for Steam Sterilization, Self-Contained**

~~» Biological Indicator for Steam Sterilization, Self-Contained, is a Biological Indicator for Steam Sterilization, Paper Carrier individually packaged in a suitable container readily penetrable by steam and designed to hold an appropriate bacteriological culture medium, so as to enable the packaged carrier, after subjection to saturated steam sterilization conditions, to be incubated in the supplied medium in a self-contained system. The supplied medium may contain a suitable indicator as a convenience for determining by a color change whether or not spores have survived. The design of the self-contained system is such that, after exposure to the specified sterilization conditions and inoculation of the medium under closed conditions as stated in the labeling, there is no loss of medium and inoculum during subsequent transport and handling, if done according to the provided instructions. The materials of which the self-contained system are made are such that there is no retention or release of any substance that may cause inhibition of growth of surviving spores under the incubation~~

~~conditions stated in the labeling.~~

Packaging and storage—~~Preserve in the original package under the conditions recommended on the label, and protect from light, from substances that may adversely affect the contained microorganisms, from excessive heat, and from moisture.~~

Expiration date—~~The expiration date is determined on the basis of stability studies and is not less than 18 months from the date of manufacture, the date of manufacture being the date on which the first determination of the total viable spore count was made.~~

Labeling—~~Label it to state that it is a Biological Indicator for Steam Sterilization, Self-Contained; to indicate the *D value* of the self-contained system, the method used to determine such *D value* (i.e., by spore count or fraction negative procedure after graded exposures to the sterilization conditions); the survival time and kill time under the specified conditions stated on the label; its particular total viable spore count, with a statement that such count has been determined after preliminary heat treatment; and its recommended storage conditions. State on the labeling that the supplied bacteriological medium will meet requirements for growth-promoting ability, the strain and ATCC number from which the spores were derived, and the instructions for spore recovery and for safe disposal of the indicator unit. Also indicate in the labeling that the stated resistance characteristics are reproducible only under steam sterilization conditions at the stated temperature and only under the exact conditions under which it was determined, that the user would not necessarily obtain the same result, and that the user should determine the suitability of the biological indicator for the particular use.~~

Identification—~~It meets the requirements of the *Identification* test under *Biological Indicator for Steam Sterilization, Paper Carrier*.~~

Resistance performance tests—

D value—~~Proceed as directed for the relevant procedure for *D Value* under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the determined *D value* is within 20% of the labeled *D value* for the selected sterilizing temperature and if the confidence limits of the estimate are within 10% of the determined *D value*.~~

Survival time and kill time—~~Follow the procedure for *Survival Time and Kill Time* in the section *Steam Sterilization, Self-Contained*, under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if all of the specimens subjected to the steam sterilization for the survival time show evidence of growth, while none of the specimens subjected to the steam sterilization for the kill time shows growth. If for either the survival time or the kill time requirement, not more than 1 specimen out of both groups fails the test, whichever is applicable, continue the corresponding test with 4 additional groups, each consisting of 10 specimens, according to the procedure described above. If all of the additional specimens subjected to steam sterilization either meet the survival requirement for the survival time test or meet the kill requirement for the kill time test, whichever is applicable, the requirements of the test are met.~~

Total viable spore count—~~Proceed as directed for *Total Viable Spore Count* under *Biological Indicators—Resistance Performance Tests* (55) using the procedure applicable to *Biological Indicator for Steam Sterilization, Paper Carrier*. The requirements of the test are met if the average number of viable spores per carrier is not less than 0.3 log of the labeled spore count~~

per carrier and does not exceed the log-labeled spore count per carrier by 0.48.

Medium suitability—

Sterility— Incubate 10 self-contained biological indicator systems at 55° to 60°, or at the optimal recovery temperature specified by the manufacturer, for 48 hours, making sure that there is no contact between the individual spore strips and the supplied medium. Examine the incubated medium visually (for change in color indicator or turbidity) and microscopically (for absence of microbial growth).

Growth promotion of medium prior to sterilization treatment— Submerge 10 self-contained units in a water bath maintained at 95° to 100° for 15 minutes. Start timing when the temperature of the container contents reach 95°. Cool rapidly in an ice-water bath (0° to 4°). Remove the units from the ice-water bath, submerge each spore strip with the self-contained medium, incubate at 55° to 60°, or at the optimal recovery temperature specified by the manufacturer, and examine visually after 48 hours for growth (for turbidity or change in color), and microscopically (for microbial growth). All the specimens under test show growth. If one or more of the specimens do not show growth, repeat the test with 20 additional units. The additional units all show growth.

Growth promotion of medium after exposure to sterilization conditions— Expose the specified number of units for both the *Survival Time* and *Kill time* stated in the labeling, as described in the section *Biological Indicator for Steam Sterilization, Self-Contained* under *Biological Indicators—Resistance Performance Tests (55)*. Incubate the spore strips submerged in the self-contained medium according to the instructions of the manufacturer. At the end of the incubation period confirm the existence of growth in each of the specimens that were exposed for each *Survival time* and the absence of growth in each of the specimens that were exposed for each *Kill time* by visual inspection (turbidity or color indicator change) and by separate microscopic examination of each specimen and confirm, where applicable, correspondence of the labeled color to the appearance of growth in the supplied medium.

Ability of medium to support growth after exposure to the sterilization conditions— Take a stated number of units (e.g., 10) after they have been exposed for each *Kill time* stated in the labeling as directed in the preceding section. Aseptically remove and pool the medium from each unit. Prepare a suspension of the indicator microorganism as directed for *Total Viable Spore Counts* under *Biological Indicator for Steam Sterilization, Paper Carrier*. Prepare a dilution of that suspension so as to contain 100 to 1000 viable microorganisms in one mL. Inoculate the pooled medium with enough suspension to contain a total of 100 to 1000 microorganisms in a 10-mL aliquot of not more than the volume from 10 units of the pooled medium. Incubate the inoculated pooled medium as directed for *Total Viable Spore Count*. Clear evidence of growth is obtained within 7 days.

Disposal— Prior to destruction or discard, sterilize it by steam at 121° for not less than 30 minutes, or by not less than an equivalent method recommended by the manufacturer. This includes test strips employed in any test procedures for the strips themselves. ■ 1S (USP39)

BRIEFING

Bisectrizole, USP 38 page 2456. On the basis of comments received, the following revisions

to the monograph are proposed:

1. The mobile phase gradient *Table 1* in the *Assay* is revised to allow the bisoctrizole isomer peak to completely elute. The Machery-Nagel Nucleosil 100 C18 AB brand or YMC PACK ODS AM brand of L1 columns are found suitable. The particle size of 5- μm is specified.
2. The *Standard solution* is replaced with the *System suitability solution* for the resolution determination in the test for *Limit of Bisoctrizole Related Compound A and Bisoctrizole Isomer*.

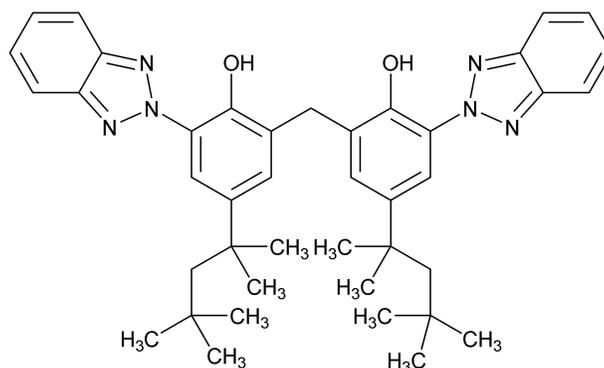
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: F. Mao.)

Correspondence Number—C139939

Comment deadline: July 31, 2015

Bisoctrizole



$\text{C}_{41}\text{H}_{50}\text{N}_6\text{O}_2$ 658.87

Phenol, 2,2'-methylenebis[6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)]-; 2,2'-Methylenebis[6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol] [103597-45-1].

DEFINITION

Bisoctrizole contains NLT 96.0% and NMT 102.0% of bisoctrizole ($\text{C}_{41}\text{H}_{50}\text{N}_6\text{O}_2$), calculated on the as-is basis.

IDENTIFICATION

- **A. Infrared Absorption** $\langle 197\text{K} \rangle$
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

Diluent: Tetrahydrofuran and 0.2% (w/v) aqueous solution of 1-pentane sulfonic acid

sodium salt (60:40)

Solution A: 0.4 g of 1-pentane sulfonic acid sodium salt, 800 mL of methanol, 200 mL of water, and 0.5 mL of phosphoric acid

Solution B: 0.4 g of 1-pentane sulfonic acid sodium salt, 1000 mL of methanol, and 0.5 mL of phosphoric acid

Mobile phase: See *Table 1*.

■ Return to original conditions and re-equilibrate the system. ■ 1S (USP39)

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
1	70	30
11	3	97
27		
■ 40 ■ 1S (USP39)	3	97
		30
28	70	■ 1S (USP39)

System suitability solution: 0.8 mg/mL of bisoctrizole from USP Bisoctrizole Resolution Mixture RS prepared as follows. Transfer USP Bisoctrizole Resolution Mixture RS to a suitable volumetric flask, dissolve in tetrahydrofuran, and dilute with *Diluent* to volume.

Standard solution: 0.8 mg/mL of USP Bisoctrizole RS prepared as follows. Transfer USP Bisoctrizole RS to a suitable volumetric flask, dissolve in tetrahydrofuran equivalent to 60% of the final volume, and dilute with *Diluent* to volume.

Sample solution: Transfer 80 mg of Bisoctrizole to a 100-mL volumetric flask. Dissolve in 60 mL of tetrahydrofuran, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 346 nm

Column: 3.0-mm × 25-cm;

■ 5-μm ■ 1S (USP39)

packing L1

Column temperature: 40°

Flow rate: 0.8 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times for bisoctrizole and the bisoctrizole isomer.]

Suitability requirements

Resolution: NLT 1.5 between bisoctrizole and the bisoctrizole isomer, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis**Samples:** *Standard solution* and *Sample solution*Calculate the percentage of bisoctrizole ($C_{41}H_{50}N_6O_2$) in the portion of Bisoctrizole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak response from the *Sample solution* r_S peak response from the *Standard solution* C_S concentration of USP Bisoctrizole RS in the *Standard solution* (mg/mL) C_U concentration of Bisoctrizole in the *Sample solution* (mg/mL)**Acceptance criteria:** 96.0%–102.0% on the as-is basis**IMPURITIES****Delete the following:**

- **Heavy Metals** (231), *Method II*: NMT 20 ppm •(Official 1-Dec-2015)

Change to read:

- **Limit of Bisoctrizole Related Compound A and Bisoctrizole Isomer**
Diluent, Solution A, Solution B, Mobile phase,
■System suitability solution, ■1S (USP39)

Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Standard stock solution A: 0.65 mg/mL of USP Bisoctrizole RS in tetrahydrofuran**Standard stock solution B:** 0.40 mg/mL of USP Bisoctrizole Related Compound A RS in tetrahydrofuran**Standard solution:** Transfer 5 mL of *Standard stock solution A* and 1.0 mL of *Standard stock solution B* to a 100-mL volumetric flask. Add 60 mL of tetrahydrofuran, and dilute with *Diluent* to volume.**System suitability****Sample:** *Standard solution*■*System suitability solution* ■1S (USP39)[Note—See *Table 2* for the relative retention times for bisoctrizole related compound A and the bisoctrizole isomer.]**Suitability requirements****Resolution:** NLT 1.5 between bisoctrizole and the bisoctrizole isomer**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of bisoctrizole related compound A in the portion of Bisoctrizole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak response of bisoctrizole related compound A from the *Sample solution* r_S peak response of bisoctrizole related compound A from the *Standard solution*

C_S concentration of USP Bisotrizole Related Compound A RS in the *Standard solution* (mg/mL)

C_U concentration of Bisotrizole in the *Sample solution* (mg/mL)

Calculate the percentage of bisotrizole isomer in the portion of Bisotrizole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of bisotrizole isomer from the *Sample solution*

r_S peak response of bisotrizole from the *Standard solution*

C_S concentration of USP Bisotrizole RS in the *Standard solution* (mg/mL)

C_U concentration of Bisotrizole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

• **Organic Impurities**

Diluent, Solution A, Solution B, Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual unspecified impurity in the portion of Bisotrizole taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of each individual impurity

r_T sum of the responses of all the peaks

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Bisotrizole related compound A ^a	0.42	0.5
Bisotrizole	1.0	—
Bisotrizole isomer ^b	1.1	4.0
Any individual unspecified impurity	—	0.10
Total impurities	—	4.0
^a 2-(2 <i>H</i> -Benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol.		
^b Phenol, 2,2-methylenebis[6-(2 <i>H</i> -benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)].		

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP Reference Standards** { 11 }
 - USP Bisotrizole RS
 - USP Bisotrizole Related Compound A RS
 - 2-(2*H*-Benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol.

$C_{20}H_{25}N_3$ 323.43

USP Bisotrizole Resolution Mixture RS

A mixture of approximately 1.5% of bisotrizole isomer [phenol, 2,2-methylenebis[6-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)]] in a matrix of bisotrizole.

BRIEFING

Bupivacaine Hydrochloride, *USP 38* page 2483. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Revise the *Acceptance criteria* in the *Definition* and *Assay* from NLT 98.5% and NMT 101.5% to NLT 98.0% and NMT 102.0%, which are typical for chromatographic procedures.
2. Replace *Identification* test A using a hazardous solvent (chloroform) with the retention time agreement from the proposed *Assay*.
3. Replace the titration procedure in the *Assay* with a validated stability-indicating HPLC procedure. The proposed procedure is based on analyses performed with the YMC-Triart C18 brand of L1 column. The typical retention time for bupivacaine is about 13 min.
4. Replace the TLC procedure in the test for *Organic Impurities* with a validated HPLC procedure that uses the same chromatographic system as that proposed for the *Assay*.
5. A lower limit of USP Ropivacaine Related Compound A RS is proposed to harmonize with the *European Pharmacopoeia*.
6. Add USP Bupivacaine Related Compound A RS, USP Bupivacaine Related Compound B RS, and USP Ropivacaine Related Compound A RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

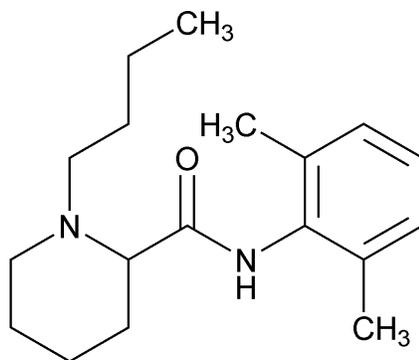
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: K.K. Seela.)

Correspondence Number—C107332

Comment deadline: July 31, 2015

Bupivacaine Hydrochloride



• HCl • H₂O

$C_{18}H_{28}N_2O \cdot HCl \cdot H_2O$ 342.90

$C_{18}H_{28}N_2O \cdot HCl$ 324.90

2-Piperidinecarboxamide, 1-butyl-*N*-(2,6-dimethylphenyl)-, monohydrochloride,

monohydrate;

(±)-1-Butyl-2',6'-pipecoloxylidide monohydrochloride, monohydrate [73360-54-0].

Anhydrous [18010-40-7].

DEFINITION

Change to read:

~~Bupivacaine Hydrochloride contains NLT 98.5% and NMT 101.5% of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$), calculated on the anhydrous basis.~~

■ Bupivacaine Hydrochloride contains NLT 98.0% and NMT 102.0% of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$), calculated on the anhydrous basis. ■ 1S (USP39)

IDENTIFICATION

Delete the following:

■ ● **A. Infrared Absorption** ~~(197S)~~

~~**Sample solution:** Dissolve 230 mg in 15 mL of water in a separator, add 1 mL of 6 N ammonium hydroxide, and extract with three 30 mL portions of chloroform. Evaporate the chloroform at room temperature with the aid of a stream of nitrogen, and dry the residue in a vacuum. Add 2 mL of chloroform to the residue, and dissolve.~~

~~**Acceptance criteria:** Meets the requirements ■ 1S (USP39)~~

Add the following:

■ ● **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

● **B. Ultraviolet Absorption** (197U)

Medium: 0.1 N hydrochloric acid

Sample solution: 500 µg/mL of Bupivacaine Hydrochloride in *Medium*

Analytical wavelength: 271 nm

Acceptance criteria: Absorptivities do not differ by more than 3.0%, calculated on the anhydrous basis.

● **C. Identification Tests—General** (191), *Chloride*

Sample solution: Dissolve 50 mg in 10 mL of water in a small separator, render alkaline with 6 N ammonium hydroxide, and extract with 10 mL of ether. Use the aqueous layer.

Acceptance criteria: Meets the requirements

ASSAY

Change to read:

● **Procedure**

~~**Sample:** 600 mg~~

~~**Titrimetric system**~~

~~**Mode:** Direct titration~~

~~**Titrant:** 0.1 N perchloric acid VS~~

Endpoint detection: ~~Visual~~

Analysis: ~~Transfer the *Sample* to a 250-mL conical flask, and dissolve in 20 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS and 3 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 32.49 mg of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$).~~

Acceptance criteria: ~~98.5%–101.5% on the anhydrous basis~~

■ **Solution A:** 10 mM ammonium bicarbonate in water. Adjust with ammonium hydroxide to a pH of 9.0.

Solution B: Acetonitrile, methanol, and isopropyl alcohol (85:10:5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
1.0	90	10
5.0	55	45
8.0	45	55
16.0	10	90
18.0	10	90
18.1	90	10
20.0	90	10

Diluent: Acetonitrile and water (20:80)

Standard solution: 0.2 mg/mL of USP Bupivacaine Hydrochloride RS in *Diluent*

Sample solution: 0.2 mg/mL of Bupivacaine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 2.0-mm × 10-cm; 1.9- μ m packing L1

Column temperature: 45^o

Flow rate: 0.25 mL/min

Injection volume: 2 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$) in the portion of Bupivacaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Bupivacaine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = concentration of Bupivacaine Hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis ■ 1S (USP39)**IMPURITIES**

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Delete the following:

- **Heavy metals, Method II** 〈 231 〉: NMT 0.001% ● (Official 1-Dec-2015)

- **Limit of Alcohol and Isopropyl Alcohol**

Standard solution A: 0.08% Alcohol in water**Standard solution B:** 0.004% Isopropyl alcohol in water**Sample solution:** 0.04 g/mL of Bupivacaine Hydrochloride in water**Chromatographic system**(See *Chromatography* 〈 621 〉, *System Suitability*.)**Mode:** GC**Detector:** Flame ionization**Column:** 4-mm × 2-m; packing S3**Temperatures****Injection port:** 200°**Detector:** 280°**Column:** 175°**Flow rate:** 40 mL/min**Injection volume:** 5 µL**Carrier gas:** Nitrogen**Analysis****Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of alcohol in the portion of Bupivacaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of alcohol from the *Sample solution*

r_S peak response of alcohol from the *Standard solution*

C_S concentration of alcohol in *Standard solution A* (mL/mL)

C_U concentration of Bupivacaine Hydrochloride in the *Sample solution* (g/mL)

Calculate the percentage of isopropyl alcohol in the portion of Bupivacaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of isopropyl alcohol from the *Sample solution*

r_S peak response of isopropyl alcohol from the *Standard solution*

C_S concentration of isopropyl alcohol in *Standard solution B* (mL/mL)

C_U concentration of Bupivacaine Hydrochloride in the *Sample solution* (g/mL)

Acceptance criteria: The sum of the content of alcohol and the content of isopropyl alcohol is NMT 2%.

Delete the following:

■● **Chromatographic Purity**

Solution A: Chloroform and isopropylamine (99:1)

Standard solution A: 20.0 mg/mL of USP Bupivacaine Hydrochloride RS in *Solution A*

Standard solution B: 100 µg/mL of USP Bupivacaine Hydrochloride RS from *Standard solution A* diluted with *Solution A*

Sample solution: 20.0 mg/mL of Bupivacaine Hydrochloride in *Solution A*

Chromatographic system

(See *Chromatography* ~~621~~, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Hexanes and isopropylamine (97:3)

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Analysis: Proceed as directed under *Chromatography* ~~621~~. Develop the chromatogram in a suitable chamber until the solvent has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and dry it in warm air. Place the plate in a closed chamber with a dish containing 1 g of iodine in a shallow layer, and allow to remain for about 5 min. Remove the plate from the chamber, spray it with 7 N sulfuric acid, and examine the chromatogram.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*, and the estimated size and intensity of any other spot of the *Sample solution* does not exceed that of the principal spot of the *Standard solution B* (0.5%). The total of the estimated sizes and intensities of all of the other spots of the *Sample solution* does not exceed four times that of the principal spot of the *Standard solution B* (2.0%). ■ 1S (USP39)

Add the following:

■● **Organic Impurities**

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 5 µg/mL each of USP Bupivacaine Related Compound A RS and USP Bupivacaine Related Compound B RS in *Diluent*

Standard solution: 5 µg/mL each of USP Bupivacaine Hydrochloride RS and USP Bupivacaine Related Compound B RS in *Diluent*

Sample solution: 1.0 mg/mL of Bupivacaine Hydrochloride in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between bupivacaine related compound A and bupivacaine related compound B, *System suitability solution*

Relative standard deviation: NMT 5.0% for bupivacaine and bupivacaine related compound B, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of bupivacaine related compound B in the portion of Bupivacaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of bupivacaine related compound B from the *Sample solution*

r_S peak response of bupivacaine related compound B from the *Standard solution*

C_S concentration of USP Bupivacaine Related Compound B RS in the *Standard solution* (mg/mL)

C_U concentration of Bupivacaine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Bupivacaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each unspecified impurity from the *Sample solution*

r_S peak response of bupivacaine hydrochloride from the *Standard solution*

C_S concentration of USP Bupivacaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U concentration of Bupivacaine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%, except the peak corresponding to ropivacaine related compound A in *Limit of Ropivacaine Related Compound A*. ■1S (USP39)

Add the following:

■● **Limit of Ropivacaine Related Compound A**

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.1 µg/mL of USP Ropivacaine Related Compound A RS in *Diluent*

Sample solution: 10 mg/mL of Bupivacaine Hydrochloride in *Diluent*

System suitability**Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 10.0%**Analysis****Samples:** *Standard solution and Sample solution*

Calculate the percentage of ropivacaine related compound A in the portion of Bupivacaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U peak response of ropivacaine related compound A from the *Sample solution*

r_S peak response of ropivacaine related compound A from the *Standard solution*

C_S concentration of USP Ropivacaine Related Compound A RS in the *Standard solution* (mg/mL)

C_U concentration of Bupivacaine Hydrochloride in the *Sample solution* (mg/mL)

M_{r1} molecular weight of ropivacaine related compound A free base, 121.18

M_{r2} molecular weight of ropivacaine related compound A hydrochloride, 157.64

Acceptance criteria: See *Table 2*.**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Bupivacaine related compound B ^{a,b}	0.54	0.5
Ropivacaine related compound A ^c	0.60	0.001
Bupivacaine	1.00	—
Individual unspecified impurity ^b	—	0.5
Total impurities ^d	—	2.0
a <i>N</i> -(2,6-Dimethylphenyl)piperidine-2-carboxamide.		

^b Results obtained in the test for *Organic Impurities*.

^c 2,6-Dimethylaniline.

^d Sum of the results obtained in the tests for *Organic Impurities* and *Limit of Ropivacaine Related Compound A*.

■ 1S (USP39)

SPECIFIC TESTS

- **pH** 〈 791 〉: 4.5–6.0, in a solution (1 in 100)
- **Water Determination** 〈 921 〉, *Method I*: 4.0%–6.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Bupivacaine Hydrochloride RS

- USP Bupivacaine Related Compound A RS

6-(Butylamino)-*N*-(2,6-dimethylphenyl)hexanamide.

C₁₈H₃₀N₂O 290.45

USP Bupivacaine Related Compound B RS

N-(2,6-Dimethylphenyl)piperidine-2-carboxamide.

C₁₄H₂₀N₂O 232.36

USP Ropivacaine Related Compound A RS

2,6-Dimethylaniline hydrochloride.

C₈H₁₁N·HCl 157.64

- 1S (USP39)

BRIEFING

Bupivacaine Hydrochloride Injection, USP 38 page 2484. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Add a stability-indicating HPLC procedure to the test for *Organic Impurities*, because the present monograph is missing an impurity test. The HPLC procedure is based on analyses performed with the YMC Triart C18 brand of L1 column. The typical retention time for the bupivacaine peak in the *Organic Impurities* test is about 13 min.
2. A more selective, single HPLC procedure is used for both the *Assay* and *Organic Impurities* test.
3. Delete *Identification* test A because it is non-value added and replace it with UV spectrum matching from the proposed *Assay*.
4. Add USP Bupivacaine Related Compound A RS and USP Bupivacaine Related Compound B RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.
5. The limit for degradation products is based on the drug substance monograph. Manufacturers with different impurity profiles and limits are encouraged to provide this information to USP for consideration.
6. Add the storage temperature to the *Packaging and Storage* section.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: K.K. Seela.)

Correspondence Number—C139207

Comment deadline: July 31, 2015

Bupivacaine Hydrochloride Injection**DEFINITION**

Bupivacaine Hydrochloride Injection is a sterile solution of Bupivacaine Hydrochloride in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$).

IDENTIFICATION

Delete the following:

■ • A. Identification—Organic Nitrogenous Bases ~~(181)~~

Sample solution: 2 mg/mL of bupivacaine hydrochloride in 0.01 N hydrochloric acid, from Injection

Analysis: Proceed as directed in the chapter beginning with "Transfer the liquid to a separator".

Acceptance criteria: Meets the requirements ■_{1S} (USP39)

Add the following:

■ • A. The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■_{1S} (USP39)

• B. The retention time of the bupivacaine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• Procedure

Buffer: 1.94 g/L of monobasic potassium phosphate and 2.48 g/L of dibasic potassium phosphate in water. Adjust, if necessary, with 1 N potassium hydroxide or 1 M phosphoric acid to a pH of 6.8.

Mobile phase: Acetonitrile and *Buffer* (65:35). Adjust, if necessary, with 1 M phosphoric acid to a pH of 7.7 ± 0.2 . Filter the solution through a membrane filter of 1 μ m or finer pore size, and degas.

Internal standard solution: 1.3 mg/mL of dibutyl phthalate in methanol

Standard solution: 0.5 mg/mL of USP Bupivacaine Hydrochloride RS, prepared as follows: In a 100 mL volumetric flask, dissolve 50 mg of USP Bupivacaine Hydrochloride RS in 10.0 mL of water, using sonication if necessary. Add 10 mL of *Internal standard solution*, and dilute with methanol to volume.

Sample solution: Nominally 0.5 mg/mL of bupivacaine hydrochloride, prepared as follows: In a 100 mL volumetric flask, transfer an amount of Injection equivalent to 50 mg of bupivacaine hydrochloride, add 10.0 mL of *Internal standard solution*, and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* ~~(621)~~, *System Suitability*.)

Mode: LC

Detector: UV 263 nm

Column: 4 mm \times 30 cm; packing L1

~~**Flow rate:** 2 mL/min~~

~~**Injection volume:** 20 μ L~~

~~**System suitability**~~

~~**Sample:** *Standard solution*~~

~~[Note—The relative retention times for bupivacaine hydrochloride and dibutyl phthalate are about 1.0 and 1.2, respectively.]~~

~~**Suitability requirements**~~

~~**Resolution:** NLT 2.0 between bupivacaine hydrochloride and dibutyl phthalate~~

~~**Relative standard deviation:** NMT 1.0% for the ratio of bupivacaine to the internal standard from three replicate injections~~

~~**Analysis**~~

~~**Samples:** *Standard solution* and *Sample solution*~~

~~Calculate the percentage of the labeled amount of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$) in the portion of Injection taken:~~

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

~~R_U peak response ratio of bupivacaine to the internal standard from the *Sample solution*~~

~~R_S peak response ratio of bupivacaine to the internal standard from the *Standard solution*~~

~~C_S concentration of USP Bupivacaine Hydrochloride RS, calculated on the anhydrous basis, in the *Standard solution* (mg/mL)~~

~~C_U nominal concentration of bupivacaine hydrochloride in the *Sample solution* (mg/mL)~~

~~**Acceptance criteria:** 93.0%–107.0%~~

- **Solution A:** 10 mM ammonium bicarbonate in water. Adjust with ammonium hydroxide to a pH of 9.0.

Solution B: Acetonitrile, isopropyl alcohol, and methanol (85:5:10)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
1.0	90	10
5.0	55	45
8.0	45	55
16.0	10	90
18.0	10	90
18.1	90	10
20.0	90	10

Diluent: Acetonitrile and water (20:80)

Standard solution: 0.2 mg/mL of USP Bupivacaine Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.2 mg/mL of bupivacaine hydrochloride in *Diluent* from Injection prepared as follows. Transfer the contents of Injection to a volumetric flask of appropriate size, dilute with *Diluent* to volume, and mix.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: 230 nm. For *Identification* test A, use a diode array detector in the range of 190–300 nm.

Column: 2.0-mm × 10-cm; 1.9-µm packing L1

Column temperature: 45°

Flow rate: 0.25 mL/min

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Bupivacaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of bupivacaine hydrochloride in the *Sample solution* (mg/mL)

■ 1S (USP39)

Acceptance criteria: 93.0%–107.0%

IMPURITIES

Add the following:

■ • Organic Impurities

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 5 µg/mL each of USP Bupivacaine Related Compound A RS and USP Bupivacaine Related Compound B RS in *Diluent*

Standard solution: 5 µg/mL of USP Bupivacaine Hydrochloride RS in *Diluent*

Sample solution: Nominally 1.0 mg/mL of bupivacaine hydrochloride in *Diluent* from Injection prepared as follows. Transfer the contents of Injection to a volumetric flask of

appropriate size, dilute with *Diluent* to volume, and mix.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between bupivacaine related compound A and bupivacaine related compound B, *System suitability solution*

Relative standard deviation: NMT 5.0% for bupivacaine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each degradation product from the *Sample solution*

r_S peak response of bupivacaine hydrochloride from the *Standard solution*

C_S concentration of USP Bupivacaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of bupivacaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Bupivacaine related compound B ^{a,b}	0.54	—
Bupivacaine related compound A ^{b,c}	0.55	—
Bupivacaine	1.00	—
Any individual degradation product	—	0.5
Total degradation products	—	2.0

^a *N*-(2,6-Dimethylphenyl)piperidine-2-carboxamide.

^b For identification only. These are process impurities monitored in the drug substance and are not included in the total degradation products.

^c 6-(Butylamino)-*N*-(2,6-dimethylphenyl)hexanamide.

■ 1S (USP39)

SPECIFIC TESTS

- **Bacterial Endotoxins Test** $\langle 85 \rangle$: NMT 2.5 USP Endotoxin Units/mg of bupivacaine hydrochloride
- **pH** $\langle 791 \rangle$: 4.0–6.5
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* $\langle 1 \rangle$.

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass. Injection labeled to contain 0.5% or less of bupivacaine hydrochloride may be packaged in 50-mL, multiple-dose containers.

■ Store at controlled room temperature. ■ 1S (USP39)

Change to read:

- **USP Reference Standards** { 11 }

USP Bupivacaine Hydrochloride RS

■ USP Bupivacaine Related Compound A RS

6-(Butylamino)-*N*-(2,6-dimethylphenyl)hexanamide.

C₁₈H₃₀N₂O 290.45

USP Bupivacaine Related Compound B RS

N-(2,6-Dimethylphenyl)piperidine-2-carboxamide.

C₁₄H₂₀N₂O 232.36

■ 1S (USP39)

USP Endotoxin RS

BRIEFING

Bupivacaine Hydrochloride in Dextrose Injection, USP 38 page 2484. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Add a stability-indicating HPLC procedure to the test for *Organic Impurities*, because the present monograph is missing an impurity test. The HPLC procedure is based on analyses performed with the YMC Triart C18 brand of L1 column. The typical retention time for the bupivacaine peak in the *Organic Impurities* test is about 13 min.
2. A more selective, single HPLC procedure is used for both the *Assay* and *Organic Impurities* test.
3. Add *Identification* test C based on UV spectrum matching from the proposed *Assay*.
4. Add USP Bupivacaine Related Compound A RS and USP Bupivacaine Related Compound B RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.
5. The limit for degradation products is based on the drug substance monograph. Manufacturers with different impurity profiles and limits are encouraged to provide this information to USP for consideration.
6. Add the storage temperature to the *Packaging and Storage* section.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: K.K. Seela.)

Correspondence Number—C139206

Comment deadline: July 31, 2015

Bupivacaine Hydrochloride in Dextrose Injection

DEFINITION

Bupivacaine Hydrochloride in Dextrose Injection is a sterile solution of Bupivacaine Hydrochloride and Dextrose in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amounts of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$) and dextrose ($C_6H_{12}O_6$). It contains no preservative.

IDENTIFICATION

- **A. Thin-Layer Chromatographic Identification Test** 〈 201 〉

Standard solution A: USP Bupivacaine Hydrochloride RS in water

Standard solution B: USP Dextrose RS in water

Standard solution C: USP Bupivacaine Hydrochloride RS in *Standard solution B* to obtain a solution with concentrations corresponding to the labeled concentrations of bupivacaine hydrochloride and dextrose in the Injection

Sample solution: Bupivacaine Hydrochloride in Dextrose Injection

Chromatographic system

Adsorbent: Chromatographic silica gel mixture; 0.25 mm

Application volume: See *Analysis*.

Developing solvent system: Butyl alcohol, dehydrated alcohol, glacial acetic acid, and water (6:1:1:2)

Spray reagents

Naphthalenediol reagent: Dissolve 20 mg of 1,3-naphthalenediol in 10 mL of dehydrated alcohol containing 0.2 mL of sulfuric acid.

Iodoplatinate reagent: Mix equal volumes of platinum chloride solution (3 in 1000) and potassium iodide solution (6 in 100).

Analysis: Separately apply 10 μ L each of *Standard solution A*, *Standard solution C*, and *Sample solution* to a portion of the chromatographic plate, and separately apply 1 μ L each of *Standard solution B* and *Sample solution* to the remaining portion of the plate. Dry the applications in a current of warm air, develop the chromatograms in the *Developing solvent system*, remove the plate from the developing chamber, and mark the solvent front. Dry the plate in warm circulating air, and examine the plate under short-wavelength UV light. *Acceptance criteria 1* should be met.

Spray the plate with *Naphthalenediol reagent*, heat at 90° for 5 min, and examine the plate. *Acceptance criteria 2* should be met.

Cool the plate, spray it with *Iodoplatinate reagent*, and examine the plate. *Acceptance criteria 3* should be met and also, bupivacaine appears as a blue-purple spot on a salmon-colored background, and the dextrose spots fade slightly.

Acceptance criteria

1: The R_F value of the principal spot from the *Sample solution* corresponds to those of *Standard solution A* and *Standard solution C*.

2: The R_F value of the principal blue-purple spot from the *Sample solution* corresponds to that from *Standard solution B*.

3: The R_F value of the bupivacaine spot from the *Sample solution* corresponds to those from *Standard solution A* and *Standard solution C*.

- **B.** The retention time of the bupivacaine peak of the *Sample solution* corresponds to that of

the *Standard solution*, as obtained in the Assay for *Bupivacaine Hydrochloride*.

Add the following:

- **C.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ■ 1S (USP39)

ASSAY

Change to read:

• **Bupivacaine Hydrochloride**

Solution A: 1.94 mg/mL of monobasic potassium phosphate and 2.48 mg/mL of dibasic potassium phosphate in water. [Note—Adjust, if necessary, with 1 N potassium hydroxide or 1 M phosphoric acid to a pH of 6.8.]

Mobile phase: Acetonitrile and *Solution A* (65:35). [Note—Adjust, if necessary, with 1 M phosphoric acid to a pH of 7.7 ± 0.2 . Pass the solution through a membrane filter of 1- μ m or finer porosity.]

Internal standard solution: 1.3 mg/mL of dibutyl phthalate in methanol

Standard solution: 0.5 mg/mL of USP Bupivacaine Hydrochloride RS prepared as follows. Dissolve 50 mg of USP Bupivacaine Hydrochloride RS in 10.0 mL of water, using sonication if necessary, in a 100-mL volumetric flask. Add 10 mL of *Internal standard solution*, and dilute with methanol to volume.

Sample solution: Nominally 0.5 mg/mL of bupivacaine hydrochloride from Injection as follows. Transfer an accurately measured volume of Injection equivalent to 50 mg of bupivacaine hydrochloride to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* ~~(621)~~, *System Suitability*.)

Mode: LC

Detector: UV 263 nm

Column: 4 mm \times 30 cm; packing L1

Flow rate: 2 mL/min

Injection size: 20 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times for dibutyl phthalate and bupivacaine hydrochloride are about 1.2 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between bupivacaine and dibutyl phthalate

Relative standard deviation: NMT 1.0% for the ratios of the bupivacaine peak to the dibutyl phthalate peak (three replicate injections)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of labeled amount of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100/L$$

~~R_S~~ peak response ratio of bupivacaine hydrochloride to the internal standard from the *Sample solution*

~~R_S~~ peak response ratio of bupivacaine hydrochloride to the internal standard from the *Standard solution*

~~C_S~~ concentration of USP Bupivacaine Hydrochloride RS, in the *Standard solution* (mg/mL)

~~C_S~~ nominal concentration of bupivacaine hydrochloride in the *Sample solution* (mg/mL)

L = label claim (mg/volume of Injection)

Acceptance criteria: ~~93.0%–107.0%~~

■ **Solution A:** 10 mM ammonium bicarbonate in water. Adjust with ammonium hydroxide to a pH of 9.0.

Solution B: Acetonitrile, methanol, and isopropyl alcohol (85:10:5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
1.0	90	10
5.0	55	45
8.0	45	55
16.0	10	90
18.0	10	90
18.1	90	10
20.0	90	10

Diluent: Acetonitrile and water (20:80)

Standard solution: 0.2 mg/mL of USP Bupivacaine Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.2 mg/mL of bupivacaine hydrochloride in *Diluent* from Injection prepared as follows. Transfer the contents of Injection to a volumetric flask of appropriate size, dilute with *Diluent* to volume, and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm. For *Identification test C*, use a diode array detector in the range of 190–300 nm.

Column: 2.0-mm × 10-cm; 1.9- μ m packing L1

Column temperature: 45°

Flow rate: 0.25 mL/min

Injection volume: 2 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Bupivacaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of bupivacaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0% ■ 1S (USP39)

• Dextrose

Analysis: Determine the angular rotation of the Injection in a suitable polarimeter tube (see *Optical Rotation* 〈 781 〉).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6$) in the portion of Injection taken:

$$\text{Result} = \{[(A \times R)/F] \times 100\} \times (100/L)$$

A = 100 mm divided by the length of the polarimeter tube (mm)

R = observed rotation (degrees)

F = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

L = label claim of dextrose (g/100 mL)

Acceptance criteria: 93.0%–107.0%

IMPURITIES

Add the following:

■ • Organic Impurities

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the Assay.

System suitability solution: 5 µg/mL each of USP Bupivacaine Related Compound A RS and USP Bupivacaine Related Compound B RS in *Diluent*

Standard solution: 5 µg/mL of USP Bupivacaine Hydrochloride RS in *Diluent*

Sample solution: Nominally 1.0 mg/mL of bupivacaine hydrochloride in *Diluent* from Injection prepared as follows. Transfer the contents of Injection to a volumetric flask of appropriate size, dilute with *Diluent* to volume, and mix.

System suitability**Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 1.5 between bupivacaine related compound A and bupivacaine related compound B, *System suitability solution***Relative standard deviation:** NMT 5.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each degradation product from the *Sample solution*

r_S peak response of bupivacaine hydrochloride from the *Standard solution*

C_S concentration of USP Bupivacaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of bupivacaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Bupivacaine related compound B ^{a,b}	0.54	—
Bupivacaine related compound A ^{b,c}	0.55	—
Bupivacaine	1.00	—
Any individual degradation product	—	0.5
Total degradation products	—	2.0
^a <i>N</i> -(2,6-Dimethylphenyl)piperidine-2-carboxamide.		

^b For identification only. These are process impurities monitored in the drug substance and are not included in the total degradation products.

^c 6-(Butylamino)-*N*-(2,6-dimethylphenyl)hexanamide.

■ 1S (USP39)

SPECIFIC TESTS

- **Bacterial Endotoxins Test** $\langle 85 \rangle$: NMT 1.8 USP Endotoxin Units/mg of bupivacaine hydrochloride
- **pH** $\langle 791 \rangle$: 4.0–6.5
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* $\langle 1 \rangle$.

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I glass.
- Store at controlled room temperature. ■ 1S (USP39)

Change to read:● **USP Reference Standards** 〈 11 〉

USP Bupivacaine Hydrochloride RS

- USP Bupivacaine Related Compound A RS

6-(Butylamino)-*N*-(2,6-dimethylphenyl)hexanamide.C₁₈H₃₀N₂O 290.45

USP Bupivacaine Related Compound B RS

N-(2,6-Dimethylphenyl)piperidine-2-carboxamide.C₁₄H₂₀N₂O 232.36

- 1S (USP39)

USP Dextrose RS

USP Endotoxin RS

BRIEFING

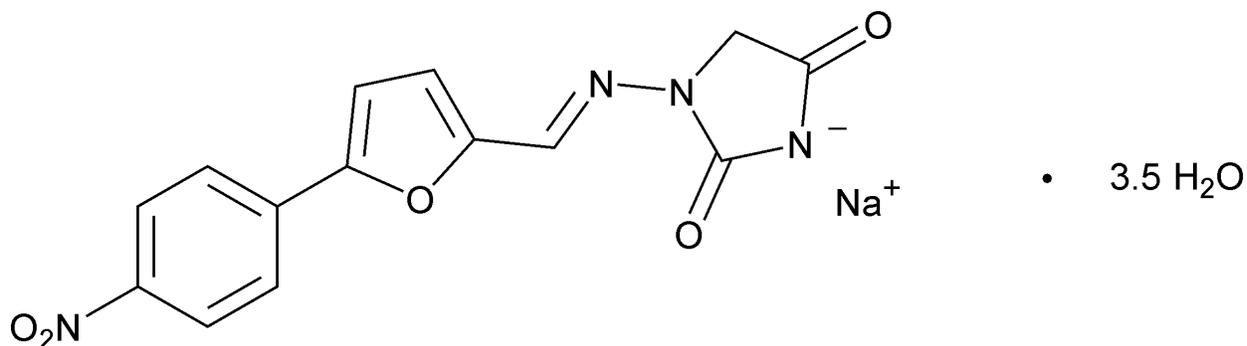
Dantrolene Sodium, *USP 38* page 2997. In preparation for the omission of the flame tests from general chapter *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to replace the use of the flame test in *Identification* test *C* with the pyroantimonate precipitation test currently described in this general chapter and to add a second multi-step precipitation reaction as *Identification* test *D*. Proposed *Identification* test *C* is consistent with 2.3.1 *Identification reactions of ions and functional groups* in the *European Pharmacopoeia*. The preparation of the *Sample solution* within the proposed *Identification* test *C* as well as the test proposed as *Identification* test *D* are adopted from the *Dantrolene Sodium* monograph published in the 2014 *British Pharmacopoeia*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: H. Joyce.)

Correspondence Number—C154839

Comment deadline: July 31, 2015**Dantrolene Sodium**



$C_{14}H_9N_4NaO_5 \cdot 3\frac{1}{2}H_2O$ 399.29

2,4-Imidazolidinedione, 1-[[[5-(4-nitrophenyl)-2-furanyl]methylene]amino]-, sodium salt, hydrate (2:7);

1-[[5-(*p*-Nitrophenyl)furfurylidene]amino]hydantoin sodium salt hydrate [24868-20-0].

DEFINITION

Dantrolene Sodium contains NLT 90.0% and NMT 96.0% of dantrolene ($C_{14}H_{10}N_4O_5$), the free acid form of Dantrolene Sodium, calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** < 197K >
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **C. Identification Tests—General** < 191 >, *Sodium* : ~~Ignite about 200 mg; the residue meets the requirements of the flame test for sodium.~~

■ **Sample solution:** To 0.1 g of Dantrolene Sodium, add 20 mL of water and 2 drops of acetic acid, shake well, and pass the resulting solution through a suitable filter. Use 2 mL of the filtrate.

Analysis

Sample: *Sample solution*

Acceptance criteria: Meets the requirements ■ 1S (*USP39*)

Add the following:

■ • D.

Solution A: Dissolve 2.7 g of methoxyphenylacetic acid in 6 mL of tetramethylammonium hydroxide solution, and add 20 mL of dehydrated alcohol.

Solution B: 158 mg/mL of ammonium carbonate in water

Sample solution: To 0.1 g of Dantrolene Sodium, add 20 mL of water and 2 drops of acetic acid, shake well, and pass the resulting solution through a suitable filter. Use the filtrate.

Analysis

Sample: *Sample solution*

Part 1: To 0.5 mL of the *Sample solution* in a suitable container, add 1.5 mL of *Solution A*, and cool in ice water for 30 min.

Part 2: Transfer the container from *Part 1* to a water bath at 20°, and stir for 5 min.

Part 3: Add 1 mL of ammonia TS to the container from *Part 2*.

Part 4: Add 1 mL of *Solution B* to the container from *Part 3*.

Acceptance criteria: The requirements for *Part 1*, *Part 2*, *Part 3*, and *Part 4* must all be met.

Part 1: A voluminous, white, crystalline precipitate is formed.

Part 2: The precipitate does not disappear.

Part 3: The precipitate dissolves completely.

Part 4: No precipitate is formed.

■ 1S (USP39)

ASSAY

Change to read:

● Procedure

Buffer: Dissolve 3.85 g of ammonium acetate in 1.0 L of water; adjusted with glacial acetic acid to a pH of 4.5 ± 0.1

Solution A: Acetonitrile, *Buffer*, and water (10:20:70)

Solution B: Acetonitrile and *Buffer* (80:20)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	60	40
20	10	90
25	10	90
25.1	90	10
35	90	10

Diluent: Acetonitrile and water (50:50)

System suitability stock solution A: 1.25 mg/mL of USP Dantrolene Sodium RS prepared as follows. Transfer a suitable amount of USP Dantrolene Sodium RS to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

System suitability stock solution B: ~~Transfer 6.3 mg each of USP Dantrolene Related Compound B RS and USP Dantrolene Related Compound C RS, accurately weighed, into a 50 mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume to obtain a solution having known concentrations of about 0.125 mg/mL each of dantrolene related compound B and dantrolene related compound C.~~

■ 0.125 mg/mL each of USP Dantrolene Related Compound B RS and USP Dantrolene Related Compound C RS prepared as follows. Transfer suitable amounts of USP Dantrolene Related Compound B RS and USP Dantrolene Related Compound C RS to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total

flask volume of glacial acetic acid, and dilute with acetone to volume. ■1S (USP39)

System suitability solution: 125 µg/mL of USP Dantrolene Sodium RS from *System suitability stock solution A* and 2.5 µg/mL each of USP Dantrolene Related Compound B RS and USP Dantrolene Related Compound C RS from *System suitability stock solution B* in *Diluent*

Standard stock solution: 1.0 mg/mL of USP Dantrolene RS prepared as follows. Transfer a suitable amount of USP Dantrolene RS to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

Standard solution: 100 µg/mL of USP Dantrolene RS from *Standard stock solution* in *Diluent*

Sample stock solution: 1.25 mg/mL of Dantrolene Sodium prepared as follows. Transfer a suitable amount of Dantrolene Sodium to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

Sample solution: 125 µg/mL of Dantrolene Sodium from *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for dantrolene related compound B, dantrolene, and dantrolene related compound C are 0.68, 1.0, and 1.24, respectively.]

Suitability requirements

Resolution: NLT 8 between dantrolene related compound C and dantrolene, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dantrolene (C₁₄H₁₀N₄O₅) in the portion of Dantrolene Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of dantrolene from the *Sample solution*

r_S peak response of dantrolene from the *Standard solution*

C_S concentration of USP Dantrolene RS in the *Standard solution* (µg/mL)

C_U concentration of Dantrolene Sodium in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–96.0% on the anhydrous basis

IMPURITIES

Delete the following:

●● **Heavy Metals, Method II** 〈 231 〉: NMT 20 ppm (Official 1-Dec-2015)

● **Limit of Dantrolene Related Compound A**

Mobile phase: Acetonitrile and water (80:20)

Standard stock solution: 17.5 µg/mL of USP Dantrolene Related Compound A RS and 50 µg/mL of USP Dantrolene Sodium RS in dimethylformamide

Standard solution: 0.35 µg/mL of USP Dantrolene Related Compound A RS and 1 µg/mL of USP Dantrolene Sodium RS from *Standard stock solution* in acetonitrile

Sample stock solution: 1.25 mg/mL of Dantrolene Sodium prepared as follows. Transfer a suitable amount of Dantrolene Sodium to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

Sample solution: 175 µg/mL of Dantrolene Sodium from *Sample stock solution* in acetonitrile

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[Note—The dantrolene peak elutes at void volume at approximately 1.5 min.]

Suitability requirements

Tailing factor: NMT 1.5 for dantrolene related compound A

Relative standard deviation: NMT 5% for dantrolene related compound A

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dantrolene related compound A in the portion of Dantrolene Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of dantrolene related compound A from the *Sample solution*

r_S peak response of dantrolene related compound A from the *Standard solution*

C_S concentration of USP Dantrolene Related Compound A RS in the *Standard solution* (µg/mL)

C_U concentration of Dantrolene Sodium in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.15%

● **Organic Impurities**

Mobile phase, Diluent, System suitability stock solution B, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution A: Use the *Standard solution* from the *Assay*.

Standard solution B: 0.25 µg/mL each of USP Dantrolene Related Compound B RS and USP

Dantrolene Related Compound C RS from *System suitability stock solution B* in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution A*

[Note—The relative retention times for dantrolene related compound B, dantrolene, and dantrolene related compound C are 0.68, 1.0, and 1.24, respectively.]

Suitability requirements

Resolution: NLT 8 between dantrolene related compound C and dantrolene, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution A*

Relative standard deviation: NMT 1.0%, *Standard solution A*

Analysis

Samples: *Sample solution* and *Standard solution B*

Calculate the percentage of dantrolene related compound B and dantrolene related compound C in the portion of Dantrolene Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of dantrolene related compound B or dantrolene related compound C from the *Sample solution*

r_S peak response of dantrolene related compound B or dantrolene related compound C from *Standard solution B*

C_S concentration of USP Dantrolene Related Compound B RS or USP Dantrolene Related Compound C RS in *Standard solution B* ($\mu\text{g/mL}$)

C_U concentration of Dantrolene Sodium in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria

Dantrolene related compound B: NMT 0.50%

Dantrolene related compound C: NMT 0.30%

SPECIFIC TESTS

- **Water Determination** { 921 }, *Method Ia*: 14.5%–17.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at room temperature.

Change to read:

- **USP Reference Standards** { 11 }

USP Dantrolene RS

■ 1-({[5-(4-Nitrophenyl)furan-2-yl]methylene}amino)imidazolidine-2,4-dione.

$\text{C}_{14}\text{H}_{10}\text{N}_4\text{O}_5$ 314.25

■ 1S (USP39)

USP Dantrolene Sodium RS

USP Dantrolene Related Compound A RS

■ 1,2-Bis-{[5-(4-nitrophenyl)furan-2-yl]methylene}hydrazine;

also known as ■ 1S (USP39)

5-(4-Nitrophenyl)furaldehyde azine.

$C_{22}H_{14}N_4O_6$

■ 430.38 ■_{1S} (USP39)

USP Dantrolene Related Compound B RS

■ *N*-Carbamoyl-*N*-({[5-(4-nitrophenyl)furan-2-yl]methylene}amino)glycine;

also known as ■_{1S} (USP39)

5-(4-Nitrophenyl)-2-furaldehyde-2-carboxymethyl semicarbazone.

$C_{14}H_{12}N_4O_6$

■ 332.27 ■_{1S} (USP39)

USP Dantrolene Related Compound C RS

5-(4-Nitrophenyl)furan-2-carbaldehyde.

$C_{11}H_7NO_4$

■ 217.18 ■_{1S} (USP39)

BRIEFING

Dipyridamole, *USP 38* page 3156. As part of USP monograph modernization efforts, the following revisions are proposed:

1. Replace the current HPLC procedure in the *Organic Impurities* test, which does not have any specified impurity, with a new procedure developed for the Dipyridamole family. The proposed procedure is specific to all the specified impurities and is based on analyses performed with the Waters Acquity UPLC BEH C18 brand of L1 column. The typical retention time for dipyridamole is about 9.5 min.
2. Add proposed *Acceptance criteria* for specified and unspecified impurities to the *Organic Impurities* section.
3. Add USP Dipyridamole Related Compound A RS, USP Dipyridamole Related Compound B RS, USP Dipyridamole Related Compound C RS, USP Dipyridamole Related Compound D RS, USP Dipyridamole Related Compound E RS, and USP Dipyridamole Related Compound F RS to the *USP Reference Standards* section to support the proposed revisions.

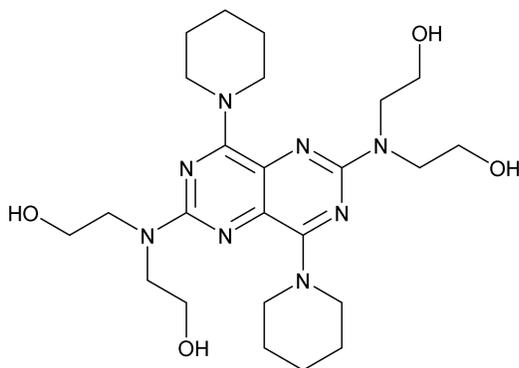
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: G. Hsu, S. Ramakrishna.)

Correspondence Number—C138902

Comment deadline: July 31, 2015

Dipyridamole



$C_{24}H_{40}N_8O_4$ 504.63

Ethanol, 2,2',2'',2'''-[(4,8-di-1-piperidinylpyrimido[5,4-*d*]pyrimidine-2,6-diyl)dinitrilo]tetrakis-

2,2',2'',2'''-[(4,8-Dipiperidinopyrimido[5,4-*d*]pyrimidine-2,6-diyl)dinitrilo]tetraethanol [58-32-2].

DEFINITION

Dipyridamole contains NLT 98.0% and NMT 102.0% of dipyridamole ($C_{24}H_{40}N_8O_4$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** { 197K }
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• Procedure

Buffer: 0.77 g/L of ammonium acetate. Adjust with glacial acetic acid to a pH of 4.0.

Mobile phase: Methanol and *Buffer* (700:300)

Standard solution: 0.2 mg/mL of USP Dipyridamole RS in methanol

Sample solution: 0.2 mg/mL of Dipyridamole in methanol

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 288 nm

Column: 4.6-mm × 25-cm; 5- μ m packing L1

Flow rate: 1.2 mL/min

Injection volume: 10 μ L

Run time: NLT 1.7 times the retention time of dipyridamole

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of dipyridamole ($C_{24}H_{40}N_8O_4$) in the portion of Dipyridamole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of dipyridamole from the *Sample solution*

r_S peak response of dipyridamole from the *Standard solution*

C_S concentration of USP Dipyridamole RS in the *Standard solution* (mg/mL)

C_U concentration of Dipyridamole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** $\langle 281 \rangle$: NMT 0.1%

Delete the following:

- **Heavy Metals, Method II** $\langle 231 \rangle$: NMT 10 ppm (Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

Mobile phase: ~~250 mg of dibasic sodium phosphate in 250 mL of water, and adjust with dilute phosphoric acid (1 in 3) to a pH of 4.6. Add 750 mL of methanol, and pass through a membrane filter of 0.5 μ m pore size.~~

Standard solution: ~~15 μ g/mL of USP Dipyridamole RS in Mobile phase~~

Sample solution A: ~~1 mg/mL of Dipyridamole in methanol~~

Sample solution B: ~~0.01 mg/mL of Dipyridamole, from Sample solution A, in methanol~~

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: ~~LC~~

Detector: ~~UV 288 nm~~

Column: ~~3.9 mm \times 30 cm; packing L1~~

Flow rate: ~~1.5 mL/min~~

Injection volume: ~~10 μ L~~

Run time: ~~NLT 10 min~~

System suitability

Sample: ~~Standard solution~~

Suitability requirements

Column efficiency: ~~NLT 1000 theoretical plates~~

Tailing factor: ~~NMT 2.0~~

Relative standard deviation: ~~NMT 2.0%~~

Analysis

Samples: ~~Sample solution A and Sample solution B~~

[Note ~~Inject Sample solution B into the chromatograph, adjusting the operating~~

parameters so that the response of the main peak (retention time about 6.5 min) obtained is about 5% full scale.]

Acceptance criteria: The sum of the responses of all secondary peaks from *Sample solution A* is NMT the response of the main peak from *Sample solution B* (1.0%).

■ **Solution A:** 10 mM ammonium formate. Adjust with formic acid to a pH of 5.0.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	80	20
2.0	80	20
17.0	5	95
19.0	5	95
21.0	80	20
25.0	80	20

Standard solution: 0.5 µg/mL each of USP Dipyrindamole RS, USP Dipyrindamole Related Compound A RS, USP Dipyrindamole Related Compound B RS, USP Dipyrindamole Related Compound C RS, USP Dipyrindamole Related Compound D RS, USP Dipyrindamole Related Compound E RS, and USP Dipyrindamole Related Compound F RS in methanol

Sample solution: 500 µg/mL of Dipyrindamole in methanol

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 295 nm

Column: 2.1-mm × 15-cm; 1.7-µm packing L1

Column temperature: 45°

Flow rate: 0.3 mL/min

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between dipyrindamole related compound D and dipyrindamole

Relative standard deviation: NMT 2.8%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Dipyrindamole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of each specified impurity from the *Sample solution*

r_S

= peak response of corresponding USP Reference Standard from the *Standard solution*

C_S

= concentration of corresponding USP Reference Standard in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= concentration of Dipyridamole in the *Sample solution* ($\mu\text{g/mL}$)

Calculate the percentage of each unspecified impurity in the portion of Dipyridamole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of each unspecified impurity from the *Sample solution*

r_S

= peak response of dipyridamole from the *Standard solution*

C_S

= concentration of USP Dipyridamole RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= concentration of Dipyridamole in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dipyridamole related compound B	0.6	0.1
Dipyridamole related compound F	0.7	0.1
Dipyridamole related compound D	0.9	0.1
Dipyridamole	—	—
Dipyridamole related compound E	1.2	0.1
Dipyridamole related compound C	1.5	0.1
Dipyridamole related compound A	1.9	0.1
Any unspecified impurity	—	0.10
Total impurities	—	1.0

■ 1S (USP39)

SPECIFIC TESTS

- **Chloride**

Sample: 500 mg

Analysis: Dissolve the *Sample* in 5 mL of alcohol and 2 mL of 2 N nitric acid. Add 1 mL of silver nitrate TS.

Acceptance criteria: No turbidity or precipitate is produced.

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at room temperature.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Dipyridamole RS

- USP Dipyridamole Related Compound A RS

2,2'-{[4,6,8-Tri(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-2-yl]azanediyl}diethanol.

C₂₅H₄₀N₈O₂ 484.64

USP Dipyridamole Related Compound B RS

2,2',2^{''},2^{'''},2^{''''},2^{'''''}-{[8-(Piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,4,6-triyl]tris(azanetriyl)}hexaethanol.

C₂₃H₄₀N₈O₆ 524.61

USP Dipyridamole Related Compound C RS

2,2'-{[6-Chloro-4,8-di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-2-yl]azanediyl}diethanol.

C₂₀H₃₀ClN₇O₂ 435.95

USP Dipyridamole Related Compound D RS

2,2'-({6-[(2-Hydroxyethyl)amino]-4,8-di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-2-yl}azanediyl)diethanol.

C₂₂H₃₆N₈O₃ 460.57

USP Dipyridamole Related Compound E RS

2,2',2^{''},2^{'''}-{[6,8-Di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,4-diyl]bis(azanetriyl)}tetraethanol.

C₂₄H₄₀N₈O₄ 504.63

USP Dipyridamole Related Compound F RS

2,2',2^{''},2^{'''}-({4-[(2-Hydroxyethyl)amino]-8-(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,6-diyl}bis(azanetriyl))tetraethanol.

C₂₁H₃₆N₈O₅ 480.56

- 1S (USP39)

BRIEFING

Dipyridamole Tablets, USP 38 page 3159. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Add an orthogonal *Identification* test *B* based on the retention time agreement in the

Assay.

2. Revise the calculation in the *Uniformity of Dosage Units* section. Change the report unit from the quantity in mg to the percentage of the label claim.
3. Add the test for *Organic Impurities* to the monograph. The proposed procedure is based on analyses performed with the Waters Acquity UPLC BEH C18 brand of L1 column. The typical retention time for dipyridamole is 9.5 min.
4. Add proposed *Acceptance criteria* for specified and unspecified degradation products.
5. Add storage requirements to the *Packaging and Storage* section.
6. Add USP Dipyridamole Related Compound A RS, USP Dipyridamole Related Compound B RS, USP Dipyridamole Related Compound C RS, USP Dipyridamole Related Compound D RS, USP Dipyridamole Related Compound E RS, and USP Dipyridamole Related Compound F RS to the *USP Reference Standards* section to support the proposed addition of the *Organic Impurities* test.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: G. Hsu , S. Ramakrishna.)

Correspondence Number—C136885

Comment deadline: July 31, 2015

Dipyridamole Tablets**DEFINITION**

Dipyridamole Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of dipyridamole ($C_{24}H_{40}N_8O_4$).

IDENTIFICATION**Change to read:**

-

■ **A.** ■ 1S (*USP39*)

Infrared Absorption { 197K }

Sample: Transfer an amount equivalent to 100 mg of dipyridamole from finely powdered Tablets to 10 mL of 0.1 N hydrochloric acid, triturate, and filter, collecting the filtrate in a beaker. Add 0.1 N sodium hydroxide until the solution is basic and a precipitate forms. Heat the mixture on a steam bath for 1 min, cool, and filter. Dry the residue at 105° for 1 h.

Acceptance criteria: Meet the requirements

Add the following:

■ • **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ■ 1S (*USP39*)

ASSAY

- **Procedure**

Mobile phase: Dissolve 250 mg of dibasic sodium phosphate in 250 mL of water, and adjust

with dilute phosphoric acid (1 in 3) to a pH of 4.6. Add 750 mL of methanol and pass through a suitable filter of 0.5- μ m pore size.

Standard solution: 15 μ g/mL of USP Dipyridamole RS in *Mobile phase*

Sample solution: Nominally 15 μ g/mL of dipyridamole prepared as follows. Transfer NLT 20 Tablets to a suitable volumetric flask. Add water equivalent to 10% of the final flask volume and sonicate for 15 min. Add methanol equivalent to 75% of the final flask volume and shake by mechanical means for 30 min. Dilute with methanol to volume and centrifuge to obtain a clear supernatant. Dilute a volume of the clear supernatant with *Mobile phase* to obtain a solution containing 15 μ g/mL of dipyridamole.

Chromatographic system

(See *Chromatography* \langle 621 \rangle , *System Suitability*.)

Mode: LC

Detector: UV 288 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 50 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dipyridamole ($C_{24}H_{40}N_8O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Dipyridamole RS in the *Standard solution* (μ g/mL)

C_U nominal concentration of dipyridamole in the *Sample solution* (μ g/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution \langle 711 \rangle

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Dipyridamole RS at a known concentration in *Medium*

Sample solution: Sample per *Dissolution* \langle 711 \rangle . Dilute with *Medium* as needed.

Instrumental conditions

Mode: UV

Analytical wavelength: 282 nm

Analysis**Samples:** *Standard solution* and *Sample solution***Tolerances:** NLT 70% (Q) of the labeled amount of dipyridamole ($C_{24}H_{40}N_8O_4$) is dissolved.**Change to read:**● **Uniformity of Dosage Units** 〈 905 〉**Procedure for content uniformity****Medium:** 1 N hydrochloric acid**Standard solution:** 10 µg/mL of USP Dipyridamole RS in *Medium***Sample solution:** Transfer 1 Tablet to a 100-mL volumetric flask, add 50 mL of *Medium*, heat in a steam bath for 5 min, and shake by mechanical means for 30 min. Cool to room temperature and dilute with *Medium* to volume. Filter, discarding the first 25 mL of the filtrate. Dilute a portion of the subsequent filtrate with *Medium* to prepare a solution of 10 µg/mL of dipyridamole.**Analytical wavelength:** 282 nm**Cell:** 1 cm**Blank:** *Medium***Instrumental conditions****Mode:** UV**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the quantity, in mg, of $C_{24}H_{40}N_8O_4$ in the Tablet taken:

$$\text{Result} = (TC/D) \times (A_U/A_S)$$

 ~~T = labeled quantity, in mg, of dipyridamole in the Tablet~~ ~~C = concentration, in µg per mL, of USP Dipyridamole RS in the *Standard solution*~~ ~~D = concentration, in µg per mL, of dipyridamole in the solution from the Tablet based on the labeled quantity per Tablet and the extent of dilution~~ ~~A_U = absorbance of the solution from the Tablet~~ ~~A_S = absorbance of the solution from the *Standard solution*~~

- Calculate the percentage of the labeled amount of dipyridamole ($C_{24}H_{40}N_8O_4$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of USP Dipyridamole RS in the *Standard solution* (µg/mL) C_U

= nominal concentration of dipyridamole in the *Sample solution* ($\mu\text{g/mL}$)

■ 1S (USP39)

Acceptance criteria: Meet the requirements

IMPURITIES

Add the following:

■ Organic Impurities

Solution A: 10 mM ammonium formate adjusted with formic acid to a pH of 5.0

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	80	20
2.0	80	20
17.0	5	95
19.0	5	95
21.0	80	20
25.0	80	20

Peak identification solution: 1 $\mu\text{g/mL}$ each of USP Dipyridamole RS, USP Dipyridamole Related Compound A RS, USP Dipyridamole Related Compound B RS, USP Dipyridamole Related Compound C RS, USP Dipyridamole Related Compound D RS, USP Dipyridamole Related Compound E RS, and USP Dipyridamole Related Compound F RS in methanol

Standard solution: 1 $\mu\text{g/mL}$ each of USP Dipyridamole RS, USP Dipyridamole Related Compound A RS, USP Dipyridamole Related Compound B RS, and USP Dipyridamole Related Compound C RS in methanol

Sample solution: Nominally 500 $\mu\text{g/mL}$ of dipyridamole in methanol from NLT 20 Tablets prepared as follows. Transfer a suitable amount of finely powdered Tablets equivalent to 50 mg of dipyridamole to a 100-mL volumetric flask. Add 60 mL of methanol, shake by mechanical means for 1 h, and dilute with methanol to volume. Centrifuge to obtain a clear supernatant and use the clear supernatant. [Note—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 295 nm

Column: 2.1-mm \times 15-cm; 1.7- μm packing L1

Column temperature: 45 $^{\circ}$

Flow rate: 0.3 mL/min

Injection volume: 2 μL

System suitability

Samples: *Peak identification solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 4.0 between dipyridamole related compound D and dipyridamole, *Peak*

identification solution

Relative standard deviation: NMT 2.8% for all the peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each specified degradation product from the *Sample solution*

r_S peak response of corresponding USP Reference Standard from the *Standard solution*

C_S concentration of corresponding USP Reference Standard in the *Standard solution* (µg/mL)

C_U nominal concentration of dipyridamole in the *Sample solution* (µg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each unspecified degradation product from the *Sample solution*

r_S peak response of dipyridamole from the *Standard solution*

C_S concentration of USP Dipyridamole RS in the *Standard solution* (µg/mL)

C_U nominal concentration of dipyridamole in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dipyridamole related compound B	0.63	0.2
Dipyridamole related compound F ^a	0.71	—
Dipyridamole related compound D ^a	0.94	—
Dipyridamole	1.0	—
Dipyridamole related compound E ^a	1.19	—
Dipyridamole related compound C	1.54	0.2
Dipyridamole related compound A	1.93	0.2
Individual unspecified impurity	—	0.2
Total impurities	—	1.0

^a Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities of the drug product.

■ 1S (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

■ Store at controlled room temperature. ■ 1S (USP39)

Change to read:

- **USP Reference Standards** { 11 }

USP Dipyrnidamole RS

■ USP Dipyrnidamole Related Compound A RS

2,2'-{[4,6,8-Tri(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-2-yl]azanediyl}diethanol.

C₂₅H₄₀N₈O₂ 484.64

USP Dipyrnidamole Related Compound B RS

2,2',2^{''},2^{'''},2^{''''},2^{'''''}-{[8-(Piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,4,6-triyl]tris(azanetriyl)}hexaethanol.

C₂₃H₄₀N₈O₆ 524.61

USP Dipyrnidamole Related Compound C RS

2,2'-{[6-Chloro-4,8-di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-2-yl]azanediyl}diethanol.

C₂₀H₃₀ClN₇O₂ 435.95

USP Dipyrnidamole Related Compound D RS

2,2'-({6-[(2-Hydroxyethyl)amino]-4,8-di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-2-yl}azanediyl)diethanol.

C₂₂H₃₆N₈O₃ 460.57

USP Dipyrnidamole Related Compound E RS

2,2',2^{''},2^{'''}-{[6,8-Di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,4-diyl]bis(azanetriyl)}tetraethanol.

C₂₄H₄₀N₈O₄ 504.63

USP Dipyrnidamole Related Compound F RS

2,2',2^{''},2^{'''}-({4-[(2-Hydroxyethyl)amino]-8-(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,6-diyl}bis(azanetriyl))tetraethanol.

C₂₁H₃₆N₈O₅ 480.56

■ 1S (USP39)

BRIEFING

Eptacog Alfa. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods of analysis, is proposed.

1. The liquid chromatographic procedure in *Identification* test *B* for *Peptide Mapping* is based on analyses performed with the Machery–Nagel Nucleosil C18 brand of L1 column.
2. The liquid chromatographic procedures in the *Assay for Concentration of Eptacog Alfa* and in the *Impurities* test for *Dimer and Related Substances of Higher Molecular Mass* are based on analyses performed with the Waters Protein-Pak 300SW brand of L59 column. The typical retention time for eptacog alfa is about 17 min.
3. The liquid chromatographic procedure in the *Impurities* test for *Degraded Heavy Chain and Oxidized Forms* is based on analyses performed with the Interchim

Uptisphere WC4, 300 Å brand of L26 column. The typical retention time for eptacog alfa is about 26 min.

- The liquid chromatographic procedure in the *Impurities* test for *Gla-Domainless Eptacog Alfa (γ-Carboxylation)* is based on analyses performed with a precolumn packed with the Bio-Rad Chelex brand of chelating ion exchange resin for removal of calcium, and the Dionex DNAPac PA-100 brand of L## analytical column. The typical retention times for eptacog alfa and Gla-domainless eptacog alfa are about 14 and 10 min, respectively.
- The liquid chromatographic procedure in the *Specific Tests for Glycan Analysis* is based on analyses performed with the Dionex brand of L## guard and analytical columns, using CarboPac PA-100 4.0 mm × 5 cm and CarboPac PA-100 4.0 mm × 25 cm, respectively.

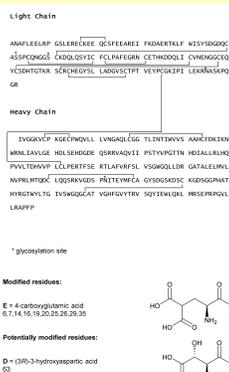
(BIO2: C. Li.)

Correspondence Number—C149307

Comment deadline: July 31, 2015

Add the following:

■ Eptacog Alfa



$C_{1982}H_{3054}N_{560}O_{618}S_{28}$ (The chemical formula includes the γ -carboxylations and modification of D (Asp) in position 63, and does not include the glycosylations.) $\approx 50,000$ (The molecular weight includes the glycosylation.) [102786–61–8].

DEFINITION

Eptacog Alfa is recombinant human coagulation factor VIIa, a recombinant form of plasma-derived activated coagulation factor VII. It is a two-chain molecule, obtained by proteolytic cleavage of the peptide bond between Arg¹⁵² and Ile¹⁵³ of single-chain coagulation factor VII. Eptacog Alfa consists of a 20 kDa light chain (N-terminal) and a 30 kDa heavy chain (C-terminal) connected by a disulfide bond.

Eptacog Alfa is produced in mammalian cells by a method based on recombinant DNA technology. It is distinguishable from the naturally occurring analogue in terms of its post-translational modifications, including its glycosylation pattern. The host cell-derived proteins (HCP) content is determined by a validated method and is below the limit approved by the competent authority. The host cell DNA content is determined by a validated method and is

below the limit approved by the competent authority. When prepared as a drug substance (eptacog alfa concentrated solution), it contains NLT 1.0 and NMT 1.7 mg/mL of eptacog alfa. It has a biological potency of NLT 44 and NMT 64 IU/ μ g of eptacog alfa. Formulation contains one or more suitable buffering and/or stabilizing agents.

[Note—Some tests refer to using the injection amount based on μ g of eptacog alfa because the concentration of eptacog alfa in the *Standard solution* or the *Sample solution* can vary. The injection volume can be approximated based on the expected protein concentration.]

IDENTIFICATION

- **A.** Meets the requirements in the *Assay for Potency*

- **B. Peptide Mapping**

Solution A: 0.65 mL/L of trifluoroacetic acid in water

Solution B: Acetonitrile, water, and trifluoroacetic acid (900: 100: 0.5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
100	50	50
105	0	100
110	0	100
110.1	100	0
125	100	0

Diluent: 50 mM tris(hydroxymethyl)aminomethane and 5 mM calcium chloride prepared as follows. Dissolve 6.06 g of tris(hydroxymethyl)aminomethane and 0.74 g of calcium chloride in 1000 mL of water. Adjust with 37% hydrochloric acid to a pH of 7.5.

Enzyme solution diluent: 0.001 N hydrochloric acid and 2 mM calcium chloride prepared as follows. Dissolve 1 mL of 1 N hydrochloric acid and 0.3 g of calcium chloride in 1000 mL of water.

Enzyme solution: 1 mg/mL of trypsin suitable for peptide mapping in *Enzyme solution diluent*

Standard solution: 1.5 mg/mL of USP Coagulation Factor VIIa RS in *Diluent*. Desalt a volume of this solution by a suitable method. [Note—For example, use a suitable gel-filtration column¹ with *Diluent* as the elution buffer.] After desalting, the concentration should be about 1.0 mg/mL. Transfer 1 mL of the desalted solution to a polypropylene tube and add 10 μ L of the *Enzyme solution*. Cap the tube and mix gently by inversion. Incubate at $37 \pm 2^\circ$ for 24 ± 2 h. At 5.5 ± 0.5 h, add an additional 10 μ L of the *Enzyme solution*. After incubation, remove the sample from the incubator and place the solution at room temperature. Add 9 μ L of glacial acetic acid and mix by inversion. Keep the solution at -15° or below until chromatographic analysis.

Sample solution: About 1.5 mL of Eptacog Alfa in *Diluent*. Desalt and digest this solution as directed in the *Standard solution*.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 215 nm
Column: 4.0 mm × 25 cm; 5- μ m packing L1
Column temperature: 30 $^{\circ}$
Flow rate: 1.0 mL/min
Injection amount: 25 μ g

System suitability

Sample: *Standard solution*

Suitability requirements

Chromatogram similarity: The chromatogram of the *Standard solution* corresponds to that of the typical chromatogram provided with the USP Certificate for USP Coagulation Factor VIIa RS.

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The chromatographic profile of the *Sample solution* corresponds to that of the *Standard solution*.

All major peaks identified in the chromatogram of the *Standard solution* are present in the chromatogram of the *Sample solution*.

No new major peaks are observed in the chromatogram of the *Sample solution* in comparison with the chromatogram of the *Standard solution*.

ASSAY**• Concentration of Eptacog Alfa**

Mobile phase: 0.2 M ammonium sulfate and 5% 2-propanol prepared as follows. Dissolve 26.4 g of ammonium sulfate in approximately 900 mL of water. Adjust first with phosphoric acid to a pH of 2.5 and then with triethylamine to a pH of 7.0. Add 50 mL of 2-propanol. Dilute with water to 1000 mL.

Standard solution: 1.5 mg/mL of USP Coagulation Factor VIIa RS in water

Sample solution: About 1.5 mg/mL of Eptacog Alfa in water

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 7.5 mm × 30 cm; 10- μ m packing L59

Autosampler temperature: 2 $^{\circ}$ –8 $^{\circ}$

Flow rate: 0.5 mL/min

Injection amount: About 30 μ g, *Sample solution*

System suitability

Sample: *Standard solution* injected at 10, 20, and 30 μ L

Suitability requirements

Tailing factor: NMT 1.3 for the monomer peak from the *Standard solution* injected at 20 μ L

Peak-to-valley ratio: NLT 1.1 for the dimer peak from the *Standard solution* injected at 20 μ L

Relative standard deviation: NMT 2.0% for the monomer peak from five replicate injections of 20 μ L of the *Standard solution*

Linearity: Inject 10, 20, and 30 μ L of the *Standard solution*. Plot the monomer peak response against the injected protein content (μ g) and perform a linear regression to

create a standard curve. The correlation coefficient calculated for the standard curve (r^2) is NLT 0.990.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration of eptacog alfa using the monomer peak response from the *Sample solution*, the standard curve, and the injection volume of the *Sample solution*.

Acceptance criteria: 1.0–1.7 mg/mL

● **Potency**

The principle of the test is to measure the ability of an eptacog alfa preparation to reduce the prolonged coagulation time of factor VII-deficient plasma. The biological activity is assessed by comparing the dose-response curve of the *Sample solution* to that of the *Standard solution*.

Use a suitable coagulation analyzer or carry out the assay with incubation tubes and reagents maintained at 37°.

Diluent: Use a suitable buffer for the coagulation test. [Note—For example, 0.05 M piperazine-1,4-bis(2-ethanesulfonic acid), 0.1 M sodium chloride, 2 mM sodium edetate, and 1% bovine albumin. Adjust to a pH of 7.2 prepared as follows. 15.12 g/L of piperazine-1,4-bis(2-ethanesulfonic acid), 5.73 g/L of sodium chloride, and 0.74 g/L of sodium edetate. Adjust with sodium hydroxide to a pH of 7.2 and add 10 g/L of bovine albumin.]

Standard solution: Prepare solutions of USP Coagulation Factor VIIa for Bioassay RS in *Diluent* at three different concentrations within the linear range. Prepare in duplicate and use the solutions immediately. [Note—An example of the linear range is 0.005–0.14 IU/mL.]

Sample solution: Prepare solutions of Eptacog Alfa in *Diluent* at three different concentrations within the linear range. Prepare in duplicate and use the solutions immediately. [Note—An example of the linear range is 0.005–0.14 IU/mL.]

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—The volumes and sequences of the reagents given below may be adapted to the tissue factor solution and apparatus used.]

To 40 μ L of each *Standard solution* or *Sample solution*, add 40 μ L of factor VII-deficient plasma, incubate for an appropriate time at 37°, and add 80 μ L of tissue factor. [Note—Use a tissue factor that contains calcium and other suitable excipients.] Measure the coagulation time (the interval between the addition of tissue factor and the formation of a fibrin clot). Calculate the activity in IU/mL using an appropriate statistical method and validity test. For example, the parallel-line assay and the statistical tests for linearity, slope, and parallelism for the sample compared to the standard have to be passed at the 95% level. The confidence limits ($P = 0.95$) must be within 80%–125% of the estimated potency.

Divide the determined activity in IU/mL by the concentration of eptacog alfa and report the result in IU/ μ g.

Acceptance criteria: 44–64 IU/ μ g

IMPURITIES

● **Degraded Heavy Chain and Oxidized Forms**

Solution A: 0.1% Trifluoroacetic acid (v/v) in water

Solution B: Acetonitrile, water, and trifluoroacetic acid (800: 200: 0.9)

Mobile phase: See *Table 2*. [Note—The main peak should elute at approximately 26 min. The percentage of *Solution B* at 0 min and 30 min can be adjusted together so that the increase in the percentage of *Solution B* should always be 13% over 30 min.]

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	54	46
30	41	59
33	41	59
33.1	0	100
38	0	100
40	54	46
50	54	46

Standard solution: 1.5 mg/mL of USP Coagulation Factor VIIa RS in water

Sample solution: About 1.5 mg/mL of Eptacog Alfa in water

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.0 mm × 25 cm; 5- μ m packing L26

Temperatures

Column: 60^o–70^o

Autosampler: 2^o–8^o

Flow rate: 1.0 mL/min

Injection amount: 30 μ g

System suitability

Sample: *Standard solution*

[Note—The retention time for eptacog alfa is about 26 min.]

Suitability requirements

The chromatogram from the *Standard solution* corresponds to the typical chromatogram provided in the USP Certificate for USP Coagulation Factor VIIa RS, with the 10 peaks being clearly visible. In the chromatogram from the *Standard solution*, identify 10 peaks according to the USP Certificate for USP Coagulation Factor VIIa RS and the descriptions provided in *Table 3*.

Table 3

Peak	Name	Typical Retention Time (min)
1	Degraded heavy chain form, amino acids 1–290	10
2	Degraded heavy chain form, amino acids 291–406	16
3–5	Oxidized forms, methionyl sulfoxide of eptacog alfa at Met ²⁹⁸ , Met ³⁰⁶ , and Met ³²⁷ , respectively	19–24

Peak	Name	Typical Retention Time (min)
6	Degraded heavy chain form, cleaved between Arg ³¹⁵ and Lys ³¹⁶	25
7	Eptacog alfa	26
8-10	Unknown	29-32

Resolution: NLT 0.9 between peaks 6 and 7

Analysis

Sample: *Sample solution*

Calculate the percentage of the degraded heavy chain and oxidized forms in the portion of Eptacog Alfa taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_{\bar{U}}$ peak response of the degraded heavy chain or oxidized forms from the *Sample solution*

$r_{\bar{T}}$ sum of all the peak responses from the *Sample solution*

Acceptance criteria

Sum of degraded heavy chain forms: NMT 11%

Sum of oxidized forms: NMT 2.2%

Gla-Domainless Eptacog Alfa (γ -Carboxylation)

Solution A: 10 mM tris(hydroxymethyl)aminomethane and 10 mM bis-tris propane prepared as follows. 1.2 g/L of tris(hydroxymethyl)aminomethane and 2.8 g/L of bis-tris propane in water. Adjust with glacial acetic acid to a pH of 9.4.

Solution B: 10 mM tris(hydroxymethyl)aminomethane, 10 mM bis-tris propane, and 1.4 M ammonium acetate, prepared as follows. 1.2 g/L of tris(hydroxymethyl)aminomethane, 2.8 g/L of bis-tris propane, and 107.9 g/L of ammonium acetate in water. Adjust with concentrated ammonia to a pH of 9.4.

Mobile phase: See Table 4.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2.5	100	0
27.5	0	100
30.5	100	0

Standard solution: 1.5 mg/mL of USP Coagulation Factor VIIa RS in water

Sample solution: About 1.5 mg/mL of Eptacog Alfa in water

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Columns

Precolumn: Chelating ion exchange resin for removal of calcium²

Analytical: 4.0 mm × 25 cm; packing L##

Temperatures

Column: 25°

Autosampler: 2°–8°

Flow rate: 1.0 mL/min

Injection amount: About 150 µg

System suitability

Sample: *Standard solution*

[Note—Gla-domainless eptacog alfa (Υ -carboxylation) is des(1-38)-eptacog alfa. The retention time for eptacog alfa is about 14 min. The relative retention time for Gla-domainless eptacog alfa (Υ -carboxylation) is about 0.7.]

Suitability requirements

Resolution: NLT 2.0 between the Gla-domainless eptacog alfa and eptacog alfa peaks

Analysis

Sample: *Sample solution*

Calculate the percentage of Gla-domainless eptacog alfa in the portion of Eptacog Alfa taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of Gla-domainless eptacog alfa from the *Sample solution*

r_T sum of the peak responses from the *Sample solution*

Acceptance criteria: NMT 5.9%

• **Dimer and Related Substances of Higher Molecular Mass**

Mobile phase, Standard solution, Sample solution, Chromatographic system, and

System suitability: Proceed as directed in the *Assay for Concentration of Eptacog Alfa*

Analysis

Sample: *Sample solution*

Calculate the percentage of dimer and related substances of higher molecular mass in the portion of Eptacog Alfa taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U sum of the peak responses with retention time less than that of the monomer from the *Sample solution*

r_T sum of the responses of all eptacog alfa-related peaks from the *Sample solution*

Acceptance criteria: NMT 1.9%

• **Non-Activated Factor VII (Single Chain)**

Sample buffer (reducing conditions): Concentrated LDS-PAGE sample buffer³ for reducing conditions containing dithiothreitol (DTT) as the reducing agent.⁴ [Note—For example, prepare by mixing 300 µL of water, 500 µL of LDS-PAGE sample buffer,³ and 200 µL of reducing agent.⁴]

Running buffer: MOPS–SDS buffer.⁵ [Note—For example, 50 mM 3-(*N*-morpholino)propanesulfonic acid, 50 mM tris(hydroxymethyl)aminomethane, 0.1% sodium dodecyl sulfate, and 1 mM EDTA. Adjust to a pH of 7.7.]

Molecular weight marker solution: Solution of molecular weight markers suitable for calibrating SDS-polyacrylamide gels in the range of 10–70 kDa

Standard solution: 800 µg/mL of USP Coagulation Factor VIIa RS in water. Mix equal

volumes of this solution and the *Sample buffer (reducing conditions)*.

Sample solution: About 800 µg/mL of Eptacog Alfa in water. Mix equal volumes of this solution and the *Sample buffer (reducing conditions)*.

Sample treatment: Boil the *Standard solution* and the *Sample solution* separately for 5 min or heat at $73 \pm 3^\circ$ for 10 min before applying to the gel.

Electrophoretic system

Mode: Polyacrylamide gel electrophoresis

Detector: Integrating densitometer

Staining: Coomassie⁶

Resolving gel: 12% Acrylamide; 1 mm thickness⁷

Application amount: 2.0–4.0 µg

System suitability

Samples: *Molecular weight marker solution*, *Standard solution*, and *Sample solution*

Suitability requirements

Molecular weight: The principal bands in the electropherogram from the *Sample solution* correspond to the position of the principle bands in the electropherogram from the *Standard solution*. [Note—The positions of the principle bands from the *Standard solution* and *Sample solution* correspond to 30 kDa for heavy chain and 20–25 kDa for light chain determined by comparison with the known molecular weight bands from the *Molecular weight marker solution*.]

Sensitivity: The band corresponding to non-activated single chain factor VII (molecular mass of 51 kDa) is visible in the electropherogram from the *Standard solution*.

Analysis

Sample: *Sample solution*

Calculate the percentage of non-activated single chain factor FVII (molecular mass of 51 kDa) in the portion of Eptacog Alfa taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_{\bar{U}}$ band response of the non-activated single chain factor VII from the *Sample solution*

$r_{\bar{T}}$ sum of the responses of all bands from the *Sample solution*

Acceptance criteria: NMT 3%

SPECIFIC TESTS

• Glycan Analysis

[Note—The procedure below can be substituted with a suitable procedure from *Oligosaccharide Analysis* (212).]

Solution A: 6 g/L of sodium hydroxide in degassed water

Solution B: 40.8 g/L of sodium acetate trihydrate and 6 g/L of sodium hydroxide in degassed water

Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	100	0

Time (min)	Solution A (%)	Solution B (%)
52	35	65
52.1	0	100
65	0	100
65.1	100	0
90	100	0

Buffer: 10 mM tris(hydroxymethyl)aminomethane prepared as follows. 1.21 g/L of tris(hydroxymethyl)aminomethane in water. Adjust with hydrochloric acid to a pH of 7.5.

Standard solution: 1.5 mg/mL of USP Coagulation Factor VIIa RS in water. Desalt a volume of this solution by a suitable method. [Note—For example, use a suitable gel-filtration column¹ with *Buffer* as the elution buffer.] After desalting, the concentration of the solution is about 1.0 mg/mL.

Sample solution: 1.5 mg/mL of Eptacog Alfa in water. Desalt a volume of this solution by a suitable method. [Note—For example, use a suitable centrifugal filter unit or gel-filtration column with *Buffer* as the elution buffer.] After desalting, the concentration of the solution is about 1.0 mg/mL.

Selective release of glycans: Transfer 500 μ L of the desalted *Sample solution* and 500 μ L of the desalted *Standard solution* to separate tubes and add 10 μ L of a 200-U/mL solution of peptide *N*-glycosidase F⁸ to each tube. Cap the tubes and incubate for 16–24 h at 37^o. Remove the protein fraction by adding 1.5 mL of ethanol (96%) prechilled at –20^o to the tubes. Mix and allow to stand at –20^o for 20–30 min. Centrifuge the tubes at 10,000 rpm for 10 min. Collect the supernatant and evaporate to dryness. [Note—For example, use a rotary evaporator.]

Labeling of glycans: Label the released glycans with 2-aminobenzamide (2-AB) using a suitable procedure.⁹ [Note—For example, add 5–15 μ L of 2-AB labeling solution to the dried glycan sample and mix well. Immediately incubate at 37^o for 16–24 h or at 65^o for 2 h. Allow to cool at room temperature for 10 min. Centrifuge briefly. Remove excess 2-AB using a suitable solid-phase exchange (SPE) cartridge.¹⁰]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Fluorescence

Excitation wavelength: 330 nm

Emission wavelength: 420 nm

Columns

Precolumn: 4.0 mm \times 5 cm; packing L##

Analytical: 4.0 mm \times 25 cm; packing L##

Temperatures

Column: 30^o

Autosampler: 2^o–8^o

Flow rate: 0.5 mL/min

Injection volume: 100 μ L

System suitability

Sample: *Standard solution*

System suitability requirements

Chromatogram similarity: The chromatogram of the *Standard solution* is similar to the typical chromatogram provided in the USP Certificate for USP Coagulation Factor VIIa RS with the 12 specified peaks (see *Table 6* for the descriptions) clearly visible.

Table 6

Peak	Charged	Structure	Typical Retention Time (min)
1	No	Core fucosylated biantennary – non sialylated (two <i>N</i> -acetylglactosamine terminals)	12
2	No	Core fucosylated biantennary – non sialylated (<i>N</i> -acetylglactosamine and galactose terminals)	15
3	No	Structure not determined	16
4	No	Core fucosylated biantennary – non sialylated (galactose and <i>N</i> -acetylglucosamine terminals)	17
5	No	Core fucosylated biantennary – non sialylated (two galactose terminals)	17.5
6	Yes	Core fucosylated biantennary – monosialylated (and one <i>N</i> -acetylglactosamine terminal)	23
7	Yes	Core fucosylated biantennary – monosialylated (and one galactose terminal)	26
8	Yes	Core fucosylated biantennary – bisialylated	38
9	Yes	High-mannose structure with one phosphate group	44
10, 11	Yes	Core fucosylated triantennary – trisialylated	47, 48
12	Yes	Structure not determined	49

Peak width at half-height: NMT 30 s for peak 8 (core fucosylated biantennary-bisialylated)

Analysis

Sample: *Sample solution*

Calculate the percentage of charged glycans in the portion of Eptacog Alfa taken:

$$\text{Result} = [A/(A + B)] \times 100$$

A = sum of the peak responses of charged glycans from the *Sample solution*

B = sum of the peak responses of uncharged glycans from the *Sample solution*

Acceptance criteria: 86%–93%

- **Bacterial Endotoxins Test** $\langle 85 \rangle$: NMT 10 USP Endotoxin Units/mg

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, protected from light. Store at -70° or below.
- **Labeling:** Label to indicate that the product is activated and is of recombinant DNA origin.
- **USP Reference Standards** $\langle 11 \rangle$
USP Coagulation Factor VIIa RS

USP Coagulation Factor VIIa for Bioassay RS

■ 1S (USP39)

¹ Available from GE Healthcare as NAP10 column, catalog number 17-0854-02, or a suitable equivalent.

² Prepare precolumn as follows. Add approximately 600 mg of styrene–divinylbenzene copolymer with iminodiacetic groups cation exchange resin (available from Bio-Rad as Chelex 100 resin, catalog number 142-2822 or a suitable equivalent) to approximately 0.5 mL of *Solution A*. Pack a Tricorn 5/50 column (available from GE Healthcare as Bio-Science AB, catalog number 28-4064-09, or a suitable equivalent) with the resin solution avoiding headspace.

³ Available from Life Technologies as NuPAGE LDS Sample buffer, catalog number NP0007, or a suitable equivalent.

⁴ Available from Life Technologies as NuPAGE Sample Reducing Agent, catalog number NP0004/NP0009, or a suitable equivalent.

⁵ Available from Life Technologies as NuPAGE MOPS SDS Running buffer, catalog number NP0001, or a suitable equivalent.

⁶ Available from Life Technologies as Colloidal Coomassie G-250 staining kit, catalog number LC6025, or a suitable equivalent.

⁷ Available from Life Technologies as NuPAGE 12% Novex, catalog number NP0341, or a suitable equivalent.

⁸ Available from Roche. Catalog number 11365185001, or a suitable equivalent. One unit is defined as the amount of enzyme that catalyzes the release of *N*-linked oligosaccharides from 1.0 nmol denatured ribonuclease B per min at 37^o, pH 7.5.

⁹ Available from Ludger Ltd. as LudgerTag™ 2-AB glycan labeling kit, catalog number LT-KAB-A2, or a suitable equivalent. Follow product guide.

¹⁰ Available from Ludger Ltd. as LudgerClean cartridges, catalog number LC-S-A6, or a suitable equivalent.

BRIEFING

Eptacog Alfa for Injection. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is proposed.

1. The liquid chromatographic procedures in the *Assay for Content of Eptacog Alfa* and in the *Impurities* test for *Dimer and Related Substances of Higher Molecular Mass* are based on analyses performed with the Waters Protein–Pak 300SW brand of L59 column. The typical retention time for eptacog alfa is about 17 min.

- The liquid chromatographic procedure in the *Impurities* test for *Degraded Heavy Chain and Oxidized Forms* is based on analyses performed with the Interchim Uptisphere WC4, 300 Å brand of L26 column. The typical retention time for eptacog alfa is about 26 min.

(BIO2: C. Li.)

Correspondence Number—C149307

Comment deadline: July 31, 2015

Add the following:

■ Eptacog Alfa for Injection

DEFINITION

Eptacog Alfa for Injection is a sterile lyophilized preparation of recombinant human coagulation factor VIIa. It contains NLT 87% and NMT 113% of the labeled amount of eptacog alfa. It has a biological potency of NLT 44 and NMT 64 IU/μg of eptacog alfa. This formulation contains one or more suitable buffering and/or stabilizing agents. [Note—The tests refer to using the injection amount based on μg of eptacog alfa because the concentration of eptacog alfa in the *Standard solution* or the *Sample solution* can vary. The injection amount as μg of eptacog alfa is listed under certain tests. The injection volume can be approximated based on the expected protein concentration.]

IDENTIFICATION

- A.** Meets the requirements in the *Assay for Potency*

ASSAY

- Content of Eptacog Alfa**

Mobile phase: 0.2 M ammonium sulfate and 5% 2-propanol prepared as follows. Dissolve 26.4 g of ammonium sulfate in approximately 900 mL of water. Adjust first with phosphoric acid to a pH of 2.5 and then with triethylamine to a pH of 7.0. Add 50 mL of 2-propanol. Dilute with water to 1000 mL.

Standard solution: 1.5 mg/mL of USP Coagulation Factor VIIa RS in water

Sample solution: Dilute Eptacog Alfa for Injection with water to obtain a concentration of 1 mg/mL of eptacog alfa.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 7.5 mm × 30 cm; 10-μm packing L59

Autosampler temperature: 2°–8°

Flow rate: 0.5 mL/min

Injection amount: About 30 μg, *Sample solution*

System suitability

Sample: *Standard solution* injected at 10, 20, and 30 μL

Suitability requirements

Tailing factor: NMT 1.3 for the monomer peak from the *Standard solution* injected at 20

μL

Peak-to-valley ratio: NLT 1.1 for the dimer peak from the *Standard solution* injected at 20 μL

Relative standard deviation: NMT 2.0% for the monomer peak from five replicate injections of 20 μL of the *Standard solution*

Linearity: Inject 10, 20, and 30 μL of the *Standard solution*. Plot the monomer peak areas against the injected eptacog alfa and perform a linear regression to create a standard curve. The correlation coefficient calculated for the standard curve (r^2) is NLT 0.990.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration of eptacog alfa using the monomer peak area in the chromatogram from the *Sample solution*, the standard curve, and the injection volume of the *Sample solution*.

Calculate the percentage of the labeled amount of eptacog alfa in the portion of Eptacog Alfa for Injection taken:

$$\text{Result} = [(C_U \times V)/L] \times 100$$

C_U = nominal concentration of eptacog alfa in the *Sample solution* (mg/mL)

V = volume of *Sample solution* (mL)

L = label claim of eptacog alfa in Eptacog Alfa for Injection (mg)

Acceptance criteria: 87%–113%

• Potency

The principle of the test is to measure the ability of an eptacog alfa preparation to reduce the prolonged coagulation time of factor VII-deficient plasma. The biological activity is assessed by comparing the dose-response curve of the *Sample solution* to that of the *Standard solution*.

Use a suitable coagulation analyzer or carry out the assay with incubation tubes and reagents maintained at 37°.

Diluent: Use a suitable buffer for the coagulation test. [Note—For example, 0.05 M piperazine-1,4-bis(2-ethanesulfonic acid), 0.1 M sodium chloride, 2 mM sodium edetate, and 1% bovine albumin. Adjust to a pH of 7.2, prepared as follows. Mix 15.12 g/L of piperazine-1,4-bis(2-ethanesulfonic acid), 5.73 g/L of sodium chloride, and 0.74 g/L of sodium edetate. Adjust with sodium hydroxide to a pH of 7.2 and add 10 g/L of bovine albumin.]

Standard solution: Prepare solutions of USP Coagulation Factor VIIa for Bioassay RS in *Diluent* at three different concentrations within the linear range. Prepare in duplicate and use the solutions immediately. [Note—An example of the linear range is 0.005–0.14 IU/mL.]

Sample solution: Prepare solutions of Eptacog Alfa for Injection in *Diluent* at three different concentrations within the linear range. Prepare in duplicate and use the solutions immediately. [Note—An example of the linear range is 0.005–0.14 IU/mL.]

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—The volumes and sequences of the reagents given below may be adapted to the

tissue factor solution and apparatus used.]

To 40 μL of each *Standard solution* or *Sample solution*, add 40 μL of factor VII-deficient plasma, incubate for an appropriate time at 37 $^{\circ}$, and add 80 μL of tissue factor. [Note—Use a tissue factor that contains calcium and other suitable excipients.] Measure the coagulation time (the interval between the addition of the tissue factor solution and the formation of a fibrin clot). Calculate the activity in IU/mL using an appropriate statistical method and validity test. For example, the parallel-line assay and the statistical tests for linearity, slope, and parallelism for the sample compared to the standard have to be passed at the 95% level. The confidence limits ($P = 0.95$) must be within 80%–125% of the estimated potency.

Divide the determined activity in IU by the determined content of eptacog alfa in μg , and report the result in IU/ μg .

Acceptance criteria: 44–64 IU/ μg

IMPURITIES

• Degraded Heavy Chain and Oxidized Forms

Solution A: 0.1% Trifluoroacetic acid (v/v) in water

Solution B: Acetonitrile, water, and trifluoroacetic acid (800: 200: 0.9)

Mobile phase: See *Table 1*. [Note—The main peak should elute at approximately 26 min. The percentage of *Solution B* at 0 min and 30 min can be adjusted together so that the increase in the percentage of *Solution B* should be 13% over 30 min.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	54	46
30	41	59
33	41	59
33.1	0	100
38	0	100
40	54	46
50	54	46

Standard solution: 1.5 mg/mL of USP Coagulation Factor VIIa RS in water

Sample solution: Dilute Eptacog Alfa for Injection with water to obtain a concentration of 1 mg/mL of eptacog alfa.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.0 mm \times 25 cm; 5- μm packing L26

Temperatures

Column: 60 $^{\circ}$ –70 $^{\circ}$

Autosampler: 2 $^{\circ}$ –8 $^{\circ}$

Flow rate: 1.0 mL/min

Injection amount: About 30 μg

System suitability**Sample:** *Standard solution*

[Note—The retention time of eptacog alfa is about 26 min.]

Suitability requirements

The chromatogram from the *Standard solution* corresponds to the typical chromatogram provided in the USP Certificate for USP Coagulation Factor VIIa RS, with the 10 specified peaks being clearly visible. In the chromatogram from the *Standard solution*, identify 10 peaks according to the USP Certificate for USP Coagulation Factor VIIa RS and the descriptions provided in *Table 2*.

Table 2

Peak	Name	Typical Retention Time (min)
1	Degraded heavy chain form, amino acids 1–290	10
2	Degraded heavy chain form, amino acids 291–406	16
3–5	Oxidized forms, methionyl sulfoxide of eptacog alfa at Met ²⁹⁸ , Met ³⁰⁶ , and Met ³²⁷ , respectively	19–24
6	Degraded heavy chain form, cleaved between Arg ³¹⁵ and Lys ³¹⁶	25
7	Eptacog alfa	26
8–10	Unknown	29–32

Resolution: NLT 0.9 between peaks 6 and 7**Analysis****Sample:** *Sample solution*

Calculate the percentage of the degraded heavy chain and oxidized forms in the portion of Eptacog Alfa for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of the degraded heavy chain or oxidized forms from the *Sample solution*

r_T sum of the peak responses from the *Sample solution*

Acceptance criteria**Sum of degraded heavy chain forms:** NMT 12.3%**Sum of oxidized forms:** NMT 4.5%

- Dimer and Related Substances of Higher Molecular Mass**

Mobile phase, Standard solution, Sample solution, Chromatographic system, and**System suitability:** Proceed as directed in the *Assay for Content of Eptacog Alfa*.**Analysis****Sample:** *Sample solution*

Calculate the percentage of dimer and related substances of higher molecular mass in the portion of Eptacog Alfa for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U sum of the peak responses with retention time less than that of the monomer from the *Sample solution*

r_T sum of the responses of all eptacog alfa-related peaks from the *Sample solution*

Acceptance criteria: NMT 8.8%

SPECIFIC TESTS

- **Water Determination** 〈 921 〉, *Method 1c*: NMT 4%
- **Bacterial Endotoxins Test** 〈 85 〉: It contains NMT 10 USP Endotoxin Units/mg.
- **pH** 〈 791 〉: 5.5–6.5
- **Sterility Tests** 〈 71 〉, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **Constituted Solution**: Meets the requirements in *Injections and Implanted Drug Products* 〈 1 〉, *Specific Tests, Completeness and clarity of solutions*

ADDITIONAL REQUIREMENTS

- **Packaging and Storage Requirements** 〈 659 〉, *Injection Packaging, Sterile solids packaging*: Meets the requirements. Protect from light.
 - **Labeling**: Label to indicate that the product is the activated form and is of recombinant DNA origin.
 - **USP Reference Standards** 〈 11 〉
 - USP Coagulation Factor VIIa RS
 - USP Coagulation Factor VIIa for Bioassay RS
- 1S (USP39)

BRIEFING

Exemestane. Because there is no existing *USP* monograph for this drug substance, a new monograph based on validated methods of analysis is proposed.

1. The liquid chromatographic procedure used in the *Assay* is based on analysis performed with the Hypersil BDS C18 brand of L1 column. The typical retention time for exemestane is about 8.5 min.
2. The liquid chromatographic procedure used in the test for *Organic Impurities* is based on analysis performed with the Waters XTerra RP18 brand of L1 column. The typical retention time for exemestane is about 23.5 min.
3. The liquid chromatographic procedure used in the *Limit of Exemestane Related Compound D* test is based on analysis performed with the Daicel Chiralcel OJ brand of L80 column. The typical retention time for exemestane is about 17 min.

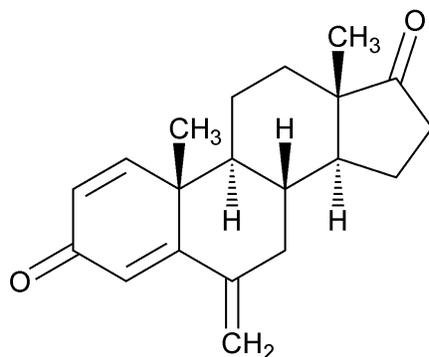
(SM3: F. Mao.)

Correspondence Number—C152104; C105513; C154957; C106738

Comment deadline: July 31, 2015

Add the following:

■ Exemestane



$C_{20}H_{24}O_2$ 296.40

Androsta-1,4-diene-3,17-dione, 6-methylene-;
6-Methyleneandrosta-1,4-diene-3,17-dione [107868-30-4].

DEFINITION

Exemestane contains NLT 97.0% and NMT 102.0% of exemestane ($C_{20}H_{24}O_2$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. Infrared Absorption** { 197K }
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• Procedure

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
15	60	40
18	10	90
28	10	90
30	60	40
35	60	40

Diluent: Acetonitrile and water (1:1)

Standard solution: 0.1 mg/mL of USP Exemestane RS in *Diluent*

Sample solution: 0.1 mg/mL of Exemestane in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 247 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 45°
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of exemestane (C₂₀H₂₄O₂) in the portion of Exemestane taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of exemestane from the *Sample solution*

r_S peak response of exemestane from the *Standard solution*

C_S concentration of USP Exemestane RS in the *Standard solution* (mg/mL)

C_U concentration of Exemestane in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.2%

- **Organic Impurities**

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See *Table 2*. Return to original conditions and re-equilibrate the system.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	75	25
30	55	45
40	5	95
45	5	95

Diluent: Acetonitrile and water (3:1)

System suitability solution: 1 mg/mL of USP Exemestane RS, 0.01 mg/mL of USP Exemestane Related Compound B RS, and 0.01 mg/mL of USP Exemestane Related Compound C RS in *Diluent*

Sensitivity solution: 0.5 µg/mL each of USP Exemestane RS, USP Exemestane Related Compound B RS, and USP Exemestane Related Compound C RS in *Diluent*

Standard solution: 5 µg/mL of USP Exemestane RS in *Diluent*

Sample solution: 1 mg/mL of Exemestane in *Diluent*. The concentration is calculated on the anhydrous and solvent-free basis.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 247 nm

Column: 4.6-mm × 25-cm; 3.5-μm packing L1

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution, Sensitivity solution, and Standard solution*

Suitability requirements

Resolution: NLT 2.0 between exemestane related compound B and exemestane related compound C; NLT 2.0 between exemestane related compound C and exemestane, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10 for the exemestane, exemestane related compound B, and exemestane related compound C peaks, *Sensitivity solution*

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of each impurity in the portion of Exemestane taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_S peak response of exemestane from the *Standard solution*

C_S concentration of USP Exemestane RS in the *Standard solution* (mg/mL)

C_U concentration of Exemestane in the *Sample solution* (mg/mL) (the concentration is calculated on the anhydrous and solvent-free basis)

F = relative response factor for each individual impurity (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any impurity peaks less than 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Exemestane related compound B	0.34	0.95	0.15
Exemestane related compound C	0.77	1.1	1.0
Exemestane	1.0	—	—
Any unspecified impurity	—	1.0	0.1

• Limit of Exemestane Related Compound D

Mobile phase: Hexane, isopropyl alcohol, and diethylamine (90: 10: 0.1)

System suitability solution: 8 mg/mL of USP Exemestane System Suitability Mixture RS in anhydrous alcohol

Standard solution: 0.04 mg/mL of USP Exemestane RS in anhydrous alcohol

Sensitivity solution: 4 μg/mL of USP Exemestane RS in anhydrous alcohol, from the *Standard solution*

Sample solution: 8 mg/mL of Exemestane in anhydrous alcohol. The concentration is

calculated on the anhydrous and solvent-free basis.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 247 nm

Column: 4.6-mm × 25-cm; 10-μm packing L80

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT than 2.0 between exemestane and exemestane related compound D, *System suitability solution*

Relative standard deviation: NMT 5%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of exemestane related compound D in the portion of Exemestane taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of exemestane related compound D from the *Sample solution*

r_S = peak response of exemestane from the *Standard solution*

C_S = concentration of USP Exemestane RS in the *Standard solution* (mg/mL)

C_U = concentration of Exemestane in the *Sample solution* (mg/mL) (the concentration is calculated on the anhydrous and solvent-free basis)

F = relative response factor for exemestane related compound D (see *Table 4*)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Exemestane	1.0	1.0	—
Exemestane related compound D	1.55	1.1	0.10

Total impurities: NMT 2.5%. Total impurities include the impurities in *Table 3* and *Table 4*.

SPECIFIC TESTS

• **Water Determination** { 921 }, *Method I*: NMT 0.3%

• **Optical Rotation** { 781S }, *Specific Rotation*

Sample solution: 10 mg/mL in methanol

Acceptance criteria: +290° to +300° on the anhydrous basis

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP Reference Standards** 〈 11 〉
 - USP Exemestane RS
 - USP Exemestane Related Compound B RS
 - 6-Hydroxymethylandrosta-1,4-diene-3,17-dione.
 - $C_{20}H_{26}O_3$ 314.42
 - USP Exemestane Related Compound C RS
 - Androsta-1,4-diene-3,17-dione.
 - $C_{19}H_{24}O_2$ 284.39
 - USP Exemestane System Suitability Mixture RS
 - Exemestane containing a small amount of exemestane related compound D (16-Methyleneandrosta-1,4-diene-3,17-dione).
 - $C_{20}H_{24}O_2$ 296.40

■ 1S (USP39)

BRIEFING

Fluticasone Propionate Lotion. Because there is no existing *USP* monograph for this dosage form, a new monograph, based on validated methods of analysis, is proposed.

1. The HPLC procedure in the *Assay* is based on analyses performed with the Inertsil ODS-3V brand of L1 column manufactured by GL Sciences. The typical retention time for fluticasone propionate in the *Assay* is about 24 min.
2. The HPLC procedure in *Organic Impurities* is based on analyses performed with the Inertsil ODS-3V brand of L1 column manufactured by GL Sciences. The typical retention time for fluticasone propionate in *Organic Impurities* is about 39 min.

(SM4: D. Min.)

Correspondence Number—C130868

Comment deadline: July 31, 2015

Add the following:**■ Fluticasone Propionate Lotion****DEFINITION**

Fluticasone Propionate Lotion is fluticasone propionate in a suitable lotion base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: 1.15 g/L of monobasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 3.5. Pass the solution through a suitable filter of 0.45- μ m pore size.

Mobile phase: Acetonitrile, methanol, and *Buffer* (13:50:37)

Standard stock solution: 0.8 mg/mL of USP Fluticasone Propionate RS, prepared as follows. Transfer 40 mg of USP Fluticasone Propionate RS to a 50-mL volumetric flask. Add 10 mL of tetrahydrofuran and sonicate to dissolve. Dilute with methanol to volume.

Standard solution: 0.04 mg/mL of USP Fluticasone Propionate RS in methanol from *Standard stock solution*

Sample solution: Nominally 0.04 mg/mL of fluticasone propionate from Lotion, prepared as follows. Transfer a portion of Lotion to a suitable volumetric flask. Add tetrahydrofuran equivalent to about 20% of the flask volume and vortex until the Lotion is dispersed. Add methanol equivalent to 60% of the flask volume and sonicate for 10 min to dissolve. Dilute with methanol to volume. Pass a portion of the solution through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: 239 nm. For *Identification test B*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 45 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: 30 min

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 5000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the portion of Lotion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of fluticasone propionate from the *Sample solution*

r_S peak response of fluticasone propionate from the *Standard solution*

C_S concentration of USP Fluticasone Propionate RS in the *Standard solution* (mg/mL)

C_U nominal concentration of fluticasone propionate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Minimum Fill** 〈 755 〉: Meets the requirements

IMPURITIES

- **Organic Impurities**

Solution A: Methanol, phosphoric acid, and water (30: 0.5: 970)

Solution B: Acetonitrile, methanol, and phosphoric acid (950: 50: 0.5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	56	44
45	56	44
49	10	90
70	10	90
71	56	44
80	56	44

System suitability solution: 0.13 mg/mL of USP Fluticasone Propionate RS and 0.38 µg/mL of USP Fluticasone Propionate Related Compound D RS in methanol

Standard stock solution: 0.25 mg/mL of USP Fluticasone Propionate RS, prepared as follows. Transfer 25 mg of USP Fluticasone Propionate RS to a 100-mL volumetric flask. Add 25 mL of tetrahydrofuran and sonicate to dissolve. Dilute with methanol to volume.

Standard solution: 0.63 µg/mL of USP Fluticasone Propionate RS in methanol from *Standard stock solution*

Sample solution: Nominally 0.13 mg/mL of fluticasone propionate from Lotion, prepared as follows. Transfer a portion of Lotion, equivalent to 1.25 mg of fluticasone propionate, to a suitable volumetric flask. Add tetrahydrofuran equivalent to 20% of the flask volume and vortex until the Lotion is dispersed. Add methanol equivalent to 40% of the flask volume and sonicate for 10 min to dissolve. Dilute with methanol to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: 239 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 40 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between fluticasone propionate related compound D and fluticasone propionate, *System suitability solution*

Relative standard deviation: NMT 5.0 from six replicate injections, *Standard solution*

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of fluticasone propionate related compound C, fluticasone propionate related compound D, or any individual unspecified impurity in the portion of Lotion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of fluticasone propionate related compound C, fluticasone propionate related compound D, or each unspecified impurity from the *Sample solution*

r_S = peak response of fluticasone propionate from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of fluticasone propionate in the *Sample solution* ($\mu\text{g/mL}$)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fluticasone propionate related compound C ^a	0.67	0.82	0.10
Fluticasone propionate related compound D	0.91	1.1	0.30
Fluticasone propionate	1.0	1.0	—
Any unspecified degradation product	—	1.0	0.10
Total impurities	—	—	1.0

^a S-Fluoromethyl 17 α -acetyloxy-6 α , 9 α -difluoro-11 β -hydroxy-16 α -methyl-3-oxoandrosta-1,4-diene-17 β -carbothioate.

SPECIFIC TESTS

- **pH** 〈 791 〉

Sample: 50 mg/mL of Lotion in water

Acceptance criteria: 4.0–6.0

- **Microbial Enumeration Tests** 〈 61 〉 and **Tests For Specified Microorganisms** 〈 62 〉:

Meets the requirements of the tests for absence of *Salmonella species*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The total aerobic microbial count is NMT 1×10^2 cfu/g, and the total combined molds and yeasts count is NMT 1×10^1 cfu/g.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature. Do not refrigerate.

- **USP Reference Standards** 〈 11 〉

USP Fluticasone Propionate RS

USP Fluticasone Propionate Related Compound D RS

S-Methyl 6 α , 9 α -difluoro-11 β -hydroxy-16 α -methyl-3-oxo-17 α -propionyloxyandrosta-

1,4-diene-17 β -carbothioate.C₂₅H₃₂F₂O₅S 482.58

■ 1S (USP39)

BRIEFING

Furosemide, USP 38 page 3626. As part of the USP monograph modernization efforts, the following revisions are proposed:

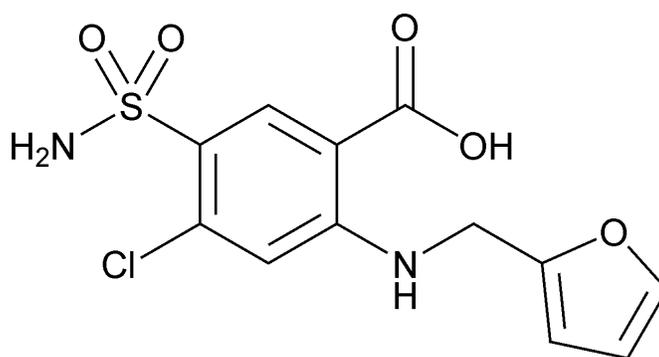
1. Revise the acceptance criteria in the *Definition* and the *Assay* from NLT 98.0% and NMT 101.0% to NLT 98.0% and NMT 102.0%, which is typical for chromatographic procedures.
2. Replace *Identification* test B, which uses ultraviolet absorptivities, with an HPLC procedure based on the retention time agreement for furosemide from the proposed *Assay* procedure.
3. Replace *Identification* test C, which uses a wet chemistry-based colorimetric procedure, with a spectroscopic procedure based on the UV spectrum match of the main peak from the proposed HPLC procedure for the *Assay* to complement the chromatographic procedure.
4. Replace the existing titration-based *Assay* procedure with a validated stability-indicating HPLC procedure. The proposed liquid chromatographic procedure is based on an analysis performed with the Waters Spherisorb ODS-1 brand of L1 column. The typical retention time for furosemide is about 23 min.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM2: C. Anthony.)

Correspondence Number—C143399

Comment deadline: July 31, 2015

Furosemide

C₁₂H₁₁ClN₂O₅S 330.74

Benzoic acid, 5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]-; 4-Chloro-*N*-furfuryl-5-sulfamoylanthranilic acid [54-31-9].

DEFINITION

Change to read:

Furosemide contains NLT 98.0% and NMT $\pm 1.0\%$

■ 102.0 ■ 1S (USP39)

of furosemide ($C_{12}H_{11}ClN_2O_5S$), calculated on the dried basis.

IDENTIFICATION

• A. Infrared Absorption (197K)

Delete the following:

■ • B. Ultraviolet Absorption (197U)

Standard solution: 8 µg/mL of USP Furosemide RS in 0.02 N sodium hydroxide

Sample solution: 8 µg/mL in 0.02 N sodium hydroxide

Analytical wavelength: 271 nm

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%. ■ 1S (USP39)

Add the following:

- • B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

Delete the following:

■ • C.

Sample solution: 0.5 mg/mL in methanol

Analysis: Transfer 1 mL of the *Sample solution* to a flask, add 10 mL of 2.5 N hydrochloric acid, and reflux on a steam bath for 15 min. Cool, and add 15 mL of 1 N sodium hydroxide and 5 mL of 1 mg/mL sodium nitrite solution. Allow the mixture to stand for 3 min, add 5 mL of a 5 mg/mL ammonium sulfamate solution, and add 5 mL of freshly prepared 1 mg/mL *N*-(1-naphthyl)ethylenediamine dihydrochloride solution.

Acceptance criteria: A red to red-violet color is produced. ■ 1S (USP39)

Add the following:

- • C. The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

ASSAY

Change to read:

• Procedure

Sample: 600 mg

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium hydroxide VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 50 mL of dimethylformamide to which has been added 3 drops of bromothymol blue TS, and which has been previously neutralized with *Titrant*.

~~Titrate with *Titrant* to a blue endpoint. Each mL of *Titrant* is equivalent to 33.07 mg of furosemide (C₁₂H₁₁ClN₂O₅S).~~

~~**Acceptance criteria:** 98.0%–101.0% on the dried basis~~

- Protect Furosemide solutions from exposure to light.

Mobile phase: Tetrahydrofuran, glacial acetic acid, and water (30:1:70)

Solution A: Acetonitrile and water (50:50)

Diluent: *Solution A* and glacial acetic acid (978:22)

System suitability solution: 20 µg/mL of USP Furosemide RS and 12 µg/mL of USP Furosemide Related Compound A RS in *Diluent*

Standard solution: 0.2 mg/mL of USP Furosemide RS in *Diluent*

Sample solution: 0.2 mg/mL of Furosemide in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 272 nm. For *Identification* test C, use a diode-array detector in the range of 200–400 nm.

Column: 4.6-mm × 25.0-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between furosemide related compound A and furosemide, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of furosemide (C₁₂H₁₁ClN₂O₅S) in the portion of Furosemide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Furosemide RS in the *Standard solution* (mg/mL)

C_U

= concentration of Furosemide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ■1S (USP39)

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Delete the following:

- **Heavy Metals, Method II** 〈 231 〉: NMT 20 ppm • (Official 1-Dec-2015)

- **Organic Impurities**

Protect Furosemide solutions from exposure to light.

Mobile phase, Solution A, Diluent, and System suitability solution: Proceed as directed in the Assay.

Standard solution: 5.0 µg/mL each of USP Furosemide Related Compound A RS and USP Furosemide Related Compound B RS in *Diluent*

Sample solution: 1.0 mg/mL of Furosemide in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detectors: 254 and 272 nm

[Note—The 2,4-dichloro-5-sulfamoylbenzoic acid impurity does not respond at 272 nm, and the 2,4-bis(furfurylamino)-5-sulfamoylbenzoic acid impurity has a very intense absorbance at 254 nm. The response for furosemide is at 254 nm.]

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 2.5 times the retention time of the furosemide peak

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.5 between furosemide and furosemide related compound A

Relative standard deviation: NMT 2.0% for furosemide

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The sum of the peak areas of peaks eluting before furosemide at 254 nm from the *Sample solution* is NMT the area of the furosemide related compound B peak at 254 nm from the *Standard solution* (0.5%). The sum of the peak areas of peaks eluting after furosemide at 272 nm from the *Sample solution* is NMT the area of the furosemide related compound A peak at 272 nm from the *Standard solution* (0.5%).

SPECIFIC TESTS

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP Reference Standards** 〈 11 〉
 - USP Furosemide RS
 - USP Furosemide Related Compound A RS
2-Chloro-4-*N*-furfurylamino-5-sulfamoylbenzoic acid.
C₁₂H₁₁ClN₂O₅S 330.74
 - USP Furosemide Related Compound B RS
4-Chloro-5-sulfamoylanthranilic acid.
C₇H₇ClN₂O₄S 250.66

BRIEFING

Gemfibrozil Tablets, *USP 38* page 3661 and *PF 40(4)* [July–Aug. 2014]. On the basis of comments received, it is proposed to revise the monograph as follows:

1. Revise the preparation of the *Standard stock solution* and the *Standard solution* in the *Dissolution* test to be consistent with the sponsor's FDA-approved drug product.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: S. Ramakrishna.)

Correspondence Number—C120986; C155820

Comment deadline: July 31, 2015

Gemfibrozil Tablets

DEFINITION

Gemfibrozil Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of gemfibrozil (C₁₅H₂₂O₃).

IDENTIFICATION

Change to read:

● **Procedure**

■ **A.** ■ 2S (*USP38*)

Sample: 100 mg of gemfibrozil from a quantity of finely ground Tablets

Analysis: Shake the *Sample* with 10 mL of 0.1 N sodium hydroxide. Filter the mixture into a 50-mL centrifuge tube, and acidify the filtrate with 3 N sulfuric acid to obtain a copious precipitate. Centrifuge, and discard the clear solution. Wash the precipitate with small portions of water, and allow it to air-dry.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the precipitate, previously dried over silica gel for 4 h, exhibits maxima only at the same wavelengths as those of a similar preparation of USP Gemfibrozil RS.

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (*USP38*)

ASSAY**Change to read:**● **Procedure**

Mobile phase: Add 10 mL of glacial acetic acid to 800 mL of methanol in a 1000-mL volumetric flask, and dilute with water to volume.

System suitability solution: 0.2 mg/mL of gemfibrozil

■ USP Gemfibrozil RS ■_{2S} (USP38)

and 0.05 mg/mL of 2,5-xylenol in *Mobile phase*

Standard stock solution: 1 mg/mL of USP Gemfibrozil RS in methanol

Standard solution: 0.2 mg/mL of USP Gemfibrozil RS from the *Standard stock solution* in *Mobile phase*

Sample stock solution: Nominally 1 mg/mL of gemfibrozil prepared as follows. Transfer the equivalent of 100 mg of gemfibrozil from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add about 80 mL of methanol and shake to dissolve. Dilute with methanol to volume and filter.

Sample solution: Nominally 0.2 mg/mL of gemfibrozil from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 276 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 0.8 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 8.0 between gemfibrozil and 2,5-xylenol, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of gemfibrozil (C₁₅H₂₂O₃) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Gemfibrozil RS in the *Standard solution* (mg/mL)

C_U nominal concentration of gemfibrozil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **Dissolution** 〈 711 〉

Medium: 0.2 M phosphate buffer prepared as follows. Dissolve 545 g of monobasic potassium phosphate in 5 L of water, add 131 g of sodium hydroxide, dilute with water to about 19.5 L, and mix well. Adjust with either 1 N phosphoric acid or 1 N sodium hydroxide to a pH of 7.5. Dilute with water to 20 L; 900 mL.

Apparatus 2: 50 rpm

Time: 30 min

Standard stock solution: 0.33 mg/mL

■ A known concentration ■ 1S (USP39)

of USP Gemfibrozil RS in *Medium* prepared as follows. Dissolve USP Gemfibrozil RS in an amount of methanol not to exceed 1% of the

■ total ■ 1S (USP39)

volume of the *Standard stock solution*. Dilute with *Medium* to volume.

Standard solution: Dilute the *Standard stock solution* with 1 N sodium hydroxide to obtain a concentration estimated to correspond to that of the filtered and diluted *Sample solution*.

■ A suitable concentration of USP Gemfibrozil RS from the *Standard stock solution*. ■ 1S (USP39)

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with 1 N sodium hydroxide to a concentration similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: 276 nm

Analysis: Calculate the percentage of the labeled amount of gemfibrozil (C₁₅H₂₂O₃) dissolved from UV absorbances of the *Sample solution* in comparison with those of the *Standard solution*.

Tolerances: NLT 80% (Q) of the labeled amount of gemfibrozil (C₁₅H₂₂O₃) is dissolved.

- **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

IMPURITIES**Add the following:**

- • **Organic Impurities**

Mobile phase, System suitability solution, and Standard stock solution: Proceed as directed in the *Assay*.

Standard solution: 0.05 mg/mL of USP Gemfibrozil RS from the *Standard stock solution* in *Mobile phase*

Sensitivity solution: 0.005 mg/mL of USP Gemfibrozil RS from the *Standard solution* in *Mobile phase*

Sample solution: Nominally 10 mg/mL of gemfibrozil prepared as follows. Transfer 500 mg of gemfibrozil from NLT 20 finely powdered Tablets to a 50-mL volumetric flask, and add about 40 mL of *Mobile phase*. Sonicate and shake for 20 min. Dilute with *Mobile phase* to volume and filter.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 276 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 3 times the retention time of gemfibrozil

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 8.0 between gemfibrozil and 2,5-xyleneol, *System suitability solution*

Relative standard deviation: NMT 2.0% for the gemfibrozil peak, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_S peak response of gemfibrozil from the *Standard solution*

C_S concentration of USP Gemfibrozil RS in the *Standard solution* (mg/mL)

C_U nominal concentration of gemfibrozil in the *Sample solution* (mg/mL)

Acceptance criteria

Individual impurities: NMT 0.17%

Total impurities: NMT 1.0%

■ 2S (USP38)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** { 11 }
USP Gemfibrozil RS

BRIEFING

Interferon beta-1a. Because there is no existing *USP* monograph for this drug substance, a new monograph based on validated methods of analysis is proposed.

1. The liquid chromatographic procedure used in the *Assay* is based on analyses performed with the Vydac 214TP Alltech brand of L26 column. The typical retention time for interferon beta-1a is 4 min.
2. The liquid chromatographic procedure used in *Identification* test C for *Peptide Mapping* and in the *Impurities* test for *Oxidized Interferon beta-1a* is based on analyses performed with the Vydac 214TP52 Alltech brand of L26 column.
3. The liquid chromatographic procedure used in the *Specific Tests* for *Analysis of N-Linked Oligosaccharides* and *Quantitation of Biantennary Sialylation* is based on analyses performed with the Shodex NH2P-50 Asahipak brand of L82 column.

(BIO1: M. Kibbey.)

Correspondence Number—C136465

*Comment deadline: July 31, 2015***Add the following:****■ Interferon beta-1a**

MSYNLLGFLQ RSSNFQCQKL LWQLNGRLEY CLKDRMNFDI PEEIKQLQQF
 QKEDAALTIY EMLQNIFAIF RQDSSSTGWN* ETIVENLLAN VYHQINHLKT
 VLEEKLEKED FTRGKLMSSL HLKRYYGRIL HYLKAKEYSH CAWTIVRVEI
 LRNFYFINRL TGYLRN

C₉₀₈H₁₄₀₆N₂₄₆O₂₅₂S₇ approximately 22500 Da
 [145258-61-3].

DEFINITION

Interferon beta-1a is a 166 amino acid glycoprotein containing a disulfide bond between Cys-31 and Cys-141 and a free cysteine at position 17. Single *N*-linked glycosylation occurs at Asn₈₀. The amino acid sequence, disulfide linkage, and carbohydrate distribution are similar to natural human fibroblast interferon. Interferon beta-1a is recombinantly produced in mammalian cells containing the human interferon DNA sequence and stored as a concentrated solution. Residual host cell proteins and host cell-derived or vector-derived DNA are measured by suitable validated methods with limits approved by the appropriate regulatory authority.

IDENTIFICATION**● A. Bioidentity**

Medium A: Dulbecco's Modified Eagle Medium containing 25 mM d-glucose,¹ as well as 10% heat-inactivated fetal bovine serum,² and 4 mM l-glutamine.³ Filter sterilize.

Medium B: Dulbecco's Modified Eagle Medium containing 25 mM d-glucose,⁴ as well as 2% heat-inactivated fetal bovine serum,⁵ and 4 mM l-glutamine.⁶ Filter sterilize.

NBB solution: An aqueous solution of 0.05% (w/v) naphthol blue black (NBB),⁷ 9% (v/v) glacial acetic acid, and 0.1 M sodium acetate.

Cell culture preparation: Prepare adherent cell cultures of human lung carcinoma cell line A549⁸ in *Medium A*, and incubate in a humidified incubator at 37° containing 5% carbon dioxide. Cells should be passaged by trypsinization about once a week and are fed four days later with *Medium A*. The day before the assay (20–24 h before assay set-up), seed assay plates by removing the media from the flasks and add sufficient volume of 37° of cell culture grade trypsin-EDTA solution⁹ to cover the flask surface. Add 2 mL of the trypsin-EDTA solution to each flask, [Note—These volumes are based on a T-150 tissue culture flask.¹⁰] rock the flasks, and then place in the 37° incubator again for 5–10 min. Remove the flasks, tap the flasks to dislodge the cells, then add 10 mL of 37° *Medium A* to each flask. Aseptically pool all trypsinized cell solutions into a suitable sterile container, dilute with *Medium A* to 1:2, and then count the cells. Adjust the cell density with

Medium A such that the final concentration is 2.5×10^5 cells/mL and mix well to ensure a single cell suspension. Seed 100 μ L of this suspension into each well of a 96-well tissue culture plate,¹¹ and incubate for 20–24 h.

Standard/Control stock solutions: Reconstitute USP Interferon beta-1a for Bioidentity RS with a suitable quantity of sterile, distilled water and then dilute further with *Medium A* to 1×10^4 Units/mL or 50,000 pg/mL. [Note—This stock may be aliquoted and frozen at -70° until day of assay performance.]

Test stock solutions: Dilute the interferon beta-1a test materials with *Medium A* to 50,000 pg/mL (10,000 Units/mL). [Note—If the *Standard/Control stock solutions* were frozen before use in the assay, then the *Test stock solutions* must also be aliquoted and frozen at -70° before evaluation in the method.]

Virus solution: Encephalomyocarditis (EMC) virus¹² is cultured in murine fibroblast L929 cells¹³ through a minimum of 4 passages. The supernatant of the virus cultures are frozen at -70° . Before qualification, the harvested virus supernatant is thawed, sonicated for 30 min, and centrifuged at $1920 \times g$ for 10 min. The centrifuged virus supernatant is pooled, aliquoted, and frozen at -70° for qualification. The virus is qualified by determining the TCID₅₀ of the virus on A549 cells. The working concentration of the virus is approximately TCID₅₀/mL = $10^{6.16}$ in *Medium B*.

Procedure: For each sample, two independent sets of interferon beta-1a *Test solutions* must be prepared for each assay plate (left block and right block are duplicate) and NLT 2 assay plates must be performed to generate an $n = 4$ for each sample (2 from each plate). On day of assay performance, dilute the 50,000 pg/mL *Standard stock solution*, the *Control stock solution*, and the *Sample stock solution* with *Medium B* to a concentration of 40 pg/mL. From this intermediate dilution, dilute 6 times serially, using 1.3-fold dilution steps, to obtain a concentration series of 30.77 pg/mL to 8.29 pg/mL. Remove the tissue culture plate containing cells from the incubator and visually inspect the plate to confirm the cells are confluent, and appear healthy and uncontaminated. Discard the media completely from the wells. Next, starting with the least concentrated solutions in row G and moving up, add 100 μ L of each concentration of *Standard solution*, *Control solution*, and *Test solution* to the appropriate well using the plate design in *Table 1*. Add 100 μ L *Medium B* to rows A and H. At a minimum, repeat this setup for a second plate of cells. Incubate the plate for 20–24 h at 37° in an incubator with 5% carbon dioxide (CO₂).

The next day, dilute the *Virus solution* with *Medium B* to the predetermined qualified dilution. Remove the plates from the incubator and completely remove the solutions in each well. Add 100 μ L of *Medium B* to all wells of row A in each plate. Add 100 μ L of *Virus solution* to all wells on the rest of the plates. Incubate the plates again for 28–30 hours at 37° in an incubator with 5% carbon dioxide (CO₂).

The next day, remove the plates from the incubator and completely remove the solutions in each well. Add 50 μ L of *NBB solution* to all wells of the plates. Incubate the plates at 37° in an incubator with 5% carbon dioxide (CO₂) for 60 ± 15 min. Remove the plates and shake out the *NBB solution* from all the wells. Add 100 μ L of phosphate buffered saline¹⁴ to every well then shake out this solution, blot the plates on a paper towel, and repeat this wash one more time. Next, add 100 μ L of 50 mM sodium hydroxide to every empty well.

Place the plates on a shaker for NLT 1 min and NMT 96 h. Read the absorbance from the plates at a wavelength of 620 nm using a suitable plate reader.

Table 1. Schematic Representation of the Final Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cells	Cells	Cells	Cells	Cells	Cells	Cells	Cells	Cells	Cells	Cells	Cells
B	TSA, #1	TSB, #1	St, #1	PC, #1	TSC, #1	TSD, #1	TSA, #1	TSB, #1	St, #1	PC, #1	TSC, #1	TSD, #1
C	TSA, #2	TSB, #2	St, #2	PC, #2	TSC, #2	TSD, #2	TSA, #2	TSB, #2	St, #2	PC, #2	TSC, #2	TSD, #2
D	TSA, #3	TSB, #3	St, #3	PC, #3	TSC, #3	TSD, #3	TSA, #3	TSB, #3	St, #3	PC, #3	TSC, #3	TSD, #3
E	TSA, #4	TSB, #4	St, #4	PC, #4	TSC, #4	TSD, #4	TSA, #4	TSB, #4	St, #4	PC, #4	TSC, #4	TSD, #4
F	TSA, #5	TSB, #5	St, #5	PC, #5	TSC, #5	TSD, #5	TSA, #5	TSB, #5	St, #5	PC, #5	TSC, #5	TSD, #5
G	TSA, #6	TSB, #6	St, #6	PC, #6	TSC, #6	TSD, #6	TSA, #6	TSB, #6	St, #6	PC, #6	TSC, #6	TSD, #6
H	VC	VC	VC	VC	VC	VC	VC	VC	VC	VC	VC	VC

Legend	
Cells	= Negative control of cells with <i>Medium B</i> ; no interferon beta-1a, no virus.
TSA	= One dilution series of <i>Test solution</i> , with the following concentrations of interferon beta-1a added to the indicated well: #1: 30.77 pg/mL; #2: 23.67 pg/mL; #3: 18.21 pg/mL; #4: 14.01 pg/mL; #5: 10.77 pg/mL; #6: 8.29 pg/mL. TSB, TSC, TSD=3 additional independent dilution series of <i>Test solution</i> , prepared as in TSA.
St	= Dilution series of <i>Standard solution</i> (concentrations same as in TSA #1 through #6).
PC	= <i>Positive control solution</i> dilution series (concentrations same as in TSA #1 through #6).
VC	= <i>Medium B</i> and cells present, followed by virus, but no interferon beta-1a.

Calculations: Each independent half-plate block of samples, standards, and controls are evaluated separately. The relative potency of the *Test solutions* and the *Control solutions* are calculated relative to the *Standard solutions* in that block using parallel line analysis.

System suitability criteria: For each individual *Control solution* compared to the *Standard solution*, the statistical tests for regression and parallelism must pass at the 95% level. The percent relative confidence interval must be NMT 50%. For the plate to be acceptable, the signal-to-noise ratio of the mean cell control signal in row A to the mean virus control signal in row H must be NLT 2.0, and the relative potency of the *Control Solution* to the *Standard solution* in that block must be NLT 80% and NMT 133% of the expected relative potency. For each individual *Test solution* compared to the *Standard solution*, the statistical tests for regression and parallelism must pass at the 95% level. The percent relative confidence interval must be NMT 50%. If any block fails based on these criteria or the *System suitability criteria* then it must be repeated until a minimum of 4 relative potencies can be combined to calculate a mean relative potency. The 95% confidence interval for the calculated mean potency must be within 64%–156% of the observed potency.

Acceptance criteria: 80%–133%

- **B.** The retention time of the interferon beta-1a main peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Peptide Mapping**
Solution A: 0.1% (w/v) trifluoroacetic acid in water. Degas.
Solution B: Prepare a 90% solution of acetonitrile in water. Add trifluoroacetic acid to 0.1% (w/v) and mix.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
70	30	70
80	100	0

Buffer solution: 0.1 M solution of tris (hydroxymethyl) aminomethane (tris) and 0.25 M

sodium chloride. Adjust with hydrochloric acid to a pH of 8.5. Filter.

Enzyme solution: Prepare an aqueous solution of 0.8 mg/mL lysyl endopeptidase from *Achromobacter lyticus*.

Standard stock solution: 1.0 mg/mL of USP Interferon beta-1a RS in *Buffer solution*

Standard solutions: Prepare duplicate tubes containing 8.8 μL of 50 mM dithiothreitol in each. Add 40 μL of *Standard stock solution* and 40 μL of *Buffer solution* to each tube. Mix and incubate for 15–60 min. Add 4.0 μL of a 20 mM EDTA solution and 4.0 μL *Enzyme solution* and gently mix, making sure not to introduce air and that all sample is at the bottom of the tube. Cap the tube and incubate at $25 \pm 2^\circ$ for 20–28 h. Add 98 μL of 8 M guanidine hydrochloride solution and 16 μL of 50 mM dithiothreitol solution. Mix and perform chromatography, injecting samples NMT 22 h later. [Note—Samples can be stored at -70° if necessary but an additional 16 μL of 50 mM dithiothreitol solution must be added before analysis if this is performed.]

Sample stock solution: 0.2 mg/mL of interferon beta-1a in *Buffer solution*. [Note—If necessary, buffer exchange by suitable means into *Buffer solution* to prepare this solution before digestion.]

Sample solutions: Prepare duplicate tubes containing 8.8 μL of 50 mM dithiothreitol in each. Add 8 μL of *Sample stock solution* and 72 μL of *Buffer solution* to each tube. Mix and incubate for 15–60 min. Add 4.0 μL of a 20 mM EDTA solution and 4.0 μL of *Enzyme solution* and gently mix, making sure not to introduce air and that all sample is at the bottom of the tube. Cap the tube and incubate at $25 \pm 2^\circ$ for 20–28 h. Add 98 μL of 8 M guanidine hydrochloride solution and 16 μL of 50 mM dithiothreitol solution. Mix and perform chromatography, injecting samples NMT 22 h later. [Note—Samples can be stored at -70° if necessary but an additional 16 μL of 50 mM dithiothreitol solution must be added before analysis if this is performed.]

Blank solutions: Prepare duplicate tubes containing 8.8 μL of 50 mM dithiothreitol in each. Add 80 μL of *Buffer solution* to each tube. Mix and incubate for 15–60 min. Add 4.0 μL of a 20 mM EDTA solution and 4.0 μL of *Enzyme solution* and gently mix, making sure not to introduce air and that all sample is at the bottom of the tube. Cap the tube and incubate at $25 \pm 2^\circ$ for 20–28 h. Add 98 μL of 8 M guanidine hydrochloride solution and 16 μL of 50 mM dithiothreitol solution. Mix and perform chromatography, injecting samples NMT 22 h later. [Note—Samples can be stored at -70° if necessary but an additional 16 μL of 50 mM dithiothreitol solution must be added before analysis if this is performed.]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 2.1-mm \times 25-cm; 5- μm packing L26

Temperatures

Column: 40°

Sample: 5°

Flow rate: 0.3 mL/min

Injection volume: 100 μL

System suitability

Samples: *Standard solutions* and *Blank solutions*

Suitability requirements

Resolution: NLT 2.0 between the AP6(ox) peak (representing amino acids 34–45 of interferon beta-1a) at half height and the AP5 peptide peak (representing amino acids 124–134 of interferon beta-1a) in the *Standard solution*. [Note—See also the chromatogram supplied with USP Interferon beta-1a RS.]

NLT 3.8 between the AP6 and AP5 peptide peaks

Interfering peaks: No significant peaks that coelute with the AP6 and AP6(ox) peaks in the *Blank solution*.

Analysis

Samples: *Standard solutions* and *Sample solutions*

[Note—Condition the chromatographic system by running a blank gradient program before injecting the digests.]

Acceptance criteria: The chromatographic profiles of the *Sample solutions* are similar to those of the *Standard solutions* and no extra peaks greater than 5% of the peak height of peptide AP9 (representing amino acids 137–166 of interferon beta-1a) are present.

ASSAY• **Procedure**

Solution A: 0.1% (w/v) trifluoroacetic acid. Degas solution before use.

Solution B: 90% (v/v) acetonitrile and 0.1% (w/v) trifluoroacetic acid. Degas solution before use.

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	60	40
5	10	90
7	10	90
9	60	40

Buffer solution: Dissolve 1.58 g of sodium acetate trihydrate and 31.60 g of l-arginine HCl in 900 mL of water, then add 0.48 mL acetic acid, followed by more water to 1 L total. Mix, filter, and confirm that the pH is 4.8 ± 0.2 .

Sample additive: 10% (w/v) polyoxyethylene–polyoxypropylene block copolymer¹⁵

Blank solution: Add 5 μ L of *Sample additive* to 500 μ L of *Buffer solution*.

Standard solution: Dilute a suitable quantity of USP Interferon beta-1a RS in *Buffer solution* to prepare a 60 μ g/mL solution. Add 10 μ L of *Sample additive* to 1 mL of this protein solution and gently mix. Before use, centrifuge at $20,000 \times g$ for 30 min at 2° – 8° , then store at this temperature before injection.

Sample solution: Dilute a suitable quantity of Interferon beta-1a in *Buffer solution* to prepare a 60 μ g/mL solution. Add 10 μ L of *Sample additive* to 1 mL of the protein solution and gently mix. Before use, centrifuge at $20,000 \times g$ for 30 min at 2° – 8° , then store at this temperature before injection.

Chromatographic System

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 2.1-mm × 5-cm; 5-μm packing L26

Temperatures

Column: 40°

Sample: 5°

Flow rate: 0.5 mL/min

Injection volume: 100 μL

System suitability

Samples: *Standard solutions, Sample solutions, and Blank solution*

Suitability requirements

Precision: NMT 3% RSD for the main peak area of the first six *Standard solution* injections before the *Sample solutions*, and NMT 3% RSD for the main peak area of the two *Standard solution* injections after the *Sample solutions*. In addition, NMT 5% difference between the means of the first (first six injections) and second set (last two injections) of *Standard solution* injections where the percent difference is calculated by subtracting the mean of the second set from the first set, divided by the average of both sets, and multiplying by 100.

Tailing factor: 1.0–2.4 for the interferon beta-1a peak in each *Standard solution* injection, except the first injection

Interfering peaks: No significant peaks may be observed in the second *Blank solution*.

Analysis

Samples: *Standard solutions and Sample solutions*

[Note—Condition the chromatographic system by running a blank gradient program before injecting the solutions.] Inject two *Blank solutions*, then six *Standard solutions*, followed by duplicate injections of up to three *Sample solutions*, followed by two *Standard solution* injections.

Integrate each chromatogram's interferon beta-1a main peak area. Calculate the concentration, in μg/mL, of interferon beta-1a in the *Sample solution* taken:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak area from each *Sample solution* injection

r_S = average peak area of bracketing *Standard solutions*

C_S = concentration of the *Standard solution* (μg/mL)

For the final result, average the *Sample solution* results.

Acceptance criteria: 90%–110%

IMPURITIES

• **Impurities with Molecular Masses Different from Those of Main Interferon beta-1a**

12% Resolving gel: Per 10 mL of solution, mix 4 mL of 30% acrylamide/bisacrylamide (29:1) solution, 3.3 mL of water, 2.5 mL of 1.5 M Tris-HCl buffer (pH 8.8), 0.1 mL of 100 g/L SDS solution, 0.1 mL of 100 g/L ammonium persulfate solution (APS), and 4 μL of tetramethylethylenediamine (TEMED). Pour into a suitable gel cassette, add a sufficient quantity of water-saturated isobutanol to cover the surface, and allow polymerization.¹⁶

Stacking gel: After polymerization of the *12% Resolving gel*, mix (per 5 mL of solution) 0.83

mL of 30% acrylamide/bisacrylamide (29:1) solution, 3.4 mL of water, 0.63 mL of 1.5 M Tris-HCl buffer (pH 8.8), 50 μ L of 100 g/L SDS solution, 50 μ L of 100 g/L APS, and 5 μ L of TEMED. Remove the isobutanol from the *12% Resolving gel* surface and then pour the stacking gel solution on top, insert a suitable gel comb without introducing air bubbles, and allow polymerization.

Buffer A: Prepare a stock solution of 151.4 g of tris, 721.0 g of glycine, and 50.0 g of sodium lauryl sulfate in 5 L of water. Immediately before use, dilute 10-fold with water. Confirm that the pH is 8.1–8.8.

Stock sample buffer: Mix 3.78 g of tris, 10 g sodium dodecyl sulfate (SDS), and 100 mg of bromophenol blue in water. Add 50.0 mL of glycerol and dilute with water to 200 mL. Add 25.0 mL of 2-mercaptoethanol. Adjust with hydrochloric acid to a pH of 6.8, and then dilute with water to 250 mL total volume.

Sample buffer: Mix equal volumes of *Stock sample buffer* with water.

Sample solution A: Concentrate the Interferon beta-1a sample with a suitable procedure to obtain a concentration of 1.5 mg/mL.

Sample solution B: Mix equal volumes of *Sample solution A* with *Stock sample buffer*.

Sample solution C: Dilute *Sample solution A* with water to obtain a concentration of 0.6 mg/mL. Mix equal volumes of this protein solution with *Stock sample buffer*.

Sample solution D: Mix 8 μ L of *Sample solution C* with 40 μ L of *Sample buffer*.

Sample solution E: Mix 15 μ L of *Sample solution D* with 35 μ L of *Sample buffer*.

Sample solution F: Mix 18 μ L of *Sample solution E* with 18 μ L of *Sample buffer*.

Sample solution G: Mix 12 μ L of *Sample solution F* with 12 μ L of *Sample buffer*.

Molecular weight markers: Mix an equal volume of a solution of relative molecular mass markers suitable for SDS-PAGE analysis in the range of 14–66 kDa¹⁷ with *Sample buffer*.

Staining solution: Dissolve 1.25 g/L of brilliant blue dye¹⁸ in a solution of glacial acetic acid, methanol, and water (1:4:5). Pass through a suitable filter.

Destaining solution: Mix glacial acetic acid, isopropanol, and water (1:1:8).

Analysis: Insert the prepared gel in a suitable electrophoresis chamber containing *Buffer A* and remove the comb. Rinse each well with *Buffer A* before loading samples. Boil all *Sample solutions* and *Molecular weight markers* for 3 min. Load 20 μ L of each *Sample solution (A through G)* and *Molecular weight marker* into individual wells of the gel. Perform the electrophoresis until the bromophenol blue dye front is near the bottom of the gel. Remove the gel from the cassette and immerse in *Staining solution* with gentle shaking at 33°–37° for 90 min. Discard the *Staining solution* and add a large excess of *Destaining solution*. Measure the distance traveled for each band in all lanes from the top of the resolving gel then divide that value by the distance traveled by the dye front to obtain the R_f for each band. Plot the logarithm of the molecular weights of each protein in the *Molecular weight markers* versus the R_f values. *Table 4* describes the apparent molecular weights of the main interferon beta-1a band as well as related substances.

Table 4

Name	Apparent Molecular Mass	Acceptance Criteria
Interferon beta-1a	23,000	—
Underglycosylated interferon beta-1a	21,000	NMT 5%
Deglycosylated interferon beta-1a	20,000	NMT 2%

System suitability requirements: The *Molecular weight markers* must be distributed across 80% of the gel, and there should be a linear relationship between the logarithm of their molecular weight and their R_F values. At least one band of approximate molecular weight of 23,000 Da (interferon beta-1a) must be present in the *Sample solution G* lane. A decrease in staining intensity is seen from *Sample solution B* through *Sample solution G*. No interferon beta-1a dimer should be visible in any of the *Sample solution* lanes (apparent molecular mass 46,000).

Acceptance criteria: Any band in *Sample solution C* that corresponds to underglycosylated interferon beta-1a must not be more intense than the interferon beta-1a band in *Sample solution E* (or 5%). Any band in *Sample solution B* that corresponds to deglycosylated interferon beta-1a must not be more intense than the interferon beta-1a band in *Sample solution E* (or 2%). Any other band (other than the two described) of lower molecular weight than interferon beta-1a must not be more intense than the interferon beta-1a band in *Sample solution F* (or 1%).

• **Oxidized Interferon beta-1a**

Mobile phase, Solution C, Enzyme solution, Standard solutions, Sample solutions, Blank solutions, Chromatographic system, and System suitability: Prepare as directed in *Identification test C, Peptide Mapping*.

Analysis: Using the results from the test for *Peptide Mapping*, locate the peaks due to the peptide fragment AP6 comprising amino acids 34–45 and its oxidized form using the *Standard solution* results and the chromatogram of oxidized interferon beta-1a digest supplied with the USP Interferon Beta-1a RS.

Calculate the percentage of oxidation of interferon beta-1a:

$$\frac{A_{34-45ox}}{A_{34-45} + A_{34-45ox}} \times 100$$

$A_{34-45ox}$ = area of the peak corresponding to the oxidized peptide fragment 34–45

A_{34-45} = area of the peak corresponding to the unoxidized peptide fragment 34–45

Acceptance criteria: NMT 6%

SPECIFIC TESTS

• **Analysis of N-Linked Oligosaccharides**

Solution A: Mix 2 mL of *Solution B* with 998 mL of water. Degas before use.

Solution B: Dissolve 19.3 g of ammonium acetate in 800 mL of water. Add 9 mL of acetic acid then dilute with water to 1000 mL. Degas before use.

Mobile phase: See *Table 5*. [Note—Additional time may be inserted after 73 min to flush the column with 100% *Solution B* at 0.75 mL/min to remove excess dye from the column. If this is necessary, the column must then be washed with 100% *Solution A* before the next sample injection.]

Table 5

Time (min)	Flow Rate (mL/min)	Solution A (%)	Solution B (%)
0	0.50	100	0
30	0.50	70	30
70	0.50	15	85
72	0.50	0	100
72.5	0.75	0	100
87	0.75	0	100
88.5	0.75	100	0
99.5	0.75	100	0
99.8	0.50	100	0
130	0.50	100	0
131	0.0	100	0

Enzyme solution: On the day of use, combine 1.0 mL of 50 mM ammonium bicarbonate with 144 μ L of 10% nonyl phenoxy polyethoxy ethanol and mix. Add 40 μ L of peptide *N*-glycosidase F¹⁹ (PNGase F) and mix.

Labeling reagent: Mix 0.3 mL of acetic acid with 0.7 mL of dimethyl sulfoxide in a 1.5 mL microfuge tube. In a fume hood, add 48 mg of anthranilic acid²⁰ (2-AA). Mix well, then add 63 mg of sodium cyanoborohydride. Mix well.

System suitability solution: Using separate vials for each glycan standard, transfer 40 μ g of NA2F complex *N*-linked glycan²¹ (BiNA0), 20 μ g of NGA2F complex *N*-linked glycan²² (BiNA0-2Gal), 60 μ g of A1F complex *N*-linked glycan²³ (BiNA1), and 80 μ g of A2F complex *N*-linked glycan²⁴ (BiNA2). Add 10 μ L of *Labeling reagent* to each tube. Mix by vortexing, then centrifuge briefly. Incubate for 3 ± 0.25 h at $45 \pm 2^\circ$. After cooling and briefly centrifuging, remove excess label on suitable cartridges²⁵ per the manufacturer's instructions. After the final acetonitrile wash, elute each labeled glycan from the cartridge using 3×0.5 mL water washes into fresh tubes. Evaporate to dryness. Reconstitute each labeled glycan standards with 100 μ L of water for every 10 μ g of starting glycan. Pool all the labeled standards, mix, and aliquot. Aliquots may be stored for up to 1 year at $-20 \pm 5^\circ$ C.

Standard solutions: Buffer exchange sufficient quantities of USP Interferon beta-1a RS by suitable methods to prepare two replicates each containing 200 μ g USP Interferon beta-1a RS in a volume of 150–300 μ L of 50 mM ammonium bicarbonate. Add 100 μ L of *Enzyme solution* and mix gently. Incubate 16–24 h at $37 \pm 2^\circ$. Recover the released glycans by reverse phase solid phase extraction using suitable cartridges²⁶ eluting with 0.5 mL of 10 % methanol. Evaporate each digest sample to dryness. Add 10 μ L of *Labeling reagent* to each tube. Mix by vortexing, then centrifuge briefly. Incubate for 3 ± 0.25 h at $45 \pm 2^\circ$. After cooling and briefly centrifuging, remove excess label on suitable cartridges²⁷ per the manufacturer's instructions. After the final acetonitrile wash, elute each labeled glycan from the cartridge using 3×0.5 mL water washes into fresh tubes. Evaporate to dryness. Before *Analysis*, reconstitute each labeled glycan sample with 50 μ L of water.

Sample solutions: Prepare two replicates for each sample. Buffer exchange by suitable methods 200 μ g of interferon beta 1a into a final volume of 150–300 μ L of 50 mM ammonium bicarbonate. Add 100 μ L of *Enzyme solution* and mix gently. Incubate 16–24 h

at $37 \pm 2^\circ\text{C}$. Recover the released glycans by reverse phase solid phase extraction using suitable cartridges²⁸ eluting with 0.5 mL of 10 % methanol. Evaporate each digest sample to dryness. Add 10 μL of *Labeling reagent* to each tube. Mix by vortexing, then centrifuge briefly. Incubate for 3 ± 0.25 h at $45 \pm 2^\circ$. After cooling and briefly centrifuging, remove excess label on suitable cartridges²⁹ per the manufacturer's instructions. After the final acetonitrile wash, elute each labeled glycan from the cartridge using 3×0.5 mL water washes into fresh tubes. Evaporate to dryness. Before *Analysis*, reconstitute each labeled glycan sample with 50 μL of water.

Blank solutions: Prepare two blanks containing 150 μL of 50 mM ammonium bicarbonate.

Add 100 μL of *Enzyme solution* and mix gently. Incubate 16–24 h at $37 \pm 2^\circ$. Perform reverse phase solid phase extraction using suitable cartridges³⁰ eluting with 0.5 mL of 10 % methanol. Evaporate each tube to dryness. Add 10 μL of *Labeling reagent* to each tube. Mix by vortexing, then centrifuge briefly. Incubate for 3 ± 0.25 h at $45 \pm 2^\circ$. After cooling and briefly centrifuging, remove excess label on suitable cartridges³¹ per the manufacturer's instructions. After the final acetonitrile wash, elute each blank from the cartridge using 3×0.5 mL water washes into fresh tubes. Evaporate to dryness. Before *Analysis*, add 50 μL of water to each tube.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Fluorescence (350-nm excitation wavelength; 430-nm emission wavelength)

Column: 4.6-mm \times 15-cm; 5- μm packing L82

Temperatures

Column: $30 \pm 3^\circ$

Sample: $2^\circ - 8^\circ$

Flow rate: 0.5 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution*, *Standard solutions*, and *Blank solutions*

Suitability requirements

Chromatogram of the *Standard solutions* must be comparable to the example chromatogram supplied with the USP Interferon beta-1a RS.

Resolution: NLT 10 between the BiNA2 glycan and BiNA1 glycan in the *System suitability solution*.

Column efficiency: NLT 10,000 based on the BiNA2 glycan peak in the *System suitability solution*.

Interfering peaks: No significant peaks that interfere with glycan quantitation in the *Blank solution*.

Analysis

Samples: *Standard solutions*, *Sample solutions*, and *Blank solutions*

Separately inject samples in the following order. Water alone, *Blank solution*, *System suitability solution*, first *Standard solution*, *Sample solutions*, second *Standard solution*, second *Blank solution*. Record the chromatograms and identify the 5 expected peaks listed in *Table 6* (same nomenclature followed in the calculations below) by comparison to the

representative chromatogram with the USP Interferon beta-1a RS. Integrate the chromatograms over the range including the BiNA0 through the BiNA3 peaks. Calculate the antennary distribution of fully sialylated form of each glycan BiNA2, TriNA3, and TriLacNA3 for each replicate, and report the average of replicate results:

$$\% \text{ BiNA2} = \frac{\text{Area of BiNA2}}{\text{Area of BiNA2} + \text{Area of TriNA3} + \text{Area of TriLacNA3}} \times 100$$

$$\% \text{ TriNA3} = \frac{\text{Area of TriNA3}}{\text{Area of BiNA2} + \text{Area of TriNA3} + \text{Area of TriLacNA3}} \times 100$$

$$\% \text{ TriLacNA3} = \frac{\text{Area of TriLacNA3}}{\text{Area of BiNA2} + \text{Area of TriNA3} + \text{Area of TriLacNA3}} \times 100$$

Table 6

Structure	Glycoform	Description
BiNA1	2A1S1F	Biantennary complex type glycan with core fucose (Bi) and one sialic acid (neuraminic acid, NA1)
BiNA2	2A2S1F	Biantennary complex type glycan with core fucose and two sialic acids
TriLacNA3	3A3S1F1L	Triantennary complex type glycan with core fucose and lactosamine repeat in one antennae, and three sialic acids
TriNA3	3A3S1F	Triantennary complex type glycan with core fucose and three sialic acids
BiNA3	2A3S1F	Biantennary complex type glycan with core fucose and three sialic acids

Acceptance criteria: Replicate chromatograms of *Sample solutions* must be comparable to each other and to the *Standard solution* chromatogram in terms of presence/absence of peaks and relative response (*Standard solution, Sample solution*).

• **Quantitation of Biantennary Sialylation**

Solution A, Solution B, Mobile phase, Enzyme solution, Labeling reagent, System suitability solution, Sample solutions, Standard solutions, Blank solutions, Chromatographic system, and System suitability: Prepare as directed in *Analysis of N-Linked Oligosaccharides*.

Analysis

Samples: *Sample solutions, Standard solutions, and Blank solution*

Separately inject samples in the following order. Water alone, *Blank solution, System suitability solution, first Standard solution, Sample solutions, second Standard solution, second Blank solution*. Record the chromatograms and identify the biantennary peaks listed in *Table 6* and by comparison to the representative chromatogram with the USP Interferon beta-1a RS.

Calculate the percentage of sialylation of biantennary glycans for each replicate, and report the average of replicate results:

$$\% \text{ Biantennary sialylation} = + \frac{(\text{Area of BiNA1}) + ((\text{Area of BiNA2} + \text{Area of BiNA3}) \times 2)}{(\text{Area of BiNA0} + \text{Area of BiNA1} + \text{Area of BiNA2} + \text{Area of BiNA3}) \times 2} \times 100$$

Acceptance criteria: NLT 90%

• **Osmolality and Osmolarity** 〈 785 〉 : 261–319 mOsmol/kg

• **Bacterial Endotoxins Test** 〈 85 〉 : It contains NMT 21 USP Endotoxin Units/mL.

- **Microbial Enumeration Tests** 〈 61 〉 and **Tests for Specified Microorganisms** 〈 62 〉: The total aerobic microbial count is NMT 10 cfu/10 mL and the total combined yeasts and molds count is NMT 10 cfu/10 mL.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, protected from light, at NMT -70° .
 - **Labeling:** The labeling states the interferon beta-1a content (mg/mL) and anti-viral activity (in Units/mL), and that the material is of recombinant origin.
 - **USP Reference Standards** 〈 11 〉
 - USP Interferon beta-1a RS
 - USP Interferon beta-1a for Bioidentity RS
- 1S (USP39)

¹ Gibco, catalog #11960-044, or suitable equivalent.

² Gibco, catalog #16000-044, or suitable equivalent.

³ Gibco, catalog #25030-081, or suitable equivalent.

⁴ Gibco, catalog #11960-044, or suitable equivalent.

⁵ Gibco, catalog #16000-044, or suitable equivalent.

⁶ Gibco, catalog #25030-081, or suitable equivalent.

⁷ Aldrich Chemical, catalog #19524-3, or suitable equivalent.

⁸ ATCC catalog #CCL-185.

⁹ Gibco, catalog #25300-054, or suitable equivalent

¹⁰ Fisher, catalog #10-126-34, or suitable equivalent.

¹¹ Corning, catalog #3595, or suitable equivalent.

¹² ATCC, catalog #VR-129B.

¹³ ATCC #CCL-1, NCTC clone 929

¹⁴ Gibco, catalog #10010-023, or suitable equivalent.

¹⁵ BASF, catalog 549919, or suitable equivalent.

16 Alternatively, commercially available 12% SDS-PAGE gels including a stacking gel may be used.

17 Sigma catalog #SDS7 or other suitable equivalent.

18 Sigma catalog #B7920 or other suitable equivalent.

19 Selectin Biosciences catalog #GE41 or suitable equivalent.

20 Fluka catalog #10680 or suitable equivalent.

21 Ludger catalog #CN-NA2F-20U or suitable equivalent.

22 Ludger catalog #CN-NGA2F-20U or suitable equivalent.

23 Ludger catalog #CN-A1F-20U or suitable equivalent.

24 Ludger catalog #CN-A2F-20U or suitable equivalent.

25 Ludger catalog #LC-S-A6 or suitable equivalent.

26 Waters catalog #WAT094225 or suitable equivalent.

27 Ludger catalog #LC-S-A6 or suitable equivalent.

28 Waters catalog #WAT094225 or suitable equivalent.

29 Ludger catalog #LC-S-A6 or suitable equivalent.

30 Waters catalog #WAT094225 or suitable equivalent.

31 Ludger catalog #LC-S-A6 or suitable equivalent.

BRIEFING

Interferon beta-1a Injection. Because there is no existing *USP* monograph for this drug product, a new monograph based on validated methods of analysis is proposed.

1. The liquid chromatographic procedure used in the *Assay* is based on analyses performed with the Vydac 214TP Alltech brand of L26 column. The typical retention time for interferon beta-1a is 4 min.
2. The liquid chromatographic procedure used in *Identification* test C for *Peptide Mapping* and in the *Impurities* test for *Oxidized Interferon beta-1a* is based on analyses performed with the Vydac 214TP52 Alltech brand of L26 column.
3. The liquid chromatographic procedure used in the *Specific Tests* for *Analysis of N-*

Linked Oligosaccharides and Quantitation of Biantennary Sialylation is based on analyses performed with the Shodex NH2P-50 Asahipak brand of L82 column.

(BIO1: M. Kibbey.)

Correspondence Number—C136467

Comment deadline: July 31, 2015

Add the following:

■ Interferon beta-1a Injection

DEFINITION

Interferon beta-1a Injection is a sterile solution of Interferon beta-1a in a suitable solvent that may include suitable buffering and stabilizing agents.

IDENTIFICATION

• **A. Bioidentity**

Medium A: Dulbecco's Modified Eagle Medium containing 25 mM d-glucose,¹ as well as 10% heat-inactivated fetal bovine serum,² and 4 mM l-glutamine.³ Filter sterilize.

Medium B: Dulbecco's Modified Eagle Medium containing 25 mM d-glucose,⁴ as well as 2% heat-inactivated fetal bovine serum,⁵ and 4 mM l-glutamine.⁶ Filter sterilize.

NBB solution: An aqueous solution of 0.05% (w/v) naphthol blue black (NBB),⁷ 9% (v/v) glacial acetic acid, and 0.1 M sodium acetate.

Cell culture preparation: Prepare adherent cell cultures of human lung carcinoma cell line A549⁸ in *Medium A* and incubate in a humidified incubator at 37° containing 5% carbon dioxide. Cells should be passaged by trypsinization about once a week and are fed 4 days later with *Medium A*. The day before the assay (20–24 h before assay set-up), seed assay plates by removing the media from the flasks and add sufficient volume of 37° cell culture grade trypsin–EDTA solution⁹ to cover the flask surface. Add 2 mL of the trypsin–EDTA solution to each flask, [Note—These volumes are based on a T-150 tissue culture flask.¹⁰] rock the flasks, and then place in the 37° incubator again for 5–10 min. Remove the flasks, tap the flasks to dislodge the cells, then add 10 mL of 37° *Medium A* to each flask. Aseptically pool all trypsinized cell solutions into a suitable sterile container, dilute with *Medium A* to 1:2, and then count the cells. Adjust the cell density with *Medium A* such that the final concentration is 2.5×10^5 cells/mL and mix well to ensure a single cell suspension. Seed 100 µL of this suspension into each well of a 96-well tissue culture plate¹¹ and incubate for 20–24 h.

Standard/Control stock solutions: Reconstitute USP Interferon beta-1a for Bioidentity RS with a suitable quantity of sterile, distilled water and then dilute further with *Medium A* to 1×10^4 Units/mL or 50,000 pg/mL. [Note—This stock may be aliquoted and frozen at –70° until day of assay performance.]

Test stock solutions: Dilute the interferon beta-1a test materials with *Medium A* to 50,000 pg/mL (10,000 Units/mL). [Note—If the *Standard/Control stock solutions* were frozen before use in the assay then the *Test stock solutions* must also be aliquoted and frozen at –70° before evaluation in the method.]

Virus solution: Encephalomyocarditis (EMC) virus¹² is cultured in murine fibroblast L929 cells¹³ through a minimum of four passages. The supernatant of the virus cultures are frozen at -70° . Before qualification, the harvested virus supernatant is thawed, sonicated for 30 min, and centrifuged at $1920 \times g$ for 10 min. The centrifuged virus supernatant is pooled, aliquoted, and frozen at -70° for qualification. The virus is qualified by determining the TCID₅₀ of the virus on A549 cells. The virus is then diluted in *Medium B* to a working concentration (approximately TCID₅₀/mL = $10^{6.16}$).

Procedure: For each sample, two independent sets of interferon beta-1a *Test solutions* must be prepared for each assay plate (left block and right block are duplicate) and NLT 2 assay plates must be performed to generate an $n = 4$ for each sample (2 from each plate). On day of assay performance, dilute the 50,000 pg/mL *Standard stock solution*, the *Control stock solution*, and the *Test stock solution* with *Medium B* to a concentration of 40 pg/mL. From this intermediate dilution, dilute six times serially, using 1.3-fold dilution steps, to obtain a concentration series of 30.77 pg/mL to 8.29 pg/mL.

Remove the tissue culture plate containing cells from the incubator and visually inspect the plate to confirm the cells are confluent, and appear healthy and uncontaminated. Discard the media completely from the wells. Next, starting with the least concentrated solutions in row G and moving up, add 100 μ L of each concentration of *Standard solution*, *Control solution*, and *Test solution* to the appropriate well using the plate design in *Table 1*. Add 100 μ L of *Medium B* to rows A and H. At a minimum, repeat this setup for a second plate of cells. Incubate the plate for 20–24 h at 37° in an incubator with 5% carbon dioxide (CO₂).

The next day, dilute the *Virus solution* with *Medium B* to the predetermined qualified dilution. Remove the plates from the incubator and completely remove the solutions in each well. Add 100 μ L of *Medium B* to all wells of row A in each plate. Add 100 μ L of *Virus solution* to all wells on the rest of the plates. Incubate the plates again for 28–30 h at 37° in an incubator with 5% carbon dioxide (CO₂).

The next day, remove the plates from the incubator and completely remove the solutions in each well. Add 50 μ L of *NBB solution* to all wells of the plates. Incubate the plates at 37° in an incubator with 5% carbon dioxide (CO₂) for 60 ± 15 min. Remove the plates and shake out the *NBB solution* from all the wells. Add 100 μ L of phosphate buffered saline¹⁴ to every well then shake out this solution, blot the plates on a paper towel, and repeat this wash one more time. Next, add 100 μ L of 50 mM sodium hydroxide to every empty well. Place the plates on a shaker for NLT 1 min and NMT 96 h. Read the absorbance from the plates at a wavelength of 620 nm using a suitable plate reader.

Table 1. Schematic Representation of the Final Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cells	Cells	Cells	Cells	Cells	Cells	Cells	Cells	Cells	Cells	Cells	Cells
B	TSA, #1	TSB, #1	St, #1	PC, #1	TSC, #1	TSD, #1	TSA, #1	TSB, #1	St, #1	PC, #1	TSC, #1	TSD, #1
C	TSA, #2	TSB, #2	St, #2	PC, #2	TSC, #2	TSD, #2	TSA, #2	TSB, #2	St, #2	PC, #2	TSC, #2	TSD, #2
D	TSA, #3	TSB, #3	St, #3	PC, #3	TSC, #3	TSD, #3	TSA, #3	TSB, #3	St, #3	PC, #3	TSC, #3	TSD, #3
E	TSA, #4	TSB, #4	St, #4	PC, #4	TSC, #4	TSD, #4	TSA, #4	TSB, #4	St, #4	PC, #4	TSC, #4	TSD, #4
F	TSA, #5	TSB, #5	St, #5	PC, #5	TSC, #5	TSD, #5	TSA, #5	TSB, #5	St, #5	PC, #5	TSC, #5	TSD, #5
G	TSA, #6	TSB, #6	St, #6	PC, #6	TSC, #6	TSD, #6	TSA, #6	TSB, #6	St, #6	PC, #6	TSC, #6	TSD, #6
H	VC	VC	VC	VC	VC	VC	VC	VC	VC	VC	VC	VC

Legend
 Cells = Negative control of cells with *Medium B*; no interferon beta-1a, no virus.
 TSA = One dilution series of *Test solution*, with the following concentrations of interferon beta-1a added to the indicated well: #1: 30.77 pg/mL; #2: 23.67 pg/mL; #3: 18.21 pg/mL; #4: 14.01 pg/mL; #5: 10.77 pg/mL; #6: 8.29 pg/mL. TSB, TSC, TSD=3 additional independent dilution series of *Test solution*, prepared as in TSA.
 St = Dilution series of *Standard solution* (concentrations same as in TSA #1 through #6).
 PC = *Positive control solution* dilution series (concentrations same as in TSA #1 through #6).
 VC= *Medium B* and cells present, followed by virus, but no interferon beta-1a.

Calculations: Each independent half-plate block of samples, standards, and controls are evaluated separately. The relative potency of the *Test solutions* and the *Control solutions* are calculated relative to the *Standard solutions* in that block using parallel line analysis.

System suitability criteria: For each individual *Control solution* compared to the *Standard solution*, the statistical tests for regression and parallelism must pass at the 95% level. The percent relative confidence interval must be NMT 50%. For the plate to be acceptable, the signal-to-noise ratio of the mean cell control signal in row A to the mean virus control signal in row H must be NLT 2.0, and the relative potency of the *Control solution* to the *Standard solution* in that block must be NLT 80% and NMT 133% of the expected relative potency. For each individual *Test solution* compared to the *Standard solution*, the statistical tests for regression and parallelism must pass at the 95% level. The percent relative confidence interval must be NMT 50%. If any block fails based on these criteria then it must be repeated until a minimum of 4 relative potencies can be combined to calculate a mean relative potency. The 95% confidence interval for the calculated mean potency must be within 64%–156% of the observed potency.

Acceptance criteria: 80%–133%

- **B.** The retention time of the interferon beta-1a main peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Peptide Mapping**
Solution A: 0.1% (w/v) trifluoroacetic acid in water. Degas.
Solution B: Prepare a 90% solution of acetonitrile in water. Add trifluoroacetic acid to 0.1% (w/v) and mix.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
70	30	70
80	100	0

Buffer solution: 0.1 M solution of tris(hydroxymethyl)aminomethane (tris) and 0.25 M sodium chloride. Adjust with hydrochloric acid to a pH of 8.5. Filter.

Enzyme solution: Prepare an aqueous solution of 0.8 mg/mL lysyl endopeptidase from *Achromobacter lyticus*.

Standard stock solution: 1.0 mg/mL of USP Interferon beta-1a RS in *Buffer solution*

Standard solutions: Prepare duplicate tubes containing 8.8 µL of 50 mM dithiothreitol in each. Add 40 µL of *Standard stock solution* and 40 µL of *Buffer solution* to each tube. Mix and incubate for 15–60 min. Add 4.0 µL of a 20 mM EDTA solution and 4.0 µL of *Enzyme*

solution and gently mix, making sure not to introduce air and that all sample is at the bottom of the tube. Cap the tube and incubate at $25 \pm 2^\circ$ for 20–28 h. Add 98 μL of 8 M guanidine hydrochloride solution and 16 μL of 50 mM dithiothreitol solution. Mix and perform chromatography, injecting samples NMT 22 h later. [Note—Samples can be stored at -70° if necessary but an additional 16 μL of 50 mM dithiothreitol solution must be added before analysis if this is performed.]

Sample stock solution: Combine a suitable number of containers and concentrate to obtain a solution of 0.2 mg/mL of interferon beta-1a in *Buffer solution*. [Note—If necessary, buffer exchange by suitable means into *Buffer solution* to prepare this solution before digestion.]

Sample solutions: Prepare duplicate tubes containing 8.8 μL of 50 mM dithiothreitol in each. Add 8 μL of *Sample stock solution* and 72 μL of *Buffer solution* to each tube. Mix and incubate for 15–60 min. Add 4.0 μL of a 20 mM EDTA solution and 4.0 μL of *Enzyme solution* and gently mix, making sure not to introduce air and that all sample is at the bottom of the tube. Cap the tube and incubate at $25 \pm 2^\circ$ for 20–28 h. Add 98 μL of 8 M guanidine hydrochloride solution and 16 μL of 50 mM dithiothreitol solution. Mix and perform chromatography, injecting samples NMT 22 h later. [Note—Samples can be stored at -70° if necessary but an additional 16 μL of 50 mM dithiothreitol solution must be added before analysis if this is performed.]

Blank solutions: Prepare duplicate tubes containing 8.8 μL of 50 mM dithiothreitol in each. Add 80 μL of *Buffer solution* to each tube. Mix and incubate for 15–60 min. Add 4.0 μL of a 20 mM EDTA solution and 4.0 μL of *Enzyme solution* and gently mix making sure not to introduce air and that all sample is at the bottom of the tube. Cap the tube and incubate at $25 \pm 2^\circ$ for 20–28 h. Add 98 μL of 8 M guanidine hydrochloride solution and 16 μL of 50 mM dithiothreitol solution. Mix and perform chromatography, injecting samples NMT 22 h later. [Note—Samples can be stored at -70° if necessary but an additional 16 μL of 50 mM dithiothreitol solution must be added before analysis if this is performed.]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 2.1-mm \times 25-cm; 5- μm packing L26

Temperatures

Column: 40°

Sample: 5°

Flow rate: 0.3 mL/min

Injection volume: 100 μL

System suitability

Samples: *Standard solutions* and *Blank solutions*

Suitability requirements

Resolution: NLT 2.0 between the AP6(ox) peak (representing amino acids 34–45 of interferon beta-1a) at half height and the AP5 peptide peak (representing amino acids 124–134 of interferon beta-1a) in the *Standard solution*. [Note—See also the chromatogram supplied with USP Interferon beta-1a RS.]

NLT 3.8 between the AP6 and AP5 peptide peaks.

Interfering peaks: No significant peaks that coelute with the AP6 and AP6(ox) peaks in the *Blank solution*

Analysis

Samples: *Standard solutions* and *Sample solutions*

[Note—Condition the chromatographic system by running a blank gradient program before injecting the digests.]

Acceptance criteria: The chromatographic profiles of the *Sample solutions* are similar to those of the *Standard solutions* and no extra peaks greater than 5% of the peak height of peptide AP9 (representing amino acids 137–166 of interferon beta-1a) are present.

ASSAY

• Procedure

Solution A: 0.1% (w/v) trifluoroacetic acid. Degas solution before use.

Solution B: 90% (v/v) acetonitrile and 0.1% (w/v) trifluoroacetic acid. Degas solution before use.

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	60	40
5	10	90
7	10	90
9	60	40

Buffer solution: Dissolve 1.58 g of sodium acetate trihydrate and 31.60 g of L-arginine HCl in 900 mL of water, then add 0.48 mL of acetic acid, followed by more water to 1 L total. Mix, filter, and confirm that the pH is 4.8 ± 0.2 .

Sample additive: 10% (w/v) polyoxyethylene-polyoxypropylene block copolymer¹⁵

Standard solution: Dilute a suitable quantity of USP Interferon beta-1a RS in *Buffer solution* to prepare a 60 µg/mL solution. Add 10 µL of *Sample additive* to 1 mL of this protein solution and gently mix. Before use, centrifuge at $20,000 \times g$ for 30 min at 2° – 8° , then store at this temperature before injection.

Sample solution: Dilute a suitable quantity of containers in *Buffer solution* to obtain a 60 µg/mL interferon beta-1a solution. Add 10 µL of *Sample additive* per 1 mL of protein solution and gently mix. Before use, centrifuge at $20,000 \times g$ for 30 min at 2° – 8° , then store at this temperature before injection.

Blank solution: Add 5 µL of *Sample additive* to 500 µL of *Buffer solution*.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 2.1-mm \times 5-cm; 5-µm packing L26

Temperatures

Column: 40°

Sample: 5°

Flow rate: 0.5 mL/min

Injection volume: 100 μ L

System suitability

Samples: *Standard solution*, *Sample solution*, and *Blank solution*

Suitability requirements

Precision: NMT 3% RSD for the main peak area of the first six *Standard solution* injections before the *Sample solutions*, and NMT 3% RSD for the main peak area of the two *Standard solution* injections after the *Sample solution*. In addition, NMT 5% difference between the means of the first (first six injections) and second set (last two injections) of *Standard solution* injections where the percent difference is calculated by subtracting the mean of the second set from the first set, divided by the average of both sets, and multiplying by 100.

Tailing factor: 1.0–2.4 for the interferon beta-1a peak in each *Standard solution* injection, except the first injection

Interfering peaks: No significant peaks may be observed in the second *Blank solution*.

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—Condition the chromatographic system by running a blank gradient program before injecting the solutions.]

Inject two *Blank solutions*, then six *Standard solutions*, followed by duplicate injections of up to three *Sample solutions*, followed by two *Standard solution* injections. Integrate each chromatogram's interferon beta-1a main peak area. Calculate the concentration, in μ g/mL, of interferon beta-1a in the *Sample solution* taken:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U peak area from each *Sample solution* injection

r_S average peak area of bracketing *Standard solutions*

C_S concentration of the *Standard solution* (μ g/mL)

For the final result, average the *Sample solution* results.

Acceptance criteria: 90%–115%

IMPURITIES

• **Impurities with Molecular Masses Different from Those of Main Interferon beta-1a**

12% Resolving gel: Per 10 mL of solution, mix 4 mL of 30% acrylamide/bisacrylamide (29:1) solution, 3.3 mL of water, 2.5 mL of 1.5 M tris-HCl buffer (pH 8.8), 0.1 mL of 100 g/L SDS solution, 0.1 mL of 100 g/L ammonium persulfate solution (APS), and 4 μ L of tetramethylethylenediamine (TEMED). Pour into a suitable gel cassette, add a sufficient quantity of water-saturated isobutanol to cover the surface, and allow polymerization.¹⁶

Stacking gel: After polymerization of the *12% Resolving gel*, prepare (per 5 mL of solution): 0.83 mL 30% acrylamide/bisacrylamide (29:1) solution, 3.4 mL water, 0.63 mL of 1.5 M tris-HCl buffer (pH 8.8), 50 μ L of 100 g/L SDS solution, 50 μ L of 100 g/L APS, and 5 μ L TEMED. Remove the isobutanol from the *12% Resolving gel* surface and then pour the *Stacking gel* solution on top, insert a suitable gel comb without introducing air bubbles, and allow polymerization.

Buffer A: Prepare a stock solution of 151.4 g of tris, 721.0 g of glycine, and 50.0 g of

sodium lauryl sulfate, in 5 L water. Immediately before use, dilute 10-fold with water. Confirm that the pH is 8.1–8.8.

Stock sample buffer: Mix 3.78 g of tris, 10 g of sodium dodecyl sulfate (SDS), and 100 mg of bromophenol blue in water. Add 50.0 mL of glycerol and dilute with water to 200 mL. Add 25.0 mL of 2-mercaptoethanol. Adjust with hydrochloric acid to a pH of 6.8, and then dilute with water to 250 mL total volume.

Sample buffer: Mix equal volumes of *Stock sample buffer* with water.

Sample solution A: Concentrate a suitable number of containers with a suitable procedure to obtain an interferon beta-1a concentration of 1.5 mg/mL.

Sample solution B: Mix equal volumes of *Sample solution A* with *Stock sample buffer*.

Sample solution C: Dilute *Sample solution A* with water to obtain a concentration of 0.6 mg/mL. Mix equal volumes of this protein solution with *Stock sample buffer*.

Sample solution D: Mix 8 μL of *Sample solution C* with 40 μL of *Sample buffer*.

Sample solution E: Mix 15 μL of *Sample solution D* with 35 μL of *Sample buffer*.

Sample solution F: Mix 18 μL of *Sample solution E* with 18 μL of *Sample buffer*.

Sample solution G: Mix 12 μL of *Sample solution F* with 12 μL of *Sample buffer*.

Molecular weight markers: Mix an equal volume of a solution of relative molecular mass markers suitable for SDS-PAGE analysis in the range of 14–66 kDa¹⁷ with *Sample buffer*.

Staining solution: Dissolve 1.25 g/L of brilliant blue dye¹⁸ in a solution of glacial acetic acid, methanol, and water (1:4:5). Filter.

Destaining solution: Mix glacial acetic acid, isopropanol, and water (1:1:8).

Analysis

Insert the prepared gel in a suitable electrophoresis chamber containing *Buffer A* and remove the comb. Rinse each well with *Buffer A* before loading samples. Boil all *Sample solutions* and *Molecular weight markers* for 3 min. Load 20 μL of each *Sample solution* (A through G) and *Molecular weight marker* into individual wells of the gel. Perform the electrophoresis until the bromophenol blue dye front is near the bottom of the gel. Remove the gel from the cassette and immerse in *Staining solution* with gentle shaking at 33°–37° for 90 min. Discard the *Staining solution* and add a large excess of *Destaining solution*. Measure the distance traveled for each band in all lanes from the top of the resolving gel then divide that value by the distance traveled by the dye front to obtain the R_F for each band. Plot the logarithm of the molecular weights of each protein in the *Molecular weight markers* versus the R_F values. *Table 4* describes the apparent molecular weights of the main interferon beta-1a band as well as related substances.

Table 4

Name	Apparent Molecular Mass	Acceptance Criteria
Interferon beta-1a	23,000	—
Underglycosylated interferon beta-1a	21,000	NMT 5%
Deglycosylated interferon beta-1a	20,000	NMT 2%

System suitability requirements: The *Molecular weight markers* must be distributed across 80% of the gel, and there should be a linear relationship between the logarithm of their molecular weight and their R_F values. At least one band of approximate molecular weight of 23,000 Da (interferon beta-1a) must be present in the *Sample solution G* lane. A

decrease in staining intensity is seen from *Sample solution B* through *Sample solution G*. No interferon beta-1a dimer should be visible in any of the *Sample solution* lanes (apparent molecular mass 46,000).

Acceptance criteria: Any band in *Sample solution C* that corresponds to underglycosylated interferon beta-1a must not be more intense than the interferon beta-1a band in *Sample solution E* (or 5%). Any band in *Sample solution B* that corresponds to deglycosylated interferon beta-1a must not be more intense than the interferon beta-1a band in *Sample solution E* (or 2%). Any other band (other than the two described) of lower molecular weight than interferon beta-1a must not be more intense than the interferon beta-1a band in *Sample solution F* (or 1%).

- **Oxidized Interferon beta-1a**

Mobile phase, Solution C, Enzyme solution, Standard solutions, Sample solutions, Blank solutions, Chromatographic system, and System suitability: Prepare as directed in *Identification test C, Peptide Mapping*.

Analysis

Using the results from the test for *Peptide Mapping*, locate the peaks due to the peptide fragment AP6 comprising amino acids 34–45 and its oxidized form using the *Standard solution* results and the chromatogram of oxidized interferon beta-1a digest supplied with the USP Interferon beta-1a RS.

Calculate the percentage of oxidation of interferon beta-1a:

$$\frac{A_{34-45ox}}{A_{34-45} + A_{34-45ox}} \times 100$$

$A_{34-45ox}$ = area of the peak corresponding to the oxidized peptide fragment 34–45

A_{34-45} = area of the peak corresponding to the unoxidized peptide fragment 34–45

Acceptance criteria: NMT 11%

SPECIFIC TESTS

- **Analysis of N-Linked Oligosaccharides**

Solution A: Mix 2 mL of *Solution B* with 998 mL water. Degas before use.

Solution B: Dissolve 19.3 g of ammonium acetate in 800 mL water. Add 9 mL of acetic acid then dilute with water to 1000 mL. Degas before use.

Mobile phase: See *Table 5*.

[Note—Additional time may be inserted after 73 min to flush the column with 100% *Solution B* at 0.75 mL/min to remove excess dye from the column. If this is necessary, the column must then be washed with 100% *Solution A* before the next sample injection.]

Table 5

Time (min)	Flow Rate (mL/min)	Solution A (%)	Solution B (%)
0	0.50	100	0
30	0.50	70	30
70	0.50	15	85
72	0.50	0	100
72.5	0.75	0	100
87	0.75	0	100
88.5	0.75	100	0
99.5	0.75	100	0
99.8	0.50	100	0
130	0.50	100	0
131	0.0	100	0

Enzyme solution: On the day of use, combine 1.0 mL of 50 mM ammonium bicarbonate with 144 μ L of 10% nonyl phenoxy polyethoxy ethanol and mix. Add 40 μ L of peptide *N*-glycosidase F¹⁹ (PNGase F) and mix.

Labeling reagent: Mix 0.3 mL of acetic acid with 0.7 mL of dimethyl sulfoxide in a 1.5 mL microfuge tube. In a fume hood, add 48 mg of anthranilic acid²⁰ (2-AA). Mix well, then add 63 mg of sodium cyanoborohydride. Mix well.

System suitability solution: Using separate vials for each glycan standard, transfer 40 μ g of NA2F complex *N*-linked glycan²¹ (BiNA0), 20 μ g of NGA2F complex *N*-linked glycan²² (BiNA0-2Gal), 60 μ g of A1F complex *N*-linked glycan²³ (BiNA1), and 80 μ g of A2F complex *N*-linked glycan²⁴ (BiNA2). Add 10 μ L of *Labeling reagent* to each tube. Mix by vortexing, then centrifuge briefly. Incubate for 3 ± 0.25 h at $45 \pm 2^\circ$. After cooling and briefly centrifuging, remove excess label on suitable cartridges²⁵ per the manufacturer's instructions. After the final acetonitrile wash, elute each labeled glycan from the cartridge using 3×0.5 mL water washes into fresh tubes. Evaporate to dryness. Reconstitute each labeled glycan standards with 100 μ L of water for every 10 μ g of starting glycan. Pool all the labeled standard, mix, and aliquot. Aliquots may be stored for up to 1 year at $-20 \pm 5^\circ$.

Standard solutions: Buffer exchange sufficient quantities of USP Interferon beta-1a RS by suitable methods to prepare two replicates each containing 200 μ g USP Interferon beta-1a RS in a volume of 150–300 μ L of 50 mM ammonium bicarbonate. Add 100 μ L of *Enzyme solution* and mix gently. Incubate 16–24 h at $37 \pm 2^\circ$. Recover the released glycans by reverse-phase solid-phase extraction using suitable cartridges²⁶ eluting with 0.5 mL of 10% methanol. Evaporate each digest sample to dryness. Add 10 μ L of *Labeling reagent* to each tube. Mix by vortexing, then centrifuge briefly. Incubate for 3 ± 0.25 h at $45 \pm 2^\circ$. After cooling and briefly centrifuging, remove excess label on suitable cartridges²⁷ per the manufacturer's instructions. After the final acetonitrile wash, elute each labeled glycan from the cartridge using 3×0.5 mL water washes into fresh tubes. Evaporate to dryness. Before *Analysis*, reconstitute each labeled glycan sample with 50 μ L of water.

Sample solutions: Prepare two replicates for each sample. Buffer exchange by suitable methods sufficient containers to produce 200 μ g of interferon beta-1a in a final volume of

150–300 μL of 50 mM ammonium bicarbonate. Add 100 μL of *Enzyme solution* and mix gently. Incubate 16–24 h at $37 \pm 2^\circ\text{C}$. Recover the released glycans by reverse-phase solid-phase extraction using suitable cartridges²⁸ eluting with 0.5 mL of 10 % methanol. Evaporate each digest sample to dryness. Add 10 μL of *Labeling reagent* to each tube. Mix by vortexing, then centrifuge briefly. Incubate for 3 ± 0.25 h at $45 \pm 2^\circ$. After cooling and briefly centrifuging, remove excess label on suitable cartridges²⁹ per the manufacturer's instructions. After the final acetonitrile wash, elute each labeled glycan from the cartridge using 3×0.5 mL water washes into fresh tubes. Evaporate to dryness. Before *Analysis*, reconstitute each labeled glycan sample with 50 μL of water.

Blank solutions: Prepare two blanks containing 150 μL of 50 mM ammonium bicarbonate.

Add 100 μL of *Enzyme solution* and mix gently. Incubate 16–24 h at $37 \pm 2^\circ$. Perform reverse-phase-solid phase extraction using suitable cartridges³⁰ eluting with 0.5 mL of 10% methanol. Evaporate each tube to dryness. Add 10 μL of *Labeling reagent* to each tube. Mix by vortexing, then centrifuge briefly. Incubate for 3 ± 0.25 h at $45 \pm 2^\circ$. After cooling and briefly centrifuging, remove excess label on suitable cartridges³¹ per the manufacturer's instructions. After the final acetonitrile wash, elute each blank from the cartridge using 3×0.5 mL water washes into fresh tubes. Evaporate to dryness. Before *Analysis*, add 50 μL of water to each tube.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Fluorescence (350-nm excitation wavelength; 430-nm emission wavelength)

Column: 4.6-mm \times 15-cm; 5- μm packing L82

Temperatures

Column: $30 \pm 3^\circ$

Sample: 2° – 8°

Flow rate: 0.5 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution*, *Standard solutions*, and *Blank solutions*

Suitability requirements

Chromatogram of the *Standard solutions* must be comparable to the example chromatogram supplied with the USP Interferon beta-1a RS.

Resolution: NLT 10 between the BiNA2 glycan and BiNA1 glycan in the *System suitability solution*.

Column efficiency: NLT 10,000 based on the BiNA2 glycan peak in the *System suitability solution*

Interfering peaks: No significant peaks that interfere with glycan quantitation in the *Blank solution*

Analysis

Samples: *Standard solutions*, *Sample solutions*, and *Blank solutions*

Separately inject samples in the following order. Water alone, *Blank solution*, *System suitability solution*, first *Standard solution*, *Sample solutions*, second *Standard solution*, second *Blank solution*. Record the chromatograms and identify the five expected peaks

listed in *Table 6* (same nomenclature followed in the calculations below) by comparison to the representative chromatogram with the USP Interferon beta-1a RS. Integrate the chromatograms over the range including the BiNA0 through the BiNA3 peaks. Calculate the antennary distribution of fully sialylated form of each glycan BiNA2, TriNA3, and TriLacNA3 for each replicate, and report the average of replicate results:

$$\% \text{ BiNA2} = \frac{\text{Area of BiNA2}}{\text{Area of BiNA2} + \text{Area of TriNA3} + \text{Area of TriLacNA3}} \times 100$$

$$\% \text{ TriNA3} = \frac{\text{Area of TriNA3}}{\text{Area of BiNA2} + \text{Area of TriNA3} + \text{Area of TriLacNA3}} \times 100$$

$$\% \text{ TriLacNA3} = \frac{\text{Area of TriLacNA3}}{\text{Area of BiNA2} + \text{Area of TriNA3} + \text{Area of TriLacNA3}} \times 100$$

Table 6

Structure	Glycoform	Description
BiNA1	2A1S1F	Biantennary complex type glycan with core fucose (Bi) and one sialic acid (neuraminic acid, NA1)
BiNA2	2A2S1F	Biantennary complex type glycan with core fucose and two sialic acids
TriLacNA3	3A3S1F1L	Triantennary complex type glycan with core fucose and lactosamine repeat in one antennae, and three sialic acids
TriNA3	3A3S1F	Triantennary complex type glycan with core fucose and three sialic acids
BiNA3	2A3S1F	Biantennary complex type glycan with core fucose and three sialic acids

Acceptance criteria: Replicate chromatograms of *Sample solutions* must be comparable to each other and to the *Standard solution* chromatogram in terms of presence/absence of peaks and relative response (*Standard solution, Sample solution*).

- **Quantitation of Biantennary Sialylation**

Solution A, Solution B, Mobile phase, Enzyme solution, Labeling reagent, System suitability solution, Standard solutions, Sample solutions, Blank solutions, Chromatographic system, and System suitability: Prepare as directed in *Analysis of N-Linked Oligosaccharides*.

Analysis

Samples: *Standard solutions, Sample solutions, and Blank solutions*

Separately inject samples in the following order. Water alone, *Blank solution, System suitability solution, first Standard solution, Sample solutions, second Standard solution, second Blank solution*. Record the chromatograms and identify the biantennary peaks listed in *Table 6* and by comparison to the representative chromatogram with the USP Interferon beta-1a RS.

Calculate the percentage of sialylation of biantennary glycans for each replicate, and report the average of replicate results in the portion of Injection taken:

$$\% \text{ Biantennary sialylation} = \frac{(\text{Area of BiNA1}) + ((\text{Area of BiNA2} + \text{Area of BiNA3}) \times 2)}{(\text{Area of BiNA0} + \text{Area of BiNA1} + \text{Area of BiNA2} + \text{Area of BiNA3}) \times 2} \times 100$$

Acceptance criteria: NLT 90%

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 96 USP Endotoxin Units/mL
- **Sterility Tests** 〈 71 〉: Meets the requirements
- **Osmolality and Osmolarity** 〈 785 〉: 261–319 mOsmol/kg
- **pH** 〈 791 〉: 4.5–5.1
- **Subvisible Particulate Matter in Therapeutic Protein Injections** 〈 787 〉: Meets the requirements for small-volume injections
- **Injections and Implanted Drug Products** 〈 1 〉: Meets the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in Type 1 glass, single-use syringes, protected from light, at 2°–8°.
 - **Labeling:** The syringe labeling states the interferon beta-1a content (µg/mL).
 - **USP Reference Standards** 〈 11 〉
 - USP Interferon beta-1a RS
 - USP Interferon beta-1a for Bioidentity RS
- 1S (USP39)

¹ Gibco, catalog #11960-044, or suitable equivalent.

² Gibco, catalog #16000-044, or suitable equivalent.

³ Gibco, catalog #25030-081, or suitable equivalent.

⁴ Gibco, catalog #11960-044, or suitable equivalent.

⁵ Gibco, catalog #16000-044, or suitable equivalent.

⁶ Gibco, catalog #25030-081, or suitable equivalent.

⁷ Aldrich Chemical, catalog #19524-3, or suitable equivalent.

⁸ ATCC catalog #CCL-185.

⁹ Gibco, catalog #25300-054, or suitable equivalent.

¹⁰ Fisher, catalog #10-126-34, or suitable equivalent.

¹¹ Corning, catalog #3595, or suitable equivalent.

¹² ATCC, catalog #VR-129B.

¹³ ATCC #CCL-1, NCTC clone 929

- 14 Gibco, catalog #10010-023, or suitable equivalent.
- 15 BASF, catalog 549919, or suitable equivalent.
- 16 Alternatively, commercially available 12% SDS-PAGE gels including a stacking gel may be used.
- 17 Sigma catalog #SDS7 or other suitable equivalent.
- 18 Sigma catalog #B7920 or other suitable equivalent.
- 19 Selectin Biosciences catalog #GE41 or suitable equivalent.
- 20 Fluka catalog #10680 or suitable equivalent.
- 21 Ludger catalog #CN-NA2F-20U or suitable equivalent.
- 22 Ludger catalog #CN-NGA2F-20U or suitable equivalent.
- 23 Ludger catalog #CN-A1F-20U or suitable equivalent.
- 24 Ludger catalog #CN-A2F-20U or suitable equivalent.
- 25 Ludger catalog #LC-S-A6 or suitable equivalent.
- 26 Waters catalog #WAT094225 or suitable equivalent.
- 27 Ludger catalog #LC-S-A6 or suitable equivalent.
- 28 Waters catalog #WAT094225 or suitable equivalent.
- 29 Ludger catalog #LC-S-A6 or suitable equivalent.
- 30 Waters catalog #WAT094225 or suitable equivalent.
- 31 Ludger catalog #LC-S-A6 or suitable equivalent.

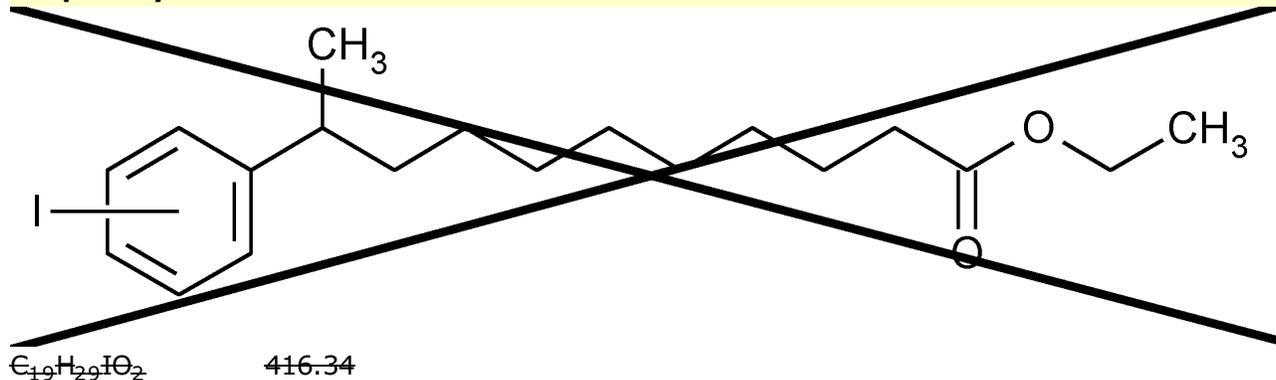
BRIEFING

Iophendylate, *USP 38* page 3916. It is proposed to omit this monograph from *USP* for the following reasons:

- This drug substance is not used in any dosage form for human use in the United States.
- This drug substance is currently not used in veterinary medicine in the United

States.

(SM4: R. Ravichandran.) Correspondence Number—C135179

*Comment deadline: July 31, 2015***Delete the following:****Iophendylate**~~Benzenedecanoic acid, iodo-*p*-methyl-, ethyl ester.~~~~Ethyl 10-(iodophenyl)undecanoate [[1320-11-2]].~~

» ~~Iophendylate is a mixture of isomers of ethyl iodophenylundecanoate, consisting chiefly of ethyl 10-(iodophenyl)undecanoate. It contains NLT 98.0 percent and NMT 102.0 percent of ethyl 10-(iodophenyl)undecanoate ($C_{19}H_{29}IO_2$).~~

Packaging and storage—~~Preserve in tight, light-resistant containers. Store at 25°; excursions permitted between 15° and 30°.~~

Identification—~~Place about 1 mL of Iophendylate, 15 mL of water, and 7 g of potassium dichromate in a round-bottom, 50-mL flask. Carefully add 10 mL of sulfuric acid, moderating the ensuing vigorous reaction by cooling the flask with tap water. When the reaction has subsided, reflux the mixture for 2 h. Pour the cooled contents of the flask into 25 mL of water, filter the mixture with suction, and wash the precipitate with a small quantity of cold water. Crystallize the precipitate from 10 mL of diluted alcohol, and sublime the solid so obtained: the sublimate of *p*-iodobenzoic acid melts between 268° and 272°.~~

Specific gravity ~~(841)~~: between 1.248 and 1.257

Refractive index ~~(831)~~: between 1.524 and 1.526

Residue on ignition ~~(281)~~: NMT 0.1%

Free acids—~~Transfer about 4 g, accurately weighed, to a small flask, and add 20 mL of alcohol. Swirl to dissolve the test specimen, add 5 drops of phenolphthalein TS, and titrate with 0.050 N alcoholic potassium hydroxide to a pink color that persists for 30 s: NMT 0.60 mL of 0.050 N alcoholic potassium hydroxide is required for neutralization, correction being made for the amount of 0.050 N alcoholic potassium hydroxide consumed by a blank (0.3% as~~

iodophenylundecanoic acid).

Free iodine—Determine its absorbance in a 4-cm cell, at 485 nm, with a suitable spectrophotometer, using water as the blank: the absorbance is not greater than 0.16 (7.5 ppm).

Saponification value—Transfer about 1 g, accurately weighed, to a 250-mL flask, add 25.0 mL of 0.5-N alcoholic potassium hydroxide VS, and reflux the mixture on a steam bath for 1 h. Cool, add 25 mL of water and 0.7 mL of phenolphthalein TS, and titrate with 0.5-N hydrochloric acid VS. The saponification value (see *Fats and Fixed Oils* ~~(401)~~) is between 132 and 142.

Assay—Dissolve about 50 mg of Iophendylate, accurately weighed, in 5 mL of toluene contained in a 125-mL separator fitted with a suitable, inert plastic stopcock. Add 15 mL of sodium biphenyl, and shake vigorously for 2 min. Extract gently with three 10-mL portions of 5-M phosphoric acid, combining the lower phases in a 125-mL iodine flask. Add 1-N sodium hypochlorite dropwise to the combined extracts until the solution turns brown, and then add an additional 0.5 mL. Shake intermittently for 3 min, add 5 mL of freshly prepared, saturated phenol solution, mix, and allow to stand for 1 min, accurately timed. Add 1 g of potassium iodide, shake for 30 s, and titrate rapidly with 0.1-N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1-N sodium thiosulfate is equivalent to 6.939 mg of ethyl 10-(iodophenyl)undecanoate ($C_{19}H_{29}IO_2$). ■ 1S (USP39)

BRIEFING

Iophendylate Injection, USP 38 page 3917. It is proposed to omit this monograph from USP for the following reasons:

- This dosage form is not used for human use in the United States.
- This drug substance is currently not used in veterinary medicine in the United States.

(SM4: R. Ravichandran.) Correspondence Number—C135180

Comment deadline: July 31, 2015

Delete the following:

■ Iophendylate Injection

» Iophendylate Injection is sterile Iophendylate.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ~~(11)~~—

USP Endotoxin RS

Bacterial endotoxins ~~(85)~~—It contains NMT 0.9 USP Endotoxin Unit per mg of iophendylate.

Other requirements—It conforms to the Definition, responds to the *Identification* test, and meets the requirements for *Specific gravity*, *Refractive index*, *Residue on ignition*, *Free acids*, *Free iodine*, *Saponification value*, and *Assay* under *Iophendylate*. It also meets the requirements under *Injections* ~~(1)~~. ■ 1S (USP39)

BRIEFING

Levamisole Hydrochloride, *USP 38* page 4058. As part of USP monograph modernization efforts, the following revisions are proposed:

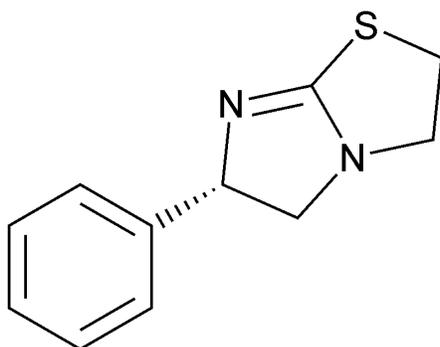
1. *Identification* test *A* is revised to reference *Infrared Absorption* 〈197〉 and to provide multiple analysis options to allow for more flexibility for the users.
2. *Identification* test *B* is revised to replace the TLC procedure with retention time agreement based on the proposed test for *Organic Impurities*.
3. The TLC procedure for *Organic Impurities* is replaced with a liquid chromatographic procedure adapted from the *European Pharmacopoeia* monograph. The LC procedure is based on analyses performed with the Hypersil BDS brand of L1 column. The typical retention time for levamisole is about 3 min.
4. The tests for *Melting Range or Temperature* and *Light Absorption* are proposed for omission.
5. A new Reference Standard, USP Levamisole System Suitability Mixture RS, used in the proposed test for *Organic Impurities*, is added to the *USP Reference Standards* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: M. Puderbaugh.)

Correspondence Number—C114616

Comment deadline: July 31, 2015

Levamisole Hydrochloride

• HCl

$C_{11}H_{12}N_2S \cdot HCl$ 240.75

Imidazo[2,1-*b*]thiazole, 2,3,5,6-tetrahydro-6-phenyl-, monohydrochloride, (*S*)-;

(-)-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]thiazole monohydrochloride [16595-80-5].

DEFINITION

Levamisole Hydrochloride contains NLT 98.5% and NMT 101.0% of levamisole hydrochloride ($C_{11}H_{12}N_2S \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

(See ~~Chromatography <621>~~, ~~Thin-Layer Chromatography~~.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Developing solvent system: Toluene, acetone, and ammonium hydroxide (60:40:1)

Analysis

Samples: ~~Identification Standard solution, Sample solution, Identification sample solution, and Diluted sample solution~~[~~Note—Identification Standard solution and Identification solution are used in Identification B.~~]

Proceed as directed for ~~Chromatography <621>~~, ~~Thin-Layer Chromatography~~. Allow the spots to dry, and develop the chromatogram in the ~~Developing solvent system~~, until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, and dry it at 105° for 15 min. Locate the spots on the plate by examination under short-wavelength UV light.

Acceptance criteria

Individual impurities: Any spot from ~~Sample solution~~, other than the one corresponding to levamisole, does not exceed, in size or intensity, the principal spot from the ~~Diluted sample solution~~, corresponding to NMT 0.5% of any individual impurity. Expose the plate to iodine vapor in a closed chamber for 15 min, and locate the spots on the plate. Any spot from the ~~Sample solution~~, other than the one corresponding to levamisole, does not exceed, in size or intensity, the principal spot from the ~~Diluted sample solution~~, corresponding to NMT 0.5% of any individual impurity.

Total impurities: NMT 1.0%

- [Note—Protect all analytical solutions from light, maintain them at NMT 25°, and inject them within 2 h of preparation.]

Solution A: 5 g/L of monobasic ammonium phosphate prepared as follows. Dissolve a suitable quantity of monobasic ammonium phosphate in 90% of the total volume of water, adjust with 1 N sodium hydroxide to a pH of 6.5, and dilute with water to volume.

Solution B: Acetonitrile

Mobile phase: See *Table 1*. Return to original conditions, and equilibrate the system for at least 4 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
8	30	70
10	30	70

System suitability solution: 10 mg/mL of USP Levamisole System Suitability Mixture RS prepared as follows. Transfer a suitable quantity of USP Levamisole System Suitability Mixture RS to an appropriate volumetric flask. Dissolve in methanol and then add 10% of the total flask volume of ammonium hydroxide. Dilute with methanol to volume.

Standard solution: 0.02 mg/mL of USP Levamisole Hydrochloride RS in methanol

Sample solution: 10 mg/mL of Levamisole Hydrochloride prepared as follows. Transfer a suitable quantity of Levamisole Hydrochloride to an appropriate volumetric flask. Dissolve

in methanol and then add 10% of the total flask volume of ammonium hydroxide. Dilute with methanol to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 10-cm; 3-μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between mercaptoethylthiazolidinone derivative and didehydro levamisole, *System suitability solution*

Tailing factor: NMT 3.5, *Standard solution*

Analysis

Samples: *System suitability solution*, *Standard solution*, and *Sample solution*

Chromatograph the *System suitability solution*, and identify the components on the basis of their relative retention times, given in *Table 2*.

Calculate the percentage of each impurity in the portion of Levamisole Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) 100$$

r_U

= peak response of each impurity from the *Sample solution*

r_S

= peak response of levamisole from the *Standard solution*

C_S

= concentration of USP Levamisole Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= concentration of Levamisole Hydrochloride in the *Sample solution* (mg/mL)

F

= relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting level for impurities is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Thiazolidinone derivative ^a	0.9	0.50	0.2
Levamisole	1.0	—	—
Styrylthiazolidin-2-imine ^b	1.4	0.59	0.2
Mercaptoethylthiazolidinone derivative ^c	1.5	0.34	0.2
Didehydro levamisole (EP, impurity D) ^d	1.6	0.77	0.2
Thiazolidinone disulfide derivative ^e	2.0	0.37	0.2
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.3
a 3-(2-Amino-2-phenylethyl)thiazolidin-2-one.			

^b (E)-3-Styrylthiazolidin-2-imine.

^c 1-(2-Mercaptoethyl)-4-phenylimidazolidin-2-one.

^d 6-Phenyl-2,3-dihydroimidazo[2,1-*b*]thiazole.

^e 1,1'-Bis(4-phenylimidazolidin-2-one-1-ylethyl)disulfide.

■ 1S (USP39)

SPECIFIC TESTS

Delete the following:

■ • **Melting Range or Temperature** ~~(741): 226°–231°~~ ■ 1S (USP39)

• **Optical Rotation** ~~(781S)~~, *Specific Rotation*

Sample solution: 50 mg/mL of Levamisole Hydrochloride in water

Acceptance criteria: -121.5° to -128.0°

• **pH** ~~(791)~~

Sample solution: Solution (1 in 20)

Acceptance criteria: 3.0–4.5

• **Loss on Drying** ~~(731)~~

Sample: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

Delete the following:

■ • **Light Absorption**

Sample solution: 1 mg/mL of Levamisole Hydrochloride in 0.2 N methanolic hydrochloric acid

Spectrometric instrumental conditions

(See *Spectrophotometry and Light Scattering* ~~(851)~~.)

Mode: UV

Cell length: 1 cm

Analytical wavelength: 310 nm

Acceptance criteria: Its absorbance is NMT 0.20 \blacksquare 1S (USP39)

- **Completeness of Solution** (641)

Sample solution: 50 mg/mL of Levamisole Hydrochloride in water

Acceptance criteria: Meets the requirements

- **Color of Solution**

Standard solution: Combine 2.5 mL of *Matching Fluid F* (see *Color and Achromicity* (631)) with 97.5 mL of 0.12 N hydrochloric acid.

Sample solution: Use the *Sample solution* from the test for *Completeness of Solution*.

Acceptance criteria: The *Sample solution* is colorless, or not more intensely colored than the *Standard solution*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light.

Change to read:

- **USP Reference Standards** (11)

USP Levamisole Hydrochloride RS

- USP Levamisole System Suitability Mixture RS

The mixture contains Levamisole Hydrochloride and the following impurities (other impurities may also be present):

3-(2-Amino-2-phenylethyl)thiazolidin-2-one.

C₁₁H₁₄N₂OS 222.31

(*E*)-3-Styrylthiazolidin-2-imine.

C₁₁H₁₂N₂S 204.29

1-(2-Mercaptoethyl)-4-phenylimidazolidin-2-one.

C₁₁H₁₄N₂OS 222.31

6-Phenyl-2,3-dihydroimidazo[2,1-*b*]thiazole.

C₁₁H₁₀N₂S 202.28

1,1'-Bis(4-phenylimidazolidin-2-one-1-ylethyl)disulfide.

C₂₂H₂₆N₄O₂S₂ 442.60 \blacksquare 1S (USP39)

BRIEFING

Levetiracetam, USP 38 page 4060. It is proposed to revise the monograph as follows:

1. Revise *Identification* test *B* to use the *Identification solution* with a lower concentration to match the concentration of the *System suitability solution*. The *Sample solution* concentration in the current monograph is 200-fold higher than the concentration of levetiracetam in the *System suitability solution* leading to difficulties in identification based on retention time.
2. Introduce an *Identification solution* in the test for *Limit of Levetiracetam R-Enantiomer* to facilitate *Identification* test *B*.

3. Rename "Organic Impurities, Procedure 1: Limit of Levetiracetam Related Compound B" as "Limit of Levetiracetam Related Compound B" to eliminate potential confusion regarding the applicability of the flexible monograph approach.
4. Rename "Organic Impurities, Procedure 2" as "Organic Impurities" to eliminate potential confusion regarding the applicability of the flexible monograph approach.

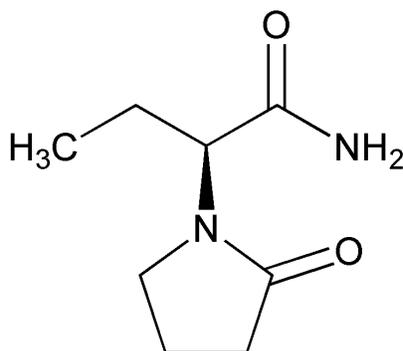
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R. Ravichandran.)

Correspondence Number—C157098

Comment deadline: July 31, 2015

Levetiracetam



$C_8H_{14}N_2O_2$ 170.21

1-Pyrrolidineacetamide, α -ethyl-2-oxo-, (α S)-;

(-)-(S)- α -Ethyl-2-oxo-1-pyrrolidineacetamide [102767-28-2].

DEFINITION

Levetiracetam contains NLT 98.0% and NMT 102.0% of levetiracetam ($C_8H_{14}N_2O_2$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. Infrared Absorption** < 197K >

Change to read:

- **B.** The retention time of the major peak for levetiracetam from the Sample solution of the *Identification solution* ■ 1S (*USP39*)

corresponds to that of the levetiracetam S-enantiomer from the *System suitability solution*, as obtained in the test for *Limit of Levetiracetam R-Enantiomer*.

ASSAY

- **Procedure**

Buffer: 2.7 g/L of monobasic potassium phosphate in water. Adjust with 2% aqueous potassium hydroxide (w/v) to a pH of 5.5.

Solution A: Acetonitrile and *Buffer* (1:19)

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
20	71	29

System suitability solution: 0.2 mg/mL of USP Levetiracetam RS and 0.08 mg/mL of USP Levetiracetam Related Compound A RS in *Solution A*. Prepare by first dissolving the required amount of USP Levetiracetam RS in a suitable volumetric flask. Add 10% of the flask volume of 0.1 N potassium hydroxide. Let the mixture react at room temperature for about 15 min, and then neutralize by adding 0.1 N hydrochloric acid at 10% of the flask volume. Add the required amount of USP Levetiracetam Related Compound A RS, sonicate to dissolve, dilute with *Solution A* to volume, and mix. [Note—Levetiracetam related compound A is included for peak identification purposes.]

Standard solution: 0.1 mg/mL of USP Levetiracetam RS in *Solution A*

Sample solution: 0.1 mg/mL of Levetiracetam in *Solution A*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 0.9 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Relative standard deviation: NMT 1.0%, for the levetiracetam peak

[Note—If system suitability criteria cannot be met, it is recommended that the column temperature be maintained at 20° to stabilize the system.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levetiracetam (C₈H₁₄N₂O₂) in the portion of Levetiracetam taken:

$$\text{Result} = [(r_U/r_S) \times (C_S/C_U) \times 100] - F$$

r_U = peak response of levetiracetam from the *Sample solution*

r_S = peak response of levetiracetam from the *Standard solution*

C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)

C_U = concentration of Levetiracetam in the *Sample solution* (mg/mL)

F = percentage of levetiracetam *R*-enantiomer from the test for *Limit of Levetiracetam R-Enantiomer*

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Delete the following:

- **Heavy Metals, Method II** 〈 231 〉: 20 ppm •(Official 1-Dec-2015)

Change to read:

- **Limit of Levetiracetam R-Enantiomer**

Mobile phase: *n*-Hexane and dehydrated alcohol (80:20)

System suitability solution: 0.1 mg/mL of USP Levetiracetam Racemic Mixture RS in *Mobile phase*

Standard solution: 0.05 mg/mL of USP Levetiracetam RS in *Mobile phase*

Sample solution: 10 mg/mL of Levetiracetam in *Mobile phase*

- **Identification solution:** 0.05 mg/mL of Levetiracetam from *Sample solution* in *Mobile phase* ■_{1S} (USP39)

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 10-μm packing L51

Flow rate: 1.0 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution*

- and *Identification solution* ■_{1S} (USP39)

[Note—The relative retention times for levetiracetam *R*-enantiomer and levetiracetam *S*-enantiomer are 0.55 and 1.0, respectively.]

- Use the chromatogram from the *Identification solution* for *Identification test B*. ■_{1S} (USP39)

]

Suitability requirements

Resolution: NLT 4.0 between the *R*- and *S*-enantiomers,

- *System suitability solution* ■_{1S} (USP39)

[Note—If a loss of resolution (less than 4.0) is observed, it is recommended that the column temperature be maintained at 25° to stabilize the system.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levetiracetam *R*-enantiomer in the portion of Levetiracetam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of levetiracetam *R*-enantiomer from the *Sample solution*

r_S peak response of levetiracetam from the *Standard solution*

C_S concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)

C_U concentration of Levetiracetam in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.8%

Change to read:

• **Organic Impurities, Procedure 1:**

■ **1S (USP39)**

Limit of Levetiracetam Related Compound B

[Note—Perform this test only if levetiracetam related compound B is a known process impurity.]

Buffer: 1.22 g of sodium 1-decanesulfonate in 1 L of water containing about 1.3 mL of phosphoric acid. Adjust with 20% (w/v) potassium hydroxide to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (3:17)

System suitability solution: 2 mg/mL of USP Levetiracetam Related Compound B RS in *Mobile phase*

Standard solution: 0.002 mg/mL of USP Levetiracetam Related Compound B RS in *Mobile phase*

Sample solution: 2.0 mg/mL of Levetiracetam in *Mobile phase*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.0 mL/min

Injection volumes

System suitability: 10 μL

Analysis: 50 μL

System suitability

Sample: *System suitability solution*

[Note—The retention time for levetiracetam related compound B is 9 min.]

Suitability requirements

Tailing factor: NMT 3.0

[Note—If a significant tailing of the levetiracetam related compound B peak is observed

(greater than 3.0), it is recommended that the column temperature be maintained at 27° to stabilize the system.]

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levetiracetam related compound B in the portion of Levetiracetam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of levetiracetam related compound B from the *Sample solution*
 r_S = peak response of levetiracetam related compound B from the *Standard solution*
 C_S = concentration of USP Levetiracetam Related Compound B RS in the *Standard solution* (mg/mL)
 C_U = concentration of Levetiracetam in the *Sample solution* (mg/mL)
 $M_{r\bar{1}}$ molecular weight of levetiracetam related compound B free base, 102.1
 $M_{r\bar{2}}$ molecular weight of levetiracetam related compound B, 138.6

Acceptance criteria: NMT 0.10%

[Note—The amount of levetiracetam related compound B measured is to be included in the total impurities in the test for *Organic Impurities, Procedure 2*

■ ■ 1S (USP39)

]

Change to read:

- **Organic Impurities, Procedure 2**

■ ■ 1S (USP39)

Buffer, Solution A, Solution B, Mobile phase, System suitability solution, and

Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.005 mg/mL of USP Levetiracetam RS in *Solution A*

Sample solution: 5 mg/mL of Levetiracetam in *Solution A*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Levetiracetam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{i\bar{1}}$ peak response of each impurity from the *Sample solution*
 r_S peak response of levetiracetam from the *Standard solution*
 C_S concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
 C_U concentration of Levetiracetam in the *Sample solution* (mg/mL)
 F = relative response factor (see *Table 2*)

[Note—Disregard any peak with a relative retention time of 0.19 or less.]

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Pyridin-2-ol ^a	0.37	1.0	0.025

- ^a Not included in the total impurities limit.
^b (S)-2-(2-Oxopyrrolidin-1-yl)butanoic acid. Included in the total impurities limit.
^c (S)-N-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide. Included in the total impurities limit only if levetiracetam related compound B is a known process impurity.

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levetiracetam acid ^b	0.62	1.2	0.3
Levetiracetam	1.00	—	—
Levetiracetam related compound A ^c	1.25	0.35	0.05
Any individual unspecified impurity	—	1.0	0.05
Total impurities	—	—	0.4

a Not included in the total impurities limit.

b (S)-2-(2-Oxopyrrolidin-1-yl)butanoic acid. Included in the total impurities limit.

c (S)-N-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide. Included in the total impurities limit only if levetiracetam related compound B is a known process impurity.

SPECIFIC TESTS

- **Water Determination** { 921 }, Method Ia: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, and store at room temperature.
- **USP Reference Standards** { 11 }
 - USP Levetiracetam RS
 - USP Levetiracetam Racemic Mixture RS
 - A 1:1 mixture of:
 - Levetiracetam S-enantiomer-(2S)-2-(2-oxopyrrolidin-1-yl)butanamide;
 - Levetiracetam R-enantiomer (2R)-2-(2-oxopyrrolidin-1-yl)butanamide.
 - USP Levetiracetam Related Compound A RS
 - (S)-N-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.
 - C₈H₁₅ClN₂O₂ 206.67
 - USP Levetiracetam Related Compound B RS
 - (S)-2-Aminobutanamide hydrochloride.
 - C₄H₁₀N₂O·HCl 138.6

BRIEFING

Lithium Carbonate, USP 38 page 4115. As part of USP monograph modernization efforts, the following changes are proposed:

1. The current *Identification* test B uses a flame test for lithium ion. It is replaced with the retention time agreement of lithium ion from the proposed ion chromatographic procedure in the *Assay*.
2. The current titration procedure in the *Assay* is replaced with an ion chromatographic procedure, which is validated using an IonPac CS16 brand of L84 cation-exchange column manufactured by ThermoScientific-Dionex. Under the chromatographic conditions, lithium ions elute at a retention time of about 10 min. The current *Acceptance criteria* is changed from NLT 99.0% to 98.0%–102.0% of lithium carbonate (Li₂CO₃) to be consistent with the chromatographic procedure. The *Definition* has been changed to reflect the revised *Acceptance criteria*.

3. The current time consuming test for *Calcium* and the flame photometric test for *Sodium* are replaced with the ion chromatographic procedure, which is the same as proposed in the *Assay*. Under the chromatographic conditions, sodium and calcium ions elute at retention times of about 16 and 22 min, respectively.
4. Add USP Lithium Carbonate RS to the *USP Reference Standards* section to support the proposed revisions to the *Assay* and the *Calcium and Sodium* test.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: W. Yang, R. Ravichandran.)
Correspondence Number—C156050

Comment deadline: July 31, 2015

Lithium Carbonate

Li₂CO₃ 73.89

Carbonic acid, dilithium salt;
Dilithium carbonate [554-13-2].

DEFINITION

Change to read:

~~Lithium Carbonate contains NLT 99.0% of lithium carbonate (Li₂CO₃), calculated on the dried basis.~~

■ Lithium Carbonate contains NLT 98.0% and NMT 102.0% of lithium carbonate (Li₂CO₃), calculated on the dried basis. ■ 1S (USP39)

IDENTIFICATION

Change to read:

- **A.** It effervesces upon the addition of an acid, yielding a colorless gas that, when passed into calcium hydroxide TS, immediately
▲ forms a white precipitate. ▲ USP38

Change to read:

- **B.** ~~When moistened with hydrochloric acid, it imparts an intense crimson color to a nonluminous flame.~~
- The retention time of the lithium peak of the *Sample solution* corresponds to the lithium peak of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

ASSAY

Change to read:

- **Procedure**

~~▲ **Sample solution:** Dissolve 0.5 g of Lithium Carbonate in 25.0 mL of 1 N hydrochloric acid VS.~~

~~**Blank:** 25.0 mL of 1 N hydrochloric acid VS~~

~~**Titrimetric system**~~

~~(See *Titrimetry* (541).)~~

~~**Mode:** Residual titration~~

~~**Titrant:** 1 N sodium hydroxide VS~~

~~**Endpoint detection:** Visual~~

~~**Indicator:** Methyl orange TS~~

~~**Analysis**~~

~~**Samples:** *Sample solution* and *Blank*~~

~~Titrate the excess acid in the *Sample solution* with *Titrant*.~~

~~Calculate the percentage of lithium carbonate (Li_2CO_3) in the portion of Lithium Carbonate taken:~~

$$\text{Result} = (V_B - V_S) \times N \times F \times (1/W) \times 100$$

~~V_B Titrant volume consumed by the *Blank* (mL)~~

~~V_S Titrant volume consumed by the *Sample solution* (mL)~~

~~N = normality of *Titrant* (mEq/mL)~~

~~F = equivalent weight of Lithium Carbonate, 36.95 mg/mEq~~

~~W = weight of Lithium Carbonate in the *Sample solution* (mg)~~

~~▲ USP38~~

Acceptance criteria: NLT 99.0% on the dried basis

■ [

Note—Polymeric containers made of high-density polyethylene (HDPE) are recommended in preparations of the *Standard solution* and the *Sample solution*.

Use water with a resistivity of NLT 18 megohm-cm to prepare the solutions.]

Solution A: 100 mM methanesulfonic acid in water

Solution B: Water

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	8	92
15	8	92
15.1	67	33
20	67	33
20.1	8	92
25	8	92

Alternatively, *Mobile phase* can be generated electrolytically using an automatic eluent generator.

[Note—It is recommended to use suitable cation trapping techniques to ensure the *Mobile phase* is free of all cationic impurities.]

Standard solution: 250 µg/mL of USP Lithium Carbonate RS, 0.3 µg/mL of sodium ions, 0.4 µg/mL of calcium ions, and 0.2 µg/mL of magnesium ions (from commercially available, NIST traceable ion chromatography standard solutions for sodium, calcium, and magnesium) in water

Sample solution: 250 µg/mL of Lithium Carbonate in water

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Conductivity with suppression

Columns

Guard: 3-mm × 5-cm; 5-µm packing L84

Analytical: 3-mm × 25-cm; 5-µm packing L84

Column temperature: 40°

Flow rate: 0.43 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[Note—Relative retention times for lithium, sodium, magnesium, and calcium ions are 1.0, 1.5, 2.0, and 2.1, respectively.]

Suitability requirements

Tailing factor: NMT 1.0 for lithium ion

Relative standard deviation: NMT 0.73% for lithium ion

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lithium carbonate (Li_2CO_3) in the portion of Lithium Carbonate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of lithium from the *Sample solution*

r_S peak response of lithium from the *Standard solution*

C_S concentration of USP Lithium Carbonate RS in the *Standard solution* (µg/mL)

C_U concentration of Lithium Carbonate in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ■1S (USP39)

IMPURITIES

● Aluminum and Iron

Sample solution: Dissolve 500 mg of Lithium Carbonate in 10 mL of water by the dropwise addition, with agitation, of hydrochloric acid.

Analysis: Boil the *Sample solution*, then cool it. To 5 mL of the solution add 6 N ammonium hydroxide until the reaction is alkaline.

Acceptance criteria: No turbidity or precipitate is observed.

Delete the following:

- ● **Calcium**

Sample solution: Suspend 5.0 g of Lithium Carbonate in 50 mL of water, and add a slight excess of 3 N hydrochloric acid. Boil the clear solution to expel carbon dioxide, add 5 mL of ammonium oxalate TS, render alkaline with 6 N ammonium hydroxide, and allow to stand for 4 h. Pass through a filtering crucible, and wash with warm water until the last washing yields no turbidity with calcium chloride TS. Place the crucible in a beaker, cover the crucible with water, add 3 mL of sulfuric acid, and heat to 70 °.

Analysis: Titrate the *Sample solution* with 0.10 N potassium permanganate to a pale pink color that persists for 30 s.

Acceptance criteria: NMT

▲3.8▲*USP38*

mL of 0.10 N potassium permanganate is consumed (0.15%). ■1S (*USP39*)

Delete the following:

- ● **Sodium**

Standard stock solution: 500 µg/mL of sodium prepared as follows. Dissolve 1.271 g of sodium chloride, previously dried at 130 ° to constant weight, in water in a 1000 mL volumetric flask. Dilute with water to volume.

Sample stock solution: 100 mg/mL of Lithium Carbonate prepared as follows. Suspend 20.0 g of Lithium Carbonate in 100 mL of water, cautiously add 50.0 mL of hydrochloric acid, transfer to a 200 mL volumetric flask, and dilute with water to volume.

Standard solution: Transfer 1 mL of *Standard stock solution* and 5 mL of *Sample stock solution* to a 100 mL volumetric flask, and dilute with water.

Sample solution: 5 mg/mL of Lithium Carbonate from *Sample stock solution* diluted with water

Instrumental conditions

Mode: Flame photometry

Analytical wavelengths: 580 and 589 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Set the flame photometer for maximum emission at 589 nm, using the *Standard solution*. Measure the emission intensities of the *Sample solution* at 580 and 589 nm.

Acceptance criteria: The difference between the intensities observed at 580 and 589 nm for the *Sample solution* does not exceed the difference between the intensities observed at 589 nm for the *Sample solution* and the *Standard solution*, respectively (0.1%).

■1S (*USP39*)

Add the following:

- ● **Calcium and Sodium**

Solution A, Solution B, Mobile phase, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

System suitability

Sample: *Standard solution*

[Note—Relative retention times for lithium, sodium, magnesium, and calcium ions are 1.0, 1.5, 2.0, and 2.1, respectively.]

Suitability requirements**Resolution:** NLT 2.5 between magnesium and calcium ions**Tailing factor:** NMT 1.5 for sodium and calcium ions**Relative standard deviation:** NMT 2.0% for sodium and calcium ions**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of sodium or calcium in the portion of Lithium Carbonate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak response of sodium or calcium from the *Sample solution* r_S peak response of sodium or calcium from the *Standard solution* C_S concentration of sodium or calcium in the *Standard solution* ($\mu\text{g/mL}$) C_U concentration of Lithium Carbonate in the *Sample solution* ($\mu\text{g/mL}$)**Acceptance criteria****Calcium:** NMT 0.15%**Sodium:** NMT 0.1%

■ 1S (USP39)

Delete the following:●● **Heavy Metals** 〈 231 〉**Sample solution:** Dissolve 1 g of Lithium Carbonate in 10 mL of 3 N hydrochloric acid, and dilute with water to 25 mL.**Acceptance criteria:** NMT 20 ppm

● (Official 1-Dec-2015)

● **Chloride and Sulfate** 〈 221 〉, *Sulfate***Standard solution:** Transfer 1 mL of 0.020 N sulfuric acid and 1 mL of 3 N hydrochloric acid to a suitable container. Dilute with water to 40 mL.**Sample solution:** Transfer 1.0 g of Lithium Carbonate to a suitable container. Dissolve in 10 mL of 3 N hydrochloric acid. Dilute with water to 40 mL.**Analysis:** To the *Standard solution* and the *Sample solution*, separately add 1 mL of barium chloride TS.**Acceptance criteria:** The turbidity produced in the *Sample solution*, after 3 min, is NMT that produced in the *Standard solution* (0.1%).● **Chloride and Sulfate** 〈 221 〉, *Chloride***Standard solution:** Transfer 0.5 mL of 0.02 N hydrochloric acid and 1.2 mL of nitric acid to a suitable container. Dilute with water to 50 mL.**Sample solution:** Transfer 500 mg of Lithium Carbonate to a suitable container. Add 1.2 mL of nitric acid. Dilute with water to 50 mL.**Analysis:** To the *Standard solution* and the *Sample solution*, separately add 1 mL of silver nitrate TS.**Acceptance criteria:** The turbidity produced in the *Sample solution* is NMT that produced in the *Standard solution* (0.07%).**Delete the following:**

▲● Insoluble Substances

Sample solution: Transfer 10 g to a 250-mL beaker, add 50 mL of water, and slowly add 50 mL of 6 N hydrochloric acid.

Analysis: Cover the *Sample solution* with a watch glass, and boil the solution for 1 h. Pass through a dried, tared filtering crucible fitted with a glass-fiber filter disk, using suction. Wash the filter with hot water until the last washing is free from chloride when tested with silver nitrate TS. Dry the crucible in an oven at 110° for 1 h.

Acceptance criteria: The weight of the residue is NMT 0.02% of the weight of Lithium Carbonate taken. ▲*USP38*

SPECIFIC TESTS**● Loss on Drying** 〈 731 〉

Analysis: Dry at 200° for 4 h.

Acceptance criteria: NMT 1.0%

Delete the following:

▲● **Alkalinity:** A saturated solution is alkaline to litmus. ▲*USP38*

ADDITIONAL REQUIREMENTS

● **Packaging and Storage:** Preserve in well-closed containers.

Add the following:**■● USP Reference Standards** 〈 11 〉

USP Lithium Carbonate RS

■1S (*USP39*)

BRIEFING

Medroxyprogesterone Acetate Injectable Suspension, *USP 38* page 4221. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Replace the current HPLC procedure in the *Assay* with a stability-indicating UHPLC method. The proposed liquid chromatographic procedure is based on analyses using the Zorbax Eclipse Plus C18 brand of L1 column. The typical retention time for medroxyprogesterone acetate is about 15.6 min.
2. Add *Identification* test *B* using a retention time agreement based on the proposed UHPLC procedure in the *Assay*.
3. Add a UHPLC procedure for the *Organic Impurities* test that is consistent with the proposed *Assay* method.
4. Update the *Packaging and Storage* section to be consistent with approved manufacturers' package inserts.
5. Add the new USP Reference Standard, USP Medroxyprogesterone Acetate Related Compound B RS, introduced by the proposed UHPLC procedure for the *Assay* and the test for *Organic Impurities*, to the *USP Reference Standards* section. Add existing USP Reference Standards, USP Medroxyprogesterone Acetate Related Compound A RS and USP Megestrol Acetate RS, to the *USP Reference Standards* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: D.A. Porter.)

Correspondence Number—C115255

Comment deadline: July 31, 2015

Medroxyprogesterone Acetate Injectable Suspension

DEFINITION

Medroxyprogesterone Acetate Injectable Suspension is a sterile suspension of Medroxyprogesterone Acetate in a suitable aqueous medium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$).

IDENTIFICATION

• A. Infrared Absorption (197K)

Sample: Transfer a portion of Injectable Suspension, equivalent to 50 mg of medroxyprogesterone acetate, to a centrifuge tube. Centrifuge, decant the supernatant, and wash the solids with two 15-mL portions of water, discarding the water washings. Dissolve the solids in 10 mL of chloroform, transfer to a small beaker, evaporate the chloroform on a steam bath, and dry the residue at 105° for 3 h.

Acceptance criteria: Meets the requirements

Add the following:

- B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■1S (*USP39*)

ASSAY

Change to read:

• Procedure

~~**Mobile phase:** 700 mL of butyl chloride, 300 mL of hexane, both previously saturated with water, and 80 mL of acetonitrile. The acetonitrile concentration may be varied to meet *System suitability* requirements and to provide elution times of about 12 and 15 min for progesterone and medroxyprogesterone acetate, respectively. Pass the solution through a membrane filter of 1 µm or less pore size.~~

~~**Internal standard solution:** 0.25 mg/mL of progesterone in *Mobile phase*~~

~~**Standard solution:** 0.4 mg/mL of USP Medroxyprogesterone Acetate RS in *Internal standard solution*~~

~~**Sample solution:** Nominally 0.4 mg/mL of medroxyprogesterone acetate in *Internal standard solution*, prepared as follows. Transfer a volume of Injectable Suspension, equivalent to 50 mg of medroxyprogesterone acetate, to a suitable container. Transfer 25 mL of chloroform into the container, shake for 20 min, and centrifuge. Transfer 4 mL of the chloroform layer into a suitable container, and evaporate to dryness. Dissolve the residue in 20 mL of *Internal standard solution*.~~

~~**Chromatographic system**~~

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 2-mm × 25-cm; 5-µm packing L3

Flow rate: The *Mobile phase* is maintained at a flow rate capable of giving the required resolution and suitable elution times.

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between progesterone and medroxyprogesterone acetate

Relative standard deviation: NMT 2.0%

■ **Solution A:** Acetonitrile, tetrahydrofuran, and water (150:50:800). Pass through a suitable filter of 0.2-µm pore size.

Solution B: Acetonitrile, tetrahydrofuran, and water (400:400:200). Pass through a suitable filter of 0.2-µm pore size.

Diluent: Acetonitrile and water (70:30)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
5.0	90	10
18.0	45	55
18.01	90	10
22.0	90	10

System suitability stock solution: 0.2 mg/mL each of USP Medroxyprogesterone Acetate RS and USP Megestrol Acetate RS prepared as follows. Add portions of each Reference Standard to a suitable volumetric flask. Add *Diluent* equivalent to 70% of the flask volume and sonicate if necessary. Dilute with *Diluent* to volume.

System suitability solution: 0.05 mg/mL each of USP Medroxyprogesterone Acetate RS and USP Megestrol Acetate RS in *Diluent* prepared from *System suitability stock solution*

Standard solution: 0.1 mg/mL of USP Medroxyprogesterone Acetate RS in *Diluent*. Sonicate to dissolve as needed. Pass through a suitable filter of 0.2-µm pore size.

Sample stock solution: Nominally 10.0 mg/mL of medroxyprogesterone acetate prepared as follows. Transfer a portion of Injectable Suspension to a suitable volumetric flask and add 70% of the flask volume of *Diluent*. Sonicate for about 10 min and dilute with *Diluent* to volume.

Sample solution: Nominally 0.1 mg/mL of medroxyprogesterone acetate in *Diluent* from *Sample stock solution*. Pass through a suitable filter of 0.2-µm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 3.0-mm × 15-cm; 1.8- μ m packing L1

Column temperature: 55 $^{\circ}$

Flow rate: 0.7 mL/min

Injection volume: 4.0 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 6.0 between the megestrol acetate and medroxyprogesterone acetate peaks, *System suitability solution*

Relative standard deviation: NMT 4.0%, *Standard solution*

■ 1S (USP39)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) in the portion of *Injectable Suspension* taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of medroxyprogesterone acetate to the internal standard from the *Sample solution*

R_S = peak area ratio of medroxyprogesterone acetate to the internal standard from the *Standard solution*

C_S = concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of medroxyprogesterone acetate in the *Sample solution* (mg/mL)

■ Calculate the percentage of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) in the portion of *Injectable Suspension* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of medroxyprogesterone acetate in the *Sample solution* (mg/mL)

■ 1S (USP39)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:**Organic Impurities**

Solution A, Solution B, Diluent, and Mobile phase: Proceed as directed in the *Assay*.

Standard stock solution: 0.2 mg/mL each of USP Medroxyprogesterone Acetate RS, USP Megestrol Acetate RS, and USP Medroxyprogesterone Acetate Related Compound B RS, and 0.1 mg/mL of USP Medroxyprogesterone Acetate Related Compound A RS prepared as follows. Add portions of each Reference Standard to a suitable volumetric flask. Add *Diluent* equivalent to 70% of the flask volume and sonicate if necessary. Dilute with *Diluent* to volume.

Standard solution: 0.05 mg/mL each of USP Medroxyprogesterone Acetate RS, USP Megestrol Acetate RS, and USP Medroxyprogesterone Acetate Related Compound B RS, and 0.025 mg/mL of USP Medroxyprogesterone Acetate Related Compound A RS in *Diluent* prepared from *Standard stock solution*

Sample solution: Nominally 5.0 mg/mL of medroxyprogesterone acetate from Injectable Suspension in *Diluent*. Sonicate if necessary. Pass through a suitable filter of 0.2- μ m pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector

UV 266 nm: For megestrol acetate, medroxyprogesterone acetate, and medroxyprogesterone related compound B

UV 282 nm: For medroxyprogesterone acetate related compound A

Column: 3.0-mm \times 15-cm; 1.8- μ m packing L1

Column temperature: 55 $^{\circ}$

Flow rate: 0.7 mL/min

Injection volume: 4.0 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times are listed in *Table 2*.]

Suitability requirements

Resolution: NLT 6.0 between the megestrol acetate and medroxyprogesterone acetate peaks

Relative standard deviation: NMT 4.0% for the medroxyprogesterone acetate, megestrol acetate, and medroxyprogesterone acetate related compound B peaks; NMT 4.5% for the medroxyprogesterone acetate related compound A peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of megestrol acetate and medroxyprogesterone acetate related compound B in the portion of Injectable Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of megestrol acetate or medroxyprogesterone acetate related compound B at 266 nm from the *Sample solution*

r_S peak response of each corresponding impurity at 266 nm from the *Standard solution*

C_S concentration of each corresponding impurity in the *Standard solution* (mg/mL)

C_U nominal concentration of medroxyprogesterone acetate in the *Sample solution* (mg/mL)

Calculate the percentage of medroxyprogesterone acetate related compound A in the portion of Injectable Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of medroxyprogesterone acetate related compound A at 282 nm from the *Sample solution*

r_S peak response of medroxyprogesterone acetate related compound A at 282 nm from the *Standard solution*

C_S concentration of USP Medroxyprogesterone Acetate Related Compound A RS in the *Standard solution* (mg/mL)

C_U nominal concentration of medroxyprogesterone acetate in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Injectable Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any degradation product at 266 nm from the *Sample solution*

r_S peak response of medroxyprogesterone acetate at 266 nm from the *Standard solution*

C_S concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (mg/mL)

C_U nominal concentration of medroxyprogesterone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
6 β -Hydroxymedroxyprogesterone acetate ^a	0.57	1.0
Homomedroxyprogesterone 13 α -epimer ^b	0.80	1.0
Medroxyprogesterone acetate related compound B	0.92	1.0
Homomedroxyprogesterone acetate ^c	0.95	1.0
Megestrol acetate	0.96	1.0
6-Epimedroxyprogesterone acetate ^d	0.98	1.0
Hydroxyprogesterone acetate 6-methylene analog ^e	0.99	1.0
Medroxyprogesterone acetate	1.0	—
Medroxyprogesterone acetate related compound A	1.16	0.5
Any individual unspecified degradation product	—	1.0
Total impurities	—	1.5

^a 6 β -Hydroxy-6 α -methyl-3,20-dioxopregn-4-ene-17-yl acetate.

^b 6 α ,13 α -Dimethyl-13(17)a-homoandrost-4-en-3,17-dione.

^c 6 α ,13 β -Dimethyl-3,17-dioxo-13(17)a-homoandrost-4-en-13a-yl acetate.

d 6 β -Methyl-3,20-dioxopregn-4-ene-17-yl acetate.

e 6-Methylene-3,20-dioxopregn-4-ene-17-yl acetate.

■ 1S (USP39)

SPECIFIC TESTS

- pH (791): 3.0–7.0
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

■ Store vials upright at controlled room temperature. ■ 1S (USP39)

Change to read:

- **USP Reference Standards** (11)

USP Medroxyprogesterone Acetate RS

■ USP Medroxyprogesterone Acetate Related Compound A RS

6 α -Methyl-3,20-dioxopregnan-17-yl acetate.

C₂₄H₃₆O₄ 388.54

USP Medroxyprogesterone Acetate Related Compound B RS

17-Hydroxy-6 α -methylpregn-4-ene-3,20-dione.

C₂₂H₃₂O₃ 344.49

USP Megestrol Acetate RS

6-Methyl-3,20-dioxopregna-4,6-diene-17-yl acetate.

C₂₄H₃₂O₄ 384.51

■ 1S (USP39)

BRIEFING

Medroxyprogesterone Acetate Tablets, USP 38 page 4222. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Replace the current HPLC procedure in the *Assay* with a stability indicating UHPLC method. The proposed liquid chromatographic procedure is based on analyses using the Zorbax Eclipse Plus C18 brand of L1 column. The typical retention time for medroxyprogesterone acetate is about 15.6 min.
2. Add *Identification test B* using a retention time agreement based on the proposed UHPLC procedure in the *Assay*.
3. Add a UHPLC procedure for the *Organic Impurities* test that is consistent with the proposed *Assay* method. There is no method for determining organic impurities in the

current monograph.

4. Update the *Packaging and Storage* section to be consistent with approved manufacturers' package inserts.
5. Add the new Reference Standard, USP Medroxyprogesterone Acetate Related Compound B RS, introduced by the proposed UHPLC procedure for the *Assay* and the test for *Organic Impurities*, to the *USP Reference Standards* section. Add existing USP Reference Standards, USP Medroxyprogesterone Acetate Related Compound A RS and USP Megestrol Acetate RS, to the *USP Reference Standards* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: D.A. Porter.)

Correspondence Number—C115256

Comment deadline: July 31, 2015

Medroxyprogesterone Acetate Tablets

DEFINITION

Medroxyprogesterone Acetate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of medroxyprogesterone acetate (C₂₄H₃₄O₄).

IDENTIFICATION

- **A. Infrared Absorption** (197K)

Sample: Triturate a number of Tablets, equivalent to about 25 mg of medroxyprogesterone acetate, with 15 mL of chloroform. Filter, evaporate the chloroform on a steam bath, and dry the residue at 105° for 3 h.

Acceptance criteria: Meet the requirements

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

ASSAY

Change to read:

- **Procedure**

~~**Mobile phase:** Acetonitrile and water (40:60)~~

~~**Standard solution:** 1 mg/mL of USP Medroxyprogesterone Acetate RS in acetonitrile~~

~~**Sample solution:** Finely powder NLT 20 Tablets. Weigh a portion of the powder, equivalent to 25 mg of medroxyprogesterone acetate, into a 50 mL glass centrifuge tube. Transfer 25 mL of acetonitrile into the tube, shake to wet the powder thoroughly, sonicate for NLT 10 min, and centrifuge. Use the clear supernatant.~~

~~**Chromatographic system**~~

~~(See *Chromatography* (621), *System Suitability*.)~~

~~**Mode:** LC~~

~~**Detector:** UV 254 nm~~~~**Column:** 4 mm × 30 cm; packing L1~~~~**Flow rate:** 2 mL/min~~~~**Injection volume:** 10 µL~~~~**System suitability**~~~~**Sample:** Standard solution~~~~**Suitability requirements**~~~~**Tailing factor:** NMT 2~~~~**Relative standard deviation:** NMT 2.0%~~

■ **Solution A:** Acetonitrile, tetrahydrofuran, and water (150:50:800). Pass through a suitable filter of 0.2-µm pore size.

Solution B: Acetonitrile, tetrahydrofuran, and water (400:400:200). Pass through a suitable filter of 0.2-µm pore size.

Diluent: Acetonitrile and water (70:30)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
5.0	90	10
18.0	45	55
18.01	90	10
22.0	90	10

System suitability stock solution: 0.2 mg/mL each of USP Medroxyprogesterone Acetate RS and USP Megestrol Acetate RS prepared as follows. Add portions of each Reference Standard to a suitable volumetric flask. Add *Diluent* equivalent to 70% of the flask volume and sonicate if necessary. Dilute with *Diluent* to volume.

System suitability solution: 0.05 mg/mL each of USP Medroxyprogesterone Acetate RS and USP Megestrol Acetate RS in *Diluent* prepared from *System suitability stock solution*

Standard solution: 0.1 mg/mL of USP Medroxyprogesterone Acetate RS in *Diluent*. Sonicate to dissolve as needed. Pass through a suitable filter of 0.2-µm pore size.

Sample solution: Nominally 0.1 mg/mL of medroxyprogesterone acetate in *Diluent* prepared as follows. Transfer a portion from NLT 20 powdered Tablets to a suitable volumetric flask and add 70% of the flask volume of *Diluent*. Sonicate to dissolve as needed and dilute with *Diluent* to volume. Pass through a suitable filter of 0.2-µm pore size.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 3.0-mm × 15-cm; 1.8-µm packing L1

Column temperature: 55°

Flow rate: 0.7 mL/min

Injection volume: 4.0 µL

System suitability**Samples:** *System suitability solution* and *Standard solution*[Note—See *Table 2* for relative retention times.]**Suitability requirements****Resolution:** NLT 6.0 between megestrol acetate and medroxyprogesterone acetate peaks, *System suitability solution***Relative standard deviation:** NMT 4.0%, *Standard solution*

■ 1S (USP39)

Analysis**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak response from the *Sample solution* r_S peak response from the *Standard solution* C_S concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (mg/mL) C_U nominal concentration of medroxyprogesterone acetate in the *Sample solution* (mg/mL)**Acceptance criteria:** 93.0%–107.0%**PERFORMANCE TESTS**• **Dissolution** 〈 711 〉**Medium:** 0.5% Sodium lauryl sulfate; 900 mL**Apparatus 2:** 50 rpm**Time:** 45 min**Mobile phase:** Acetonitrile and water (60:40)**Sodium lauryl sulfate stock solution:** Transfer 180.0 g of sodium lauryl sulfate to a 2000-mL volumetric flask. Add 1500 mL of water and stir until dissolved. [Note—Several hours of stirring are required.] Dilute with water to volume.**Standard stock solution:** 70 mg of USP Medroxyprogesterone Acetate RS in 140 mL of *Sodium lauryl sulfate stock solution*. Dilute with water to 250 mL. [Note—It may be necessary to sonicate the solution to mix in the Reference Standard before diluting with water.] Prepare the *Standard stock solution* fresh daily.**Standard solution:** Transfer a 20-mL aliquot of *Standard stock solution* to a 1-L volumetric flask. Add 40 mL of *Sodium lauryl sulfate stock solution* and dilute with water to volume. This solution is stable for up to 7 days.**Sample solution:** Withdraw 15 mL of the solution under test and filter, discarding the first 5 mL of the filtrate.**Chromatographic system**(See *Chromatography* 〈 621 〉, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4-mm × 8-cm; packing L7**Flow rate:** 1.5 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of medroxyprogesterone acetate ($\text{C}_{24}\text{H}_{34}\text{O}_4$) dissolved using the responses from the *Standard solution* and *Sample solution*.

Tolerances: NLT 50% (Q) of the labeled amount of medroxyprogesterone acetate ($\text{C}_{24}\text{H}_{34}\text{O}_4$) is dissolved.

• **Uniformity of Dosage Units** 〈 905 〉

Procedure for content uniformity

Diluent: Alcohol and water (3:1)

Standard solution: 15 $\mu\text{g}/\text{mL}$ of USP Medroxyprogesterone Acetate RS in *Diluent*

Sample solution: Nominally 15 $\mu\text{g}/\text{mL}$ of medroxyprogesterone acetate in *Diluent* prepared as follows. Transfer 1 Tablet to a volumetric flask, dilute with *Diluent* to volume, and shake for 15 min. Filter and quantitatively dilute a portion of the filtrate as needed.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: Maximum at about 242 nm

Cell: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of medroxyprogesterone acetate ($\text{C}_{24}\text{H}_{34}\text{O}_4$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_{\bar{U}}$ absorbance of the *Sample solution*

$A_{\bar{S}}$ absorbance of the *Standard solution*

$C_{\bar{S}}$ concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* ($\mu\text{g}/\text{mL}$)

$C_{\bar{U}}$ nominal concentration of medroxyprogesterone acetate in the *Sample solution* ($\mu\text{g}/\text{mL}$)

Acceptance criteria: Meet the requirements

IMPURITIES

Add the following:

■ • **Organic Impurities**

Solution A, Solution B, Diluent, and Mobile phase: Proceed as directed in the *Assay*.

Standard stock solution: 0.2 mg/mL each of USP Medroxyprogesterone Acetate RS, USP Megestrol Acetate RS, and USP Medroxyprogesterone Acetate Related Compound B RS,

and 0.1 mg/mL of USP Medroxyprogesterone Acetate Related Compound A RS prepared as follows. Add portions of each Reference Standard to a suitable volumetric flask. Add *Diluent* equivalent to 70% of the flask volume and sonicate if necessary. Dilute with *Diluent* to volume.

Standard solution: 0.05 mg/mL each of USP Medroxyprogesterone Acetate RS, USP Megestrol Acetate RS, and USP Medroxyprogesterone Acetate Related Compound B RS, and 0.025 mg/mL of USP Medroxyprogesterone Acetate Related Compound A RS, in *Diluent* prepared from *Standard stock solution*. Pass through a suitable filter of 0.2- μ m pore size.

Sample solution: Nominally 5.0 mg/mL of medroxyprogesterone acetate prepared as follows. Add a portion from NLT 20 finely powdered Tablets to a suitable volumetric flask and add *Diluent* equivalent to 75% of the flask volume. Sonicate for about 10 min, then dilute with *Diluent* to volume. Pass through a suitable filter of 0.2- μ m pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector

UV 266 nm: For megestrol acetate, medroxyprogesterone acetate, and medroxyprogesterone related compound B

UV 282 nm: For medroxyprogesterone acetate related compound A

Column: 3.0-mm \times 15-cm; 1.8- μ m packing L1

Column temperature: 55 $^{\circ}$

Flow rate: 0.7 mL/min

Injection volume: 4.0 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times are listed in *Table 2*.]

Suitability requirements

Resolution: NLT 6.0 between megestrol acetate and medroxyprogesterone acetate peaks

Relative standard deviation: NMT 4.5% for the medroxyprogesterone acetate, megestrol acetate, and medroxyprogesterone acetate related compound B peaks; NMT 5.0% for the medroxyprogesterone acetate related compound A peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of megestrol acetate and medroxyprogesterone acetate related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of megestrol acetate or medroxyprogesterone acetate related compound B at 266 nm from the *Sample solution*

r_S peak response of each corresponding Reference Standard at 266 nm from the *Standard solution*

C_S concentration of each corresponding Reference Standard in the *Standard solution* (mg/mL)

C_U nominal concentration of medroxyprogesterone acetate in the *Sample solution* (mg/mL)

Calculate the percentage of medroxyprogesterone acetate related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of medroxyprogesterone acetate related compound A at 282 nm from the *Sample solution*

r_S peak response of each corresponding Reference Standard at 282 nm from the *Standard solution*

C_S concentration of each corresponding Reference Standard in the *Standard solution* (mg/mL)

C_U nominal concentration of medroxyprogesterone acetate in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any degradation product from the *Sample solution*

r_S peak response of medroxyprogesterone acetate from the *Standard solution*

C_S concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (mg/mL)

C_U nominal concentration of medroxyprogesterone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
6 β -Hydroxymedroxyprogesterone acetate ^a	0.57	1.0
Homomedroxyprogesterone 13 α -epimer ^b	0.80	1.0
Medroxyprogesterone acetate related compound B	0.92	1.0
Homomedroxyprogesterone acetate ^c	0.95	1.0
Megestrol acetate	0.96	1.0
6-Epimedroxyprogesterone acetate ^d	0.97	1.0
Hydroxyprogesterone acetate 6-methylene analog ^e	0.99	1.0
Medroxyprogesterone acetate	1.0	—
Medroxyprogesterone acetate related compound A	1.16	0.5
Any individual unspecified degradation product	—	1.0
Total impurities	—	1.5

^a 6 β -Hydroxy-6 α -methyl-3,20-dioxopregn-4-ene-17-yl acetate.

^b 6 α ,13 α -Dimethyl-13(17) α -homoandrost-4-en-3,17-dione.

^c 6 α ,13 β -Dimethyl-3,17-dioxo-13(17) α -homoandrost-4-en-13 α -yl acetate.

^d 6 β -Methyl-3,20-dioxopregn-4-ene-17-yl acetate.

e 6-Methylene-3,20-dioxopregn-4-ene-17-yl acetate.

■ 1S (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in well-closed containers.
- Store at controlled room temperature. ■ 1S (USP39)

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Medroxyprogesterone Acetate RS

- USP Medroxyprogesterone Acetate Related Compound A RS

6 α -Methyl-3,20-dioxopregnan-17-yl acetate.

C₂₄H₃₆O₄ 388.54

USP Medroxyprogesterone Acetate Related Compound B RS

17-Hydroxy-6 α -methylpregn-4-ene-3,20-dione.

C₂₂H₃₂O₃ 344.49

USP Megestrol Acetate RS

6-Methyl-3,20-dioxopregna-4,6-diene-17-yl acetate.

C₂₄H₃₂O₄ 384.51

■ 1S (USP39)

BRIEFING

Mesna, USP 38 page 4273. In preparation for the omission of the flame tests from general chapter *Identification Tests—General* 〈 191 〉, proposed in PF 41(2) [Mar.–Apr. 2015], it is proposed to replace the use of the flame test in *Identification* test B with the pyroantimonate precipitation test currently described in the general chapter. This test is also consistent with test A in section 2.3.1—*Identification Reactions of Ions and Functional Groups* in the *European Pharmacopoeia*, and is employed in the EP monograph for *Mesna*. In addition, it is proposed to make the following changes on the basis of comments received:

1. Delete the third chemical name for Mesna.
2. Revise the *Diluent* used in the preparation of *Sulfate standard solution* in the test for *Limit of Sulfate* to address the solubility issue. The proposed *Diluent* is consistent with that in the EP monograph for *Mesna*.
3. Add *Sample solution* to the pH test. The proposed *Sample solution* is consistent with that in the EP monograph for *Mesna*.
4. Definitions of the concentration of *Sample solution* in the test for *Organic Impurities* are clarified by removing “nominal.”
5. The relative response factor for individual unspecified impurities is clarified.
6. Update all chemical names for impurities to be consistent with USAN naming convention, and include the appropriate acronyms. The molecular weights for mesna

related compound A and mesna related compound B are also updated.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

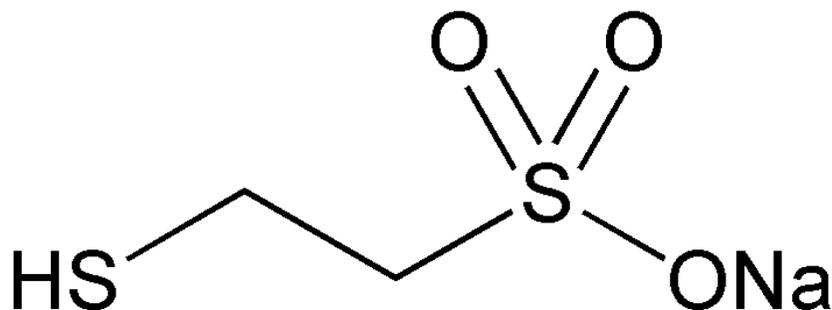
(SM2: H. Cai.)

Correspondence Number—C149855

Comment deadline: July 31, 2015

Mesna

Change to read:



$C_2H_5NaO_3S_2$ 164.18

Ethanesulfonic acid, 2-mercapto-, monosodium salt;

Sodium 2-mercaptoethanesulfonate

Sodium 2-sulphanylethanesulfonate

■ ■ 1S (USP39)

[19767-45-4].

DEFINITION

Mesna contains NLT 96.0% and NMT 102.0% of mesna ($C_2H_5NaO_3S_2$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈 197K 〉

Delete the following:

- ● **B. Identification Tests—General, Sodium** 〈 191 〉: A solution meets the requirements of the flame test. ■ 1S (USP39)

Add the following:

- ● **B. Identification Tests—General** 〈 191 〉, *Sodium*
Acceptance criteria: Meet the requirements ■ 1S (USP39)

ASSAY● **Procedure**

Sample solution: 120 mg of Mesna in 10 mL of water

Analysis: To the *Sample solution* add 10 mL of 1 M sulfuric acid and 10 mL of 0.1 N iodine VS. Titrate with 0.1 N sodium thiosulfate VS, adding 1 mL of starch TS near the endpoint.

Perform a blank determination, and make any necessary corrections (see *Titrimetry* { 541 }). Each mL of sodium thiosulfate is equivalent to 16.42 mg of mesna ($C_2H_5NaO_3S_2$).

Acceptance criteria: 96.0%–102.0% on the dried basis

IMPURITIES● **Limit of Chloride**

Chloride standard solution: 8.24 $\mu\text{g/mL}$ of sodium chloride in water

Sample solution: 200 mg/mL of Mesna in carbon dioxide-free water

Analysis: To 1 mL of the *Sample solution* and 15 mL of water add 1 mL of 2 M nitric acid.

Add the resulting solution to 1 mL of silver nitrate solution (17 g in 1000 mL), and allow to stand for 5 min, protected from light. To 10 mL of the *Chloride standard solution* add 5 mL of water and 1 mL of 2 M nitric acid. To this solution add 1 mL of silver nitrate solution (17 g in 1000 mL) and allow to stand for 5 min, protected from light. When viewed against a dark background, the *Sample solution* is not more turbid than the *Chloride standard solution*.

Acceptance criteria: NMT 250 ppm

Change to read:● **Limit of Sulfate**

~~**Sulfate standard solution:** 1.81 mg/mL of potassium sulfate in alcohol. Immediately before use, dilute with alcohol to 100 times its volume, and mix.~~

■ **Diluent:** 30% (v/v) ethanol in water

Sulfate standard stock solution: 1.81 mg/mL of potassium sulfate in *Diluent*

Sulfate standard solution: 0.0181 mg/mL of potassium sulfate in *Diluent*, prepared immediately before use from *Sulfate standard stock solution* ■ 1S (USP39)

Sample solution: Add 5.0 mL of the *Sample solution* prepared as directed in the test for *Limit of Chloride* to a 30-mL volumetric flask, and dilute with water to volume.

Analysis: Add 3 mL of a 250-g/L solution of barium chloride to 4.5 mL of *Sulfate standard solution*. Shake and allow to stand for 1 min. To 2.5 mL of this solution add 15 mL of the *Sample solution* and 0.5 mL of acetic acid. Use 15 mL of this mixture for comparison with 15 mL of the *Sulfate standard solution*, prepared in the same manner, but using the *Sulfate standard solution* instead of the *Sample solution*. After 5 min, any opalescence in the *Sample solution* is not more intense than that in the *Sulfate standard solution*.

Acceptance criteria: NMT 300 ppm

Delete the following:

●● **Heavy Metals, Method I** { 231 } : 10 ppm ● (Official 1-Dec-2015)

Change to read:● **Organic Impurities**

Mobile phase: In a 1000-mL volumetric flask dissolve 2.94 g of potassium dihydrogen

phosphate, 2.94 g of dipotassium hydrogen phosphate, and 2.6 g of tetrabutylammonium hydrogen sulfate in about 600 mL of water. Adjust with phosphoric acid to a pH of 2.3, add 335 mL of methanol, and dilute with water to volume.

System suitability solution: 0.18 mg/mL and 0.004 mg/mL of USP Mesna RS and USP Mesna Related Compound A RS, respectively, in *Mobile phase*

Standard solution 1: 8 µg/mL and 120 µg/mL of USP Mesna Related Compound A RS and USP Mesna Related Compound B RS, respectively, in *Mobile phase*

Standard solution 2: 12 µg/mL of USP Mesna RS in *Mobile phase*

Sample solution: 4 mg/mL of Mesna in *Mobile phase*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 10-µm packing L1

Flow rate: 1 mL/min

Run time: Four times the elution time for mesna

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for mesna and mesna related compound A are about 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 3.0 between mesna and mesna related compound A

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

[Note—Identify the peaks using the relative retention times provided in *Table 1*.]

Calculate the percentage of mesna related compound A in the portion of Mesna taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mesna related compound A from the *Sample solution*

r_S = peak response of mesna related compound A from *Standard solution 1*

C_S = concentration of USP Mesna Related Compound A RS in *Standard solution 1* (mg/mL)

C_U = nominal

■ ■ 1S (USP39)

concentration of Mesna in the *Sample solution* (mg/mL)

Calculate the percentage of mesna related compound B in the portion of Mesna taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mesna related compound B from the *Sample solution*

r_S = peak response of mesna related compound B from *Standard solution 1*

C_S = concentration of USP Mesna Related Compound B RS in *Standard solution 1* (mg/mL)

C_U = nominal

■ ■ 1S (USP39)

concentration of Mesna in the *Sample solution* (mg/mL)

Calculate the percentage of any specified impurities (2-(Carbamimidoylsulphonyl)ethanesulfonic

~~acid; 2-[[[(Guanidino)(imino)methyl]sulphonyl]ethanesulfonic acid; and 2-(4,6-Diamino-1,3,5-triazin-2-yl)sulphonyl]ethanesulfonic acid)~~

■ (thiuronium ethanesulfonic acid, guanidinthiuronium ethanesulfonic acid, and mesna triazine analog) ■ 1S (USP39)

and any unspecified impurities in the portion of Mesna taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any specified or unspecified individual impurity from the *Sample solution*

r_S = peak response of mesna from *Standard solution 2*

C_S = concentration of USP Mesna RS in *Standard solution 2* (mg/mL)

C_U = nominal

■ ■ 1S (USP39)

■ concentration of Mesna in the *Sample solution* (mg/mL)

F = relative response factors (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-(Carbamimidoylsulfonyl)ethanesulfonic acid ■ Thiuronium ethanesulfonic acid ^a ■ 1S (USP39)	0.6	100	0.3
2-[[[(Guanidino)(imino)methyl]sulphonyl]ethanesulfonic acid ■ Guanidinthiuronium ethanesulfonic acid ^b ■ 1S (USP39)	0.6	100	0.3
2-(4,6-Diamino-1,3,5-triazin-2-yl)sulfonyl]ethanesulfonic acid ■ Mesna triazine analog ^c ■ 1S (USP39)	0.8	100	0.3
Mesna	1.0	—	—
Mesna related compound A ^a ■ ■ 1S (USP39)	1.4	—	0.2
Mesna related compound B ^b ■ ■ 1S (USP39)	2.3	—	3.0
Individual unspecified impurities	—	■ 1.0 ■ 1S (USP39)	0.1
Total unspecified impurities	—	—	0.3

- a ~~2-(Acetylsulfanyl)ethanesulfonic acid.~~
- b ~~2,2-(Disulfanediy)bis(ethanesulfonic acid).~~
-
- a 2-(Carbamimidoylthio)ethane-1-sulfonic acid.
- b 2-[(N-Carbamidoylcarbamimidoyl)thio]ethane-1-sulfonic acid.
- c 2-((4,6-Diamino-1,3,5-triazin-2-yl)thio)ethane-1-sulfonic acid.
- 1S (USP39)

SPECIFIC TESTS

- **Loss on Drying**

Sample: 1 g

Analysis: Dry the *Sample* under vacuum at a pressure not exceeding 1 mm of mercury at 60° over phosphorus pentoxide for 2 h.

Acceptance criteria: NMT 1.0%

Change to read:

- pH $\langle 791 \rangle$ 4.5–6.0

■ **Sample solution:** 100 mg/mL of Mesna in carbon dioxide-free water

Acceptance criteria: 4.5–6.0 ■ 1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in a tight container, and store at room temperature.

Change to read:

- **USP Reference Standards $\langle 11 \rangle$**

USP Mesna RS

USP Mesna Related Compound A RS

~~2-(Acetylsulfanyl)ethanesulfonic acid.~~

■ 2-(Acetylthio)ethane-1-sulfonic acid. ■ 1S (USP39)

$C_4H_8O_4S_2$ — 184.23

■ 184.22 ■ 1S (USP39)

USP Mesna Related Compound B RS

~~2,2-(Disulfanediy)bis(ethanesulfonic acid).~~

■ 2,2'-Disulfanediybis(ethane-1-sulfonic acid). ■ 1S (USP39)

$C_4H_{10}O_6S_4$ — 282.38

■ 282.36 ■ 1S (USP39)

BRIEFING

Methylprednisolone Sodium Succinate, *USP 38* page 4356. In preparation for the omission of the flame tests from *Identification Tests—General* 〈191〉, proposed in *PF 41(2)* [Mar.—Apr. 2015], it is proposed to delete the reference to 〈191〉 in *Identification* test *C* and include a complete description of the flame test in the monograph. Manufacturers are encouraged to submit a replacement wet chemistry or an instrumental procedure for consideration by the Expert Committee.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: M. Koleck.)

Correspondence Number—C154900

Comment deadline: July 31, 2015

Methylprednisolone Sodium Succinate

$C_{26}H_{33}NaO_8$ 496.53

Pregna-1,4-diene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-11,17-dihydroxy-6-methyl-, monosodium salt, (6 α ,11 β)-;

11 β ,17,21-Trihydroxy-6 α -methylpregna-1,4-diene-3,20-dione 21-(sodium succinate) [2375-03-3].

DEFINITION

Methylprednisolone Sodium Succinate contains NLT 97.0% and NMT 103.0% of methylprednisolone sodium succinate ($C_{26}H_{33}NaO_8$), calculated on the dried basis.

IDENTIFICATION

● **A. Infrared Absorption**

Sample: 100 mg of Methylprednisolone Sodium Succinate

Analysis: Transfer the *Sample* to a separator, dissolve in 10 mL of water, add 1 mL of 3 N hydrochloric acid, and extract immediately with 50 mL of chloroform. Filter the chloroform extract through cotton, evaporate on a steam bath to dryness, and dry under vacuum at 60° for 3 h.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the residue so obtained exhibits maxima only at the same wavelengths as those of a similar preparation of USP Methylprednisolone Hemisuccinate RS.

● **B. Ultraviolet Absorption** 〈197U〉

Standard solution: 20 μ g/mL of USP Methylprednisolone Hemisuccinate RS in methanol

Sample solution: 20 μ g/mL of Methylprednisolone Sodium Succinate in methanol

Analytical wavelength: 243 nm

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

Delete the following:

- **C. Identification Tests—General, Sodium** 〈191〉: Meets the requirements of the flame test ■1S (*USP39*)

Add the following:

- C. The sample imparts an intense yellow color to a nonluminous flame. ■1S (USP39)

ASSAY● **Procedure**

Solution A: 5 mg/mL of blue tetrazolium in alcohol

Solution B: Alcohol and tetramethylammonium hydroxide TS (9:1)

Standard solution: Proceed as directed for *Assay for Steroids* (351), *Standard Preparation*, preparing 12.5 µg/mL of USP Methylprednisolone Hemisuccinate RS in alcohol.

Sample solution: 12.5 µg/mL of Methylprednisolone Sodium Succinate in alcohol

Blank: Alcohol

Instrumental conditions

Mode: Vis

Analytical wavelength: 525 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Pipet 20.0 mL of the *Blank*, *Standard solution*, and *Sample solution* into three different glass-stoppered, 50-mL conical flasks. Add 2.0 mL of *Solution A*, and mix. To each flask add 4.0 mL of *Solution B*. Mix, and allow to stand in the dark for 90 min. Add 1.0 mL of glacial acetic acid, and mix. Without delay, determine the absorbances of the *Standard solution* and the *Sample solution* against the *Blank*.

Calculate the percentage of methylprednisolone sodium succinate (C₂₆H₃₃NaO₈) in the portion of Methylprednisolone Sodium Succinate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Methylprednisolone Hemisuccinate RS in the *Standard solution* (µg/mL)

C_U = concentration of Methylprednisolone Sodium Succinate in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of methylprednisolone sodium succinate, 496.53

M_{r2} = molecular weight of methylprednisolone hemisuccinate, 474.54

Acceptance criteria: 97.0%–103.0% on the dried basis

OTHER COMPONENTS● **Sodium Content**

Sample solution: Dissolve, with gentle heating, about 1 g of Methylprednisolone Sodium Succinate in 75 mL of glacial acetic acid. Add 20 mL of dioxane, then add 1 drop of crystal violet TS.

Titrimetric system

Mode: Direct

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: Titrate with *Titrant* to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 2.299 mg of sodium (Na).

Acceptance criteria: 4.49%–4.77% on the dried basis

SPECIFIC TESTS

- **Optical Rotation** 〈 781S 〉, *Specific Rotation*

Sample solution: 10 mg/mL of Methylprednisolone Sodium Succinate in alcohol

Acceptance criteria: +96° to +104°

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 3.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

- **USP Reference Standards** 〈 11 〉

USP Methylprednisolone Hemisuccinate RS

BRIEFING

Metoprolol Succinate, *USP 38* page 4370. As part of the USP monograph modernization effort, it is proposed to make the following changes:

1. Replace the existing *Organic Impurities, Procedure 1* by TLC with a hydrophilic interaction chromatograph (HILIC) method coupled with a charged aerosol detector (CAD) for the organic impurities without UV chromophore. The HILIC procedure is based on analysis performed with the Halo Penta-HILIC brand of L## column manufactured by Advanced Materials Technologies. The typical retention time for the metoprolol peak in the test is 2.3 min.
2. Add the required *A* designation to the existing *Identification* test by FT-IR. Add *Identification* test *B* based on the retention time agreement in the *Assay*.
3. Delete the test for *Clarity and Color of Solution* as it is non-value added.
4. Two new Reference Standards are added to the *USP Reference Standards* section to support the proposed revision in *Impurities*.

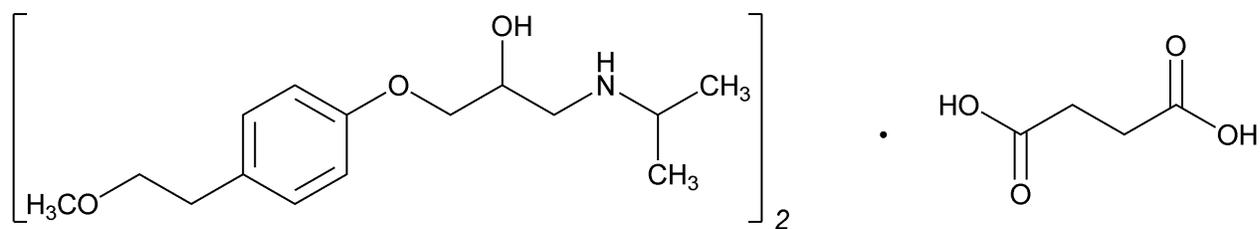
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: D. Min.)

Correspondence Number—C142873

Comment deadline: July 31, 2015

Metoprolol Succinate



$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$ 652.82

2-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, (\pm)-, butanedioate (2:1) (salt); (\pm)-1-(Isopropylamino)-3-[*p*-(2-methoxyethyl)phenoxy]-2-propanol succinate (2:1) (salt) [98418-47-4].

DEFINITION

Metoprolol Succinate contains NLT 98.0% and NMT 102.0% of metoprolol succinate [$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$], calculated on the dried basis.

IDENTIFICATION

Change to read:

•

■ A. ■ 1S (USP39)

Infrared Absorption \langle 197K \rangle

Add the following:

- • B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

ASSAY

• Procedure

Solution A: 1.3 mg/mL of sodium dodecyl sulfate in aqueous phosphoric acid, 0.1% (w/v)

Mobile phase: Acetonitrile and *Solution A* (40:60)

System suitability solution: 5 μ g/mL each of USP Metoprolol Succinate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, USP Metoprolol Related Compound C RS, and USP Metoprolol Related Compound D RS in *Mobile phase*

Standard solution: 0.08 mg/mL of USP Metoprolol Succinate RS in *Mobile phase*

Sample stock solution: 0.8 mg/mL of Metoprolol Succinate in *Mobile phase*

Sample solution: 0.08 mg/mL of Metoprolol Succinate from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* \langle 621 \rangle , *System Suitability*.)

Mode: LC

Detector: UV 223 nm

Column: 4-mm \times 12.5-cm; 4- μ m packing L7

Column temperature: 30°

Flow rate: 0.9 mL/min

Injection volume: 10 µL

Run time: NLT 1.5 times the retention time of the metoprolol peak

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for metoprolol related compound C, metoprolol related compound B, metoprolol related compound A, and metoprolol are 0.6, 0.7, 0.8, and 1.0, respectively. The relative retention times for the two diastereomers of metoprolol related compound D are 5.0 and 5.2.]

Suitability requirements

Resolution: NLT 2.5 between metoprolol related compound A and metoprolol related compound B; NLT 1.5 between metoprolol related compound B and metoprolol related compound C, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of metoprolol succinate [(C₁₅H₂₅NO₃)₂·C₄H₆O₄] in the portion of Metoprolol Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Metoprolol Succinate RS in the *Standard solution* (mg/mL)

C_U = concentration of Metoprolol Succinate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

Delete the following:

- **Heavy Metals, Method I** (231): NMT 10 ppm • (Official 1-Dec-2015)

Delete the following:

- **Organic Impurities, Procedure 1**

Sample solution: 50 mg/mL of Metoprolol Succinate in methanol

Standard solution: 0.1 mg/mL of Metoprolol Succinate from *Sample solution* in methanol

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Ethyl acetate and methanol (80:20)

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter. Place two 50 mL beakers, each containing 30 mL of ammonium hydroxide, on the bottom of a chromatographic chamber that is lined with filter paper and contains the *Developing solvent system*, and allow to equilibrate for 1 h. Position the plate in the chromatographic chamber, and develop the chromatogram until the solvent front has moved about two thirds of the length of the plate. Remove the plate from the chamber, mark the solvent front, and dry the plate for 3 h in a current of warm air. Place the plate in a chamber containing iodine vapor, and allow to react for at least 15 h. Compare the intensities of the brown spots appearing on the chromatogram.

Acceptance criteria: Any secondary spot obtained from the *Sample solution* is not more intense than the corresponding spot obtained from the *Standard solution*. NMT 0.2%.

■ 1S (USP39)

Add the following:**■ • Content of Metoprolol Related Compound H and Metoprolol Related Compound I**

Buffer: 6.31 g/L of ammonium formate in water. Adjust with formic acid to a pH of 3.2.

Pass the solution through a suitable filter of 0.22- μ m pore size.

Mobile phase: Acetonitrile and *Buffer* (85:15)

Diluent: Acetonitrile and water (85:15)

System suitability solution: 0.1 mg/mL of USP Metoprolol Succinate RS and 0.01 mg/mL each of USP Metoprolol Related Compound H RS and USP Metoprolol Related Compound I RS in *Diluent*

Standard solution: 2 μ g/mL each of USP Metoprolol Related Compound H RS and USP Metoprolol Related Compound I RS in *Diluent*

Sample solution: 2 mg/mL of Metoprolol Succinate in *Diluent*

[Note—Sonication may be needed to aid dissolution.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Charged aerosol

Nitrogen pressure: 35 psi

Range: 100 pA

Column: 4.6-mm \times 15-cm; 5- μ m packing L##

Flow rate: 0.8 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between metoprolol related compound H and metoprolol related compound I, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of metoprolol related compound H and metoprolol related compound I in the portion of Metoprolol Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of the corresponding impurity from the *Sample solution*

r_S peak response of the corresponding impurity from the *Standard solution*

C_S concentration of the corresponding USP Impurity RS in the *Standard solution* (mg/mL)

C_U concentration of Metoprolol Succinate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Metoprolol ^a	1.0	—
Succinic acid ^a	1.5	—
Metoprolol related compound I ^b	2.7	0.2
Metoprolol related compound H ^c	3.0	0.2
a Included for identification only.		
b Isopropylaminoglycerol.		
c Bisopropylaminoglycerol.		

■ 1S (USP39)

Change to read:

- **Organic Impurities, Procedure 2**

■ 1S (USP39)

Solution A, Mobile phase, System suitability solution, and Chromatographic system:

Proceed as directed in the *Assay*.

Standard solution: 1.0 µg/mL of USP Metoprolol Succinate RS in *Mobile phase*

Sample solution: 1 mg/mL of Metoprolol Succinate in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for metoprolol related compound C, metoprolol related compound B, metoprolol related compound A, metoprolol are 0.6, 0.7, 0.8, 1.0, respectively. The relative retention times for the two diastereomers of metoprolol related compound D are 5.0 and 5.2.]

Suitability requirements

Resolution: NLT 2.5 between metoprolol related compound A and metoprolol related compound B; NLT 1.5 between metoprolol related compound B and metoprolol related compound C, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Metoprolol Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U individual peak response of related impurities from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Metoprolol Succinate RS in the *Standard solution* (mg/mL)

C_U concentration of Metoprolol Succinate in the *Sample solution* (mg/mL)

[Note—The sum of the peak responses for the two diastereomers of metoprolol related compound D is used in the above calculation to report the amount of metoprolol related compound D.]

Acceptance criteria

Individual impurities: NMT 0.1% of any single impurity

Total impurities: NMT 0.5%

SPECIFIC TESTS

- **pH** 〈 791 〉

Sample solution: 65 mg/mL

Acceptance criteria: 7.0–7.6

Delete the following:

- ~~**Clarity and Color of Solution**~~

~~**Sample solution:** 20 mg/mL of Metoprolol Succinate in water~~

~~**Instrumental conditions**~~

~~**Mode:** UV-Vis~~

~~**Analytical wavelength:** 440 nm~~

~~**Cell:** 5-cm~~

~~**Blank:** Water~~

~~**Acceptance criteria 1:** The *Sample solution* is not less clear than water.~~

~~**Acceptance criteria 2:** The absorbance of the *Sample solution* is NMT 0.1. ■ 1S (USP39)~~

- **Loss on Drying** 〈 731 〉

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers at controlled room temperature.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Metoprolol Related Compound A RS

(±)1-(Ethylamino)-3-[4-(2-methoxyethyl)phenoxy]-propan-2-ol.

C₁₄H₂₃NO₃ 253.34

USP Metoprolol Related Compound B RS

(±)1-Chloro-2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]-propane.

$C_{12}H_{17}ClO_3$ 244.71

USP Metoprolol Related Compound C RS

(±)4-[2-Hydroxy-3-(1-methylethyl)aminopropoxy]benzaldehyde.

$C_{13}H_{19}NO_3$ 237.29

USP Metoprolol Related Compound D RS

(±) *N,N*-Bis[2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl](1-methylethyl)amine.

$C_{27}H_{41}NO_6$ 475.62

■ USP Metoprolol Related Compound H RS

1,3-Bis(Isopropylamino)propan-2-ol.

$C_9H_{22}N_2O$ 174.29

USP Metoprolol Related Compound I RS

3-(Isopropylamino)propane-1,2-diol.

$C_6H_{15}NO_2$ 133.19 ■ 1S (USP39)

USP Metoprolol Succinate RS

BRIEFING

Metoprolol Succinate Extended-Release Tablets, *USP 38* page 4371. As part of the USP monograph modernization effort, it is proposed to revise the monograph as follows:

1. Add the test for *Organic Impurities* to the monograph. The proposed HPLC procedure in the test for *Organic Impurities* was validated using the Agilent Zorbax StableBond C8 brand of L7 column in which metoprolol elutes at about 8 min.
2. Add *Identification* test C by retention time agreement in the *Assay*.
3. Add the pore size of the HPLC column used in the test for *Uniformity of Dosage Units* based on the original submission document.
4. Add three new Reference Standards to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: D. Min.)

Correspondence Number—C143421

Comment deadline: July 31, 2015

Metoprolol Succinate Extended-Release Tablets

DEFINITION

Metoprolol Succinate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metoprolol succinate [$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$].

IDENTIFICATION

● A. Infrared Absorption { 197K }

Sample solution: Equivalent to 200 mg of metoprolol succinate from 1 or more Tablets in a stoppered centrifuge tube. Add 40 mL of pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*) and 40 mL of methylene chloride, and shake

for 5 min. Centrifuge, filter, and use the aqueous phase as the *Sample solution*.

Sample: Transfer 3 mL of the *Sample solution* to a separator. Add 2 mL of ammonium hydroxide, and extract with 20 mL of methylene chloride. Filter the methylene chloride phase. Grind 1 mL of the filtrate with 300 mg of potassium bromide, dry in a current of warm air, and prepare a disk.

Acceptance criteria: The IR spectrum of the *Sample* exhibits maxima only at the same wavelengths as those obtained from a similar preparation of USP Metoprolol Succinate RS (presence of metoprolol).

• **B. Infrared Absorption** 〈 197K 〉

Sample: Transfer 5 mL of the *Sample solution* prepared in *Identification* test A to a glass-stoppered test tube. Add 2 mL of 5 N hydrochloric acid, and extract with 5 mL of ether. Filter the ether phase. Grind 2 mL of the filtrate with 300 mg of potassium bromide, dry in a current of warm air, and prepare a disk.

Acceptance criteria: The IR spectrum of the *Sample* exhibits maxima only at the same wavelengths as those obtained from a similar preparation of succinic acid (presence of succinate).

Add the following:

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

ASSAY

• **Procedure**

Analysis: Determine the mean percentage value of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ from the Tablets analyzed in the test for *Uniformity of Dosage Units*.

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** 〈 711 〉

Test 1

Medium: pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 500 mL

Apparatus 2: 50 rpm

Times: 1, 4, 8, and 20 h

Buffer, Mobile phase, and Standard solution: Proceed as directed in the test for *Uniformity of Dosage Units*.

Analysis: Proceed as directed in the test for *Uniformity of Dosage Units*, except use 5.0 mL of a filtered portion of the solution under test as the *Sample solution*, and use *Medium* as the blank, in comparison with a *Standard solution* with a known concentration of USP Metoprolol Succinate RS in the same *Medium*.

Acceptance criteria: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
1	NMT 25

Time (h)	Amount Dissolved (%)
4	20–40
8	40–60
20	NLT 80

The percentages of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ dissolved at the times specified conform to *Dissolution* $\langle 711 \rangle$, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: Simulated gastric fluid without enzyme, pH 1.2; 500 mL

Apparatus 2: 75 rpm

Times: 1, 4, 8, and 20 h

Buffer: 1 M monobasic sodium phosphate, 1 M phosphoric acid, and water (50:8:942). If necessary, adjust with 1 M monobasic sodium phosphate or 1 M phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (250:750)

Standard solution: Prepare a solution of USP Metoprolol Succinate RS in *Medium* as directed in *Table 2*.

Table 2

Tablet Strength (mg as metoprolol succinate)	Concentration (mg/mL)
200	0.380
100	0.190
50	0.095
25	0.048

Sample solution: Pass the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm \times 12.5-cm; 4- μ m packing L7

Flow rate: 1 mL/min

Injection volume: See *Table 3*.

Table 3

Tablet Strength (mg as metoprolol succinate)	Volume (μ L)
25	40
50	20
100	10
200	5

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1500 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i), in mg/mL, of metoprolol succinate dissolved in *Medium* at each time point (i):

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U peak response of metoprolol from the *Sample solution*

r_S peak response of metoprolol from the *Standard solution*

C_S concentration of USP Metoprolol Succinate RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ dissolved (Q_i), at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i concentration of metoprolol succinate in the portion of sample withdrawn at time point (i) (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

V_S volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 20
2	4	20–40
3	8	55–85
4	20	NLT 80

The percentages of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ dissolved at the times specified conform to *Dissolution* { 711 }, *Acceptance Table 2*.

Change to read:

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

Procedure for content uniformity

Buffer: Mix 50 mL of 1 M monobasic sodium phosphate and 8.0 mL of 1 M phosphoric acid, and dilute with water to 1000 mL. If necessary, adjust with 1 M monobasic

potassium phosphate or 1 M phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (250:750)

Standard solution: 0.05 mg/mL of USP Metoprolol Succinate RS in *Mobile phase*

Sample stock solution: Nominally 1 mg/mL of metoprolol succinate prepared as follows.

Transfer 1 Tablet to a suitable volumetric flask, add about 5 mL of water, and allow the Tablet to disintegrate. Add a volume of alcohol to fill 30% of the flask volume, and shake for 30 min. Add a portion of 0.1 N hydrochloric acid to fill 50% of the flask volume, and shake for an additional 30 min. Dilute with 0.1 N hydrochloric acid to volume. Filter, and discard the first 10 mL of the filtrate.

Sample solution: Nominally 0.05 mg/mL of metoprolol succinate from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4-mm × 12.5-cm;

■ 5- μ m ■ 1S (USP39)

packing L7

Flow rate: 1 mL/min

Injection volume: 40 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metoprolol succinate

[(C₁₅H₂₅NO₃)₂·C₄H₆O₄] in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of metoprolol from the *Sample solution*

r_S peak response of metoprolol from the *Standard solution*

C_S concentration of USP Metoprolol Succinate RS in the *Standard solution* (mg/mL)

C_U nominal concentration of metoprolol succinate in the *Sample solution* (mg/mL)

IMPURITIES

Add the following:

■ • Organic Impurities

Solution A: 1.3 g/L of sodium dodecyl sulfate in 0.1% (w/v) phosphoric acid

Mobile phase: Acetonitrile and *Solution A* (40:60)

System suitability solution: 5 μ g/mL each of USP Metoprolol Succinate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, and USP Metoprolol Related Compound C RS in *Mobile phase*

Standard solution: 2.5 μ g/mL each of USP Metoprolol Succinate RS and USP Metoprolol Related Compound C RS in *Mobile phase*

Sample solution: Nominally 1 mg/mL of metoprolol succinate from Tablets prepared as follows. Transfer a portion of finely powdered Tablets (NLT 20), equivalent to 50 mg of metoprolol succinate, to a 50-mL volumetric flask. Dilute with *Mobile* phase to the volume and sonicate for 20 min. Centrifuge. Use the supernatant.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 223 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The run time is 1.3 times the retention time of the metoprolol peak.]

Suitability requirements

Resolution: NLT 1.5 between metoprolol related compound A and metoprolol related compound B; NLT 2.5 between metoprolol related compound B and metoprolol related compound C, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of metoprolol related compound C in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of metoprolol related compound C from the *Sample solution*

r_S peak response of metoprolol from the *Standard solution*

C_S concentration of USP Metoprolol Related Compound C RS in the *Standard solution* (μg/mL)

C_U nominal concentration of metoprolol succinate in the *Sample solution* (μg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each unspecified impurity from the *Sample solution*

r_S peak response of metoprolol from the *Standard solution*

C_S concentration of USP Metoprolol Succinate RS in the *Standard solution* (μg/mL)

C_U nominal concentration of metoprolol succinate in the *Sample solution* (μg/mL)

Acceptance criteria: See *Table 5*. Disregard peaks below 0.1%.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Metoprolol related compound C ^a	0.64	0.2
Metoprolol related compound B ^{b,c}	0.74	—
Metoprolol related compound A ^{c,d}	0.83	—
Metoprolol	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities	—	0.5
a (±)-4-[2-Hydroxy-3-(isopropylamino)propoxy]benzaldehyde.		

b (±)-1-Chloro-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.

c Process impurities controlled in the drug substance. Included for identification only.

d (±)-1-(Ethylamino)-3-[4-(2-methoxyethyl)phenoxy]-propan-2-ol.

■ 1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
- **Labeling:** Label it to indicate the content of metoprolol succinate and its equivalent, expressed as metoprolol succinate [(C₁₅H₂₅NO₃)₂·C₄H₆O₆]. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

- **USP Reference Standards** < 11 >

■ USP Metoprolol Related Compound A RS

(±)-1-(Ethylamino)-3-[4-(2-methoxyethyl)phenoxy]-propan-2-ol.

C₁₄H₂₃NO₃ 253.34

USP Metoprolol Related Compound B RS

(±)-1-Chloro-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.

C₁₂H₁₇ClO₃ 244.71

USP Metoprolol Related Compound C RS

(±)-4-[2-Hydroxy-3-(isopropylamino)propoxy]benzaldehyde.

C₁₃H₁₉NO₃ 237.29

■ 1S (USP39)

USP Metoprolol Succinate RS

BRIEFING

Oxcarbazepine Tablets, *USP 38* page 4689. It is proposed to revise the preparation of the *Standard solution* concentration in *Dissolution Test 1* to allow the use of a single standard

solution for all strengths.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R. Ravichandran.)

Correspondence Number—C157607

Comment deadline: July 31, 2015

Oxcarbazepine Tablets

DEFINITION

Oxcarbazepine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$).

IDENTIFICATION

- **A. Infrared Absorption** { 197K }

Sample: Weigh about 840 mg of crushed Tablet powder, and add to a 50-mL volumetric flask. Add 45 mL of chloroform, and shake the flask for 30 min on a mechanical shaker. Add chloroform to volume, centrifuge, and collect the supernatant in a Petri dish.

Evaporate the supernatant on a water bath at 60°. Dry the residue, and crush the residue thoroughly with potassium bromide in the ratio of 1:100.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Add 2 mL of triethylamine. Adjust with phosphoric acid to a pH of 6.0.

Diluent: Methanol and water (80:20)

Mobile phase: Methanol, acetonitrile, and *Buffer* (22:16:62)

Standard stock solution: 0.5 mg/mL of USP Oxcarbazepine RS in *Diluent*. Sonication may be used to aid in dissolution.

Standard solution: 0.1 mg/mL of USP Oxcarbazepine RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: Nominally equivalent to 1.2 mg/mL of oxcarbazepine from a portion of finely powdered (NLT 20) Tablets, prepared as follows. Transfer a weighed quantity of powdered Tablets, equivalent to 600 mg of oxcarbazepine, to a 500-mL volumetric flask. Add *Diluent* to fill 50% of the final volume. Sonicate for 15 min with intermittent swirling, cool to room temperature, and dilute with *Diluent* to volume. Pass this solution through a suitable 2- μ m glass filter, and discard the first portion of the filtrate.

Sample solution: 0.1 mg/mL of oxcarbazepine in *Mobile phase* from a portion of the filtrate obtained from the *Sample stock solution*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Temperatures

Sample: 5°

Column: 50°

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxcarbazepine (C₁₅H₁₂N₂O₂) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)

C_U nominal concentration of oxcarbazepine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** 〈 711 〉

Test 1

Medium

For Tablets labeled to contain 150 mg: 0.3% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated

For Tablets labeled to contain 300 mg: 0.6% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated

For Tablets labeled to contain 600 mg: 1.0% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated

Apparatus 2: 60 rpm

Times: 30 and 60 min

Standard stock solution: 0.35 mg/mL of USP Oxcarbazepine RS in methanol

Standard solution: Dilute the *Standard stock solution* with the corresponding *Medium* according to the Tablet strength, to obtain a final concentration of $(L/900)$ µg/mL, where L is the label claim in mg/Tablet.

■ to obtain a final concentration of 0.0175 mg/mL of USP Oxcarbazepine RS. ■ 1S (USP39)

Sample solution: Use portions of the solution under test passed through a suitable filter of

0.45- μm pore size. The volume of the solution under test withdrawn must be replaced by the same volume of corresponding *Medium*. Dilute with the appropriate *Medium* if necessary, according to the Tablet strength, to obtain a final concentration similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 256 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxcarbazepine ($\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}_2$) dissolved at 30 min (Q_{30}):

$$Q_{30} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

Calculate the percentage of oxcarbazepine ($\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}_2$) dissolved at 60 min (Q_{60}):

$$Q_{60} = [(A_U/A_S) \times (C_S/L) \times D \times V \times 100] + [Q_{30} \times (V_S/V)]$$

$A_{\bar{U}}$ absorbance of the *Sample solution*

$A_{\bar{S}}$ absorbance of the *Standard solution*

$C_{\bar{S}}$ concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

D = dilution factor of the *Sample solution*

V = volume of *Medium*, 900 mL

V_S = volume of the solution under test withdrawn (mL)

Tolerances: NLT 70% (Q) of the labeled amount of oxcarbazepine is dissolved in 30 min, and NLT 80% (Q) of the labeled amount of oxcarbazepine is dissolved in 60 min.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium

For Tablets labeled to contain 150 mg: 0.3% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated

For Tablets labeled to contain 300 mg: 0.6% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated

For Tablets labeled to contain 600 mg: 1.0% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated

Apparatus 2: 60 rpm

Times: 30 and 60 min

Standard stock solution: 3.3 mg/mL of USP Oxcarbazepine RS in methanol. [Note—This solution is stable for 22 h at 10 $^{\circ}$.]

Standard solution: Dilute the *Standard stock solution* with the corresponding *Medium*, according to the Tablet strength, to obtain a final concentration of ($L/900$)

•mg/mL, where L is the label claim in mg/Tablet. •(ERR 1-Jun-2014)

Sample solution: Use portions of the solution under test passed through a suitable filter of 0.45- μm pore size. The volume of the solution under test withdrawn must be replaced by the same volume of corresponding *Medium*.

Instrumental conditions**Mode:** UV-Vis**Analytical wavelength:** 304 nm**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of oxcarbazepine ($C_{15}H_{12}N_2O_2$) dissolved at 30 min (Q_{30}):

$$Q_{30} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

Calculate the percentage of oxcarbazepine ($C_{15}H_{12}N_2O_2$) dissolved at 60 min (Q_{60}):

$$Q_{60} = [(A_U/A_S) \times (C_S/L) \times D \times V \times 100] + [Q_{30} \times (V_S/V)]$$

 A_U absorbance of the *Sample solution* A_S absorbance of the *Standard solution* C_S concentration of the *Standard solution* (mg/mL) L = label claim (mg/Tablet) D = dilution factor of the *Sample solution* V = volume of *Medium*, 900 mL V_S volume of the solution under test withdrawn (mL)**Tolerances**

For Tablets labeled to contain 150 or 300 mg: NLT 70% (Q) of the labeled amount of oxcarbazepine is dissolved in 30 min, and NLT 80% (Q) of the labeled amount of oxcarbazepine is dissolved in 60 min.

For Tablets labeled to contain 600 mg: NLT 50% (Q) of the labeled amount of oxcarbazepine is dissolved in 30 min, and NLT 80% (Q) of the labeled amount of oxcarbazepine is dissolved in 60 min.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

[Note—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. If methoxydibenzazepine is a potential degradation product, *Procedure 1* is recommended. If carbamazepinedione or dibenzazepinodione is a potential degradation product, *Procedure 2* is recommended.]

Change to read:

- **Organic Impurities, Procedure 1**

Buffer and Chromatographic system: Proceed as directed in the *Assay*.**Diluent:** Methanol and water (60:40)**Mobile phase:** Methanol, acetonitrile, and *Buffer* (29:21:75)**System suitability solution:** 0.5 mg/mL of USP Oxcarbazepine RS and 1.0 µg/mL of USP Carbamazepine RS in *Mobile phase***Standard solution:** 0.5 µg/mL of USP Oxcarbazepine RS in *Mobile phase***Sample stock solution:** Nominally equivalent to 1.2 mg/mL of oxcarbazepine from a portion of finely powdered (NLT 20) Tablets, prepared as follows. Transfer a weighed quantity of powdered Tablets, equivalent to 600 mg of oxcarbazepine, to a 500-mL volumetric flask.

Add *Diluent* to fill 50% of the final volume. Sonicate for 15 min with intermittent swirling, cool to room temperature, and dilute with *Diluent* to volume. Pass this solution through a suitable 2- μ m glass filter, and discard the first portion of the filtrate.

Sample solution: 0.5 mg/mL of oxcarbazepine from the *Sample stock solution* in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 8.0 between oxcarbazepine and carbamazepine, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of oxcarbazepine from the *Standard solution*

C_S = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxcarbazepine in the *Sample solution* (mg/mL)

F = relative response factor for the corresponding impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxcarbazepine	1.0	1.0	—
Carbamazepine	1.6	1.5	0.5
Dibenzazepinone ^a	2.0	1.0	0.05
•Methoxycarbamazepine ^b (ERR 1-Jun-2014)	2.3	1.3	0.05
Any unspecified individual degradation product	—	1.0	0.10
Total impurities	—	—	0.75

a 10(11*H*)-Oxo-5*H*-dibenz[*b,f*]azepine.
b 10-Methoxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide.

• Organic Impurities, Procedure 2

Buffer A: 4.2 g of *tris*(hydroxymethyl)amino methane and 0.2 g of edetate disodium in 1 L of water

Buffer B: 18 g of *tris*(hydroxymethyl)amino methane and 0.9 g of edetate disodium in 1 L of water

Diluent: Acetonitrile and 1.8 g/L of ascorbic acid in water (1:99)

Solution A: Acetonitrile, tetrahydrofuran, and *Buffer A* (5:10:85)

Solution B: Acetonitrile, tetrahydrofuran, and *Buffer B* (70:10:20)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	95	5
33.0	30	70
33.1	95	5
45.0	95	5

System suitability stock solution: 1 µg/mL of USP Oxcarbazepine Related Compound C RS and 12 µg/mL of USP Carbamazepine RS in acetonitrile. Sonication may be used to aid in dissolution. [Note—The water bath temperature should not exceed 23°.]

System suitability solution: 0.05 µg/mL of USP Oxcarbazepine Related Compound C RS, 0.6 µg/mL of USP Carbamazepine RS, and 0.06 mg/mL of USP Oxcarbazepine RS prepared as follows. Transfer a suitable volume of *System suitability stock solution* to a volumetric flask containing 50% of the final volume of *Diluent*. Allow the solution to reach ambient temperature, and dilute with acetonitrile to volume.

Standard stock solution: 12 µg/mL of USP Carbamazepine RS in acetonitrile. Sonication may be used to aid in dissolution. [Note—The water bath temperature should not exceed 23°.]

Standard solution: 0.6 µg/mL of USP Carbamazepine RS from *Standard stock solution* prepared as follows. Transfer a suitable volume of *Standard stock solution* to a flask containing 50% of the final volume of *Diluent* and 20% of the final volume of acetonitrile. Allow the solution to reach ambient temperature, and dilute with acetonitrile to volume.

Sample stock solution: 1.5 mg/mL of oxcarbazepine from a portion of finely powdered (NLT 20) Tablets prepared as follows. Transfer a weighed quantity of powdered Tablets, equivalent to about 375 mg of oxcarbazepine, to a 250-mL volumetric flask. Add 150 mL of acetonitrile, and sonicate for 15 min. Shake for 15 min, and dilute with acetonitrile to volume. Mix, and allow the suspension to settle for 30 min. Use the supernatant. [Note—The water bath temperature should not exceed 23°.]

Sample solution: 0.3 mg/mL of oxcarbazepine from the *Sample stock solution* prepared as follows. Transfer a suitable volume of *Sample stock solution* to a volumetric flask containing 50% of the final volume of *Diluent* and 20% of the final volume of acetonitrile. Allow the solution to warm to ambient temperature, and dilute with acetonitrile to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.0-mm × 25-cm; 5-µm packing L1

Flow rate: 0.5 mL/min

Injection volume: 20 µL

Temperatures

Sample: 5°

Column: 35°

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between oxcarbazepine related compound C and carbamazepine, *System suitability solution*

Relative standard deviation: NMT 15%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of carbamazepine from the *Standard solution*

C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxcarbazepine in the *Sample solution* (mg/mL)

F = relative response factor for the corresponding impurity (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Carbamazepinedione ^a	0.72	0.70	0.2
Oxcarbazepine	1.0	1.0	—
Oxcarbazepine related compound C ^b	1.3	—	—
Carbamazepine	1.4	1.0	0.5
Dibenzazepinodione ^c	1.7	2.8	0.2
Any unspecified individual degradation product	—	1.0	0.1
Total impurities	—	—	1.0

^a 10,11-Dioxo-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide.

^b For system suitability and identification purposes only. Process impurity. Not included in total.

^c 5H-Dibenzo[b,f]azepine-10,11-dione.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.
- **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.
- **USP Reference Standards** { 11 }
 - USP Carbamazepine RS
 - USP Oxcarbazepine RS
 - USP Oxcarbazepine Related Compound C RS

Acridin-9(10*H*)-one.
 $C_{13}H_9NO$ 195.22

BRIEFING

Oxtriphylline Delayed-Release Tablets, *USP* 38 page 4696. It is proposed to omit the monograph for the following reasons. No drug products formulated as defined under Oxtriphylline Delayed-Release Tablets are currently marketed in the United States. The drug product is currently not used in veterinary medicine in the United States.

(SM4 : M. Koleck.) Correspondence Number—C127643

Comment deadline: July 31, 2015

Delete the following:

■ Oxtriphylline Delayed-Release Tablets

~~» Oxtriphylline Delayed-Release Tablets contain an amount of oxtriphylline equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).~~

~~**Packaging and storage**—Preserve in tight containers.~~

~~**Labeling**—Label the Tablets to state both the content of oxtriphylline and the content of anhydrous theophylline. The label indicates that the Tablets are enteric-coated.~~

~~**USP Reference standards** ~~(11)~~—
 USP Oxtriphylline RS~~

~~**Identification**—~~

~~**A:** The retention time exhibited by theophylline in the chromatogram of the *Assay preparation* corresponds to that of theophylline in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.~~

~~**B:** Transfer a quantity of finely ground Tablets, equivalent to about 100 mg of oxtriphylline, to a suitable test tube, add 10 mL of methanol, shake on a vortex mixer for several minutes, and filter to obtain the test solution. Apply 10 μ L of the test solution and 10 μ L of a Standard solution of *USP Oxtriphylline RS* in methanol containing 10 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ~~(621)~~) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the applications to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, alcohol, and formic acid (88:10:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light: the principal spot obtained from the test solution corresponds in color, size, and R_f value to that obtained from the Standard solution.~~

~~**Disintegration** ~~(701)~~—Test Tablets as directed for *Enteric-Coated Tablets* (see *Disintegration* ~~(701)~~): the tablets do not disintegrate after 30 minutes of agitation in simulated gastric fluid TS; continue agitation in simulated gastric fluid TS for an additional 30 minutes: the tablets may disintegrate during this period; if all of the tablets have not~~

disintegrated, place the basket in simulated intestinal fluid TS, and operate the apparatus: all of the tablets disintegrate within 90 minutes (2.5 hours total disintegration time).

Uniformity of dosage units ~~(905)~~: meet the requirements

Assay

Mobile phase— Dissolve 6.8 g of monobasic potassium phosphate in water to make 1000 mL, and adjust with 0.1 N potassium hydroxide to a pH of 5.8 ± 0.1 . Prepare a filtered and degassed mixture of this solution and methanol (4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ~~(621)~~).

Standard preparation— Dissolve an accurately weighed quantity of *USP Oxtriphylline RS* in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation— Place 10 Tablets in a 1000-mL volumetric flask, and add about 700 mL of water. Heat on a steam bath, with occasional shaking, until the Tablets have disintegrated. Cool to room temperature, dilute with water to volume, mix, and filter. Transfer an accurately measured volume of this specimen solution, equivalent to about 20 mg of Oxtriphylline, to a 200-mL volumetric flask, dilute with water to volume, and mix.

System suitability preparation— Dissolve suitable quantities of *USP Oxtriphylline RS* and theobromine in water to obtain a solution containing about 0.6 mg and 0.3 mg per mL, respectively. Dilute this solution quantitatively, and stepwise if necessary, with water to obtain a solution containing about 60 µg of *USP Oxtriphylline RS* per mL and about 30 µg of theobromine per mL.

Chromatographic system (see *Chromatography* ~~(621)~~)—The liquid chromatograph is equipped with a 275-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the resolution, R , between the theobromine and theophylline peaks is not less than 3.0, and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%. The relative retention times are about 0.7 for theobromine and 1.0 for theophylline.

Procedure— Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [Note—The major peaks recorded in the chromatograms represent the theophylline moiety of oxtriphylline.] Calculate the quantity, in mg, of $C_7H_8N_4O_2$ per Tablet taken by the formula:

$$(180.17 / 283.33)(20C / V)(r_U / r_S)$$

in which 180.17 and 283.33 are the molecular weights of anhydrous theophylline and oxtriphylline, respectively, C is the concentration, in µg per mL, of *USP Oxtriphylline RS* in the *Standard preparation*, V is the volume, in mL, of specimen solution taken for the *Assay preparation*, and r_U and r_S are the theophylline peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. ■1S (USP39)

BRIEFING

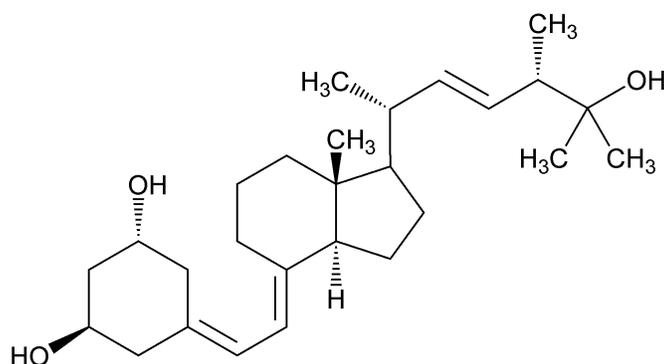
Paricalcitol, *USP 38* page 4761. The currently official monograph contains a test for *Organic Impurities* but does not list specified impurities. As a part of USP's modernization process, the monograph is revised to update the analytical procedure and to include acceptance criteria for specified impurities. The liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with the Halo C18 brand of L1 column. The typical retention time for paricalcitol is about 12 min.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: A. Wise, E. Gonikberg.)
Correspondence Number—C138779

Comment deadline: July 31, 2015

Paricalcitol



$C_{27}H_{44}O_3$ 416.64

19-Nor-1- α ,25-dihydroxyvitamin D_2 ;

(1 α ,3 β ,7 E ,22 E)-19-Nor-9,10-secoergosta-5,7,22-triene-1,3,25-triol;

(7 E ,22 E)-19-Nor-9,10-secoergosta-5,7,22-triene-1 α ,3 β ,25-triol [131918-61-1].

DEFINITION

Paricalcitol contains NLT 97.0% and NMT 103.0% of paricalcitol ($C_{27}H_{44}O_3$), calculated on the dried basis.

[**Caution**—Handle Paricalcitol with exceptional care because it is very potent. Care should be taken to prevent inhaling particles of Paricalcitol and exposing the skin to it.]

IDENTIFICATION

- **A. Infrared Absorption** $\langle 197K \rangle$
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

[Note—Protect paricalcitol solutions from light.]

Mobile phase: Methanol and water (4:1)

Diluent: Methanol and water (1:1)

Standard solution: Dilute USP Paricalcitol Solution RS with *Diluent* to obtain a solution containing 5.0 µg/mL of paricalcitol.

Sample solution: Transfer an accurately weighed amount of Paricalcitol to a suitable volumetric flask, add dehydrated alcohol (approximately 1 mL of dehydrated alcohol per each 0.5 mg of paricalcitol), sonicate to dissolve, and dilute with *Diluent* to volume. Further dilute this solution with *Diluent* to obtain a solution containing 5.0 µg/mL of paricalcitol.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of paricalcitol (C₂₇H₄₄O₃) in the portion of Paricalcitol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of paricalcitol in the *Standard solution* (µg/mL)

C_U concentration of Paricalcitol in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

Change to read:

• Organic Impurities

[Note—

■ Unless otherwise specified, ■ 1S (USP39)

protect paricalcitol solutions from light.]

Diluent: Dehydrated alcohol and water (1:1)

Butylparaben solution: 0.25 mg/mL of butylparaben in *Diluent*

Solution A: Use a filtered and degassed mixture of acetonitrile and water (5:95), and add 1 drop of phosphoric acid per L of solution.

Solution B: Filtered and degassed acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
30	47	53
40	47	53
45	0	100
50	0	100
51	100	0
60	100	0

Standard solution: Dilute USP Paricalcitol Solution RS with *Diluent* to obtain a solution containing 0.1 µg/mL of paricalcitol.

Sample stock solution: 200 µg/mL of Paricalcitol in dehydrated alcohol

Sample solution: A mixture of *Sample stock solution* and water (1:1)

System suitability solution: Combine 1 mL of *Butylparaben solution* and 1 mL of *Sample stock solution* in a 100 mL volumetric flask, and dilute with *Diluent* to volume. Transfer 1 mL of this solution to a 10 mL volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ~~621~~, *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6 mm × 25 cm; 5 µm packing L1

Flow rate: 2 mL/min

Injection volume: 100 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for butylparaben and paricalcitol in the *System suitability solution* are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 12.0 between paricalcitol and butylparaben, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Diluent*, *Standard solution*, and *Sample solution*

Measure the peak responses, disregarding any peaks corresponding to those from the *Diluent*.

Calculate the percentage of each impurity in the portion of Paricalcitol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of paricalcitol from the *Standard solution*

C_S = concentration of paricalcitol in the *Standard solution* (µg/mL)

C_U = concentration of Paricalcitol in the *Sample solution* (µg/mL)

Acceptance criteria

Any individual impurity: ~~NMT 0.1%~~

Total impurities: ~~NMT 0.5%~~

- **Diluent:** Dehydrated alcohol and water (50:50)
- Solution A:** Acetonitrile and water (5:95)
- Solution B:** Acetonitrile and methanol (75:25)
- Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	42	58
11 ^a	42	58
20	0	100
27	0	100
27.1	42	58
30	42	58

^a Determine the retention time of the paricalcitol peak using the *Standard solution*. Adjust the start of the gradient to be 1.0 ± 0.1 min prior to the retention time of paricalcitol and accordingly adjust the remaining gradient times.

System suitability stock solution: Prepare a 50- $\mu\text{g}/\text{mL}$ solution of paricalcitol from USP Paricalcitol Solution RS in dehydrated alcohol. Using a colorless glass container, expose the solution to ultraviolet light at 254 nm. Paricalcitol undergoes partial degradation to 7Z-paricalcitol. A degradation of at least 0.2% of paricalcitol to 7Z-paricalcitol [(7Z,22E)-19-nor-9,10-secoergosta-5,7,22-triene-1 α ,3 β ,25-triol] must be obtained, based on the corresponding peaks. If it is not obtained, expose the solution to ultraviolet light again.

System suitability solution: *System suitability stock solution* and water (1:1)

Standard stock solution: 5 $\mu\text{g}/\text{mL}$ of paricalcitol from USP Paricalcitol Solution RS in *Diluent*

Standard solution: 0.15 $\mu\text{g}/\text{mL}$ of paricalcitol from *Standard stock solution* in *Diluent*

Sensitivity solution: 0.05 $\mu\text{g}/\text{mL}$ of paricalcitol from *Standard solution* in *Diluent*

Sample stock solution: 200 $\mu\text{g}/\text{mL}$ of Paricalcitol in dehydrated alcohol

Sample solution: 100 $\mu\text{g}/\text{mL}$ of Paricalcitol from *Sample stock solution* in water

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6-mm \times 10-cm; 2.7- μm packing L1

Column temperature: 30 $^{\circ}$

Flow rate: 0.9 mL/min

Injection volume: 25 μL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[Note—The relative retention times for paricalcitol and 7Z-paricalcitol are 1.0 and 1.06, respectively.]

Suitability requirements

Resolution: NLT 1.5 between paricalcitol and 7Z-paricalcitol, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Paricalcitol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U

= peak response of each impurity from the *Sample solution*

r_S

= peak response of paricalcitol from the *Standard solution*

C_S

= concentration of paricalcitol in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= concentration of Paricalcitol in the *Sample solution* ($\mu\text{g/mL}$)

F

= relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Paricalcitol	1.0	—	—
22Z-Paricalcitol ^a	1.23	0.64	0.15
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	0.5
^a (7E,22Z)-19-Nor-9,10-secoergosta-5,7,22-triene-1 α ,3 β ,25-triol.			

■ 1S (USP39)

SPECIFIC TESTS

• Loss on Drying

(See *Thermal Analysis* < 891 > .)

Sample: 8 mg of Paricalcitol

Analysis: Determine the percentage of volatile substances by thermogravimetric analysis

on an appropriately calibrated instrument. Heat at a rate of 5^o/min between ambient

temperature and 150^o in an atmosphere of nitrogen at a flow rate of 40 mL/min. Determine the accumulated loss in weight from the thermogram.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store under argon in a freezer.

- **USP Reference Standards** 〈 11 〉

- USP Paricalcitol RS

- USP Paricalcitol Solution RS

BRIEFING

Paricalcitol Capsules. Because there is no existing *USP* monograph for this dosage form, a new monograph, based on validated methods of analysis, is proposed.

1. The liquid chromatographic procedure in the *Assay* is based on analyses performed using the Waters Symmetry C18 brand of L1 column. The Sulpeco LC-18 DB and Alltech Altima C18 brands of L1 column are also suitable for this procedure. The typical retention time for paricalcitol is about 7.5 min. An internal standard is employed to compensate for displacement and dilution effects.
2. The liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed using the Waters Symmetry C18 brand of L1 column. The Alltech Altima C18 brand of L1 column is also suitable for this procedure. The typical retention time for paricalcitol is about 35 min. An internal standard is employed because the sample preparation includes an extraction procedure.
3. Degradation solutions A and B described in the proposal are similar to the ones in the currently official *Paricalcitol Injection* monograph. The same specified unidentified degradation products (related compounds A–I) are formed. Because the analytical procedures in the two monographs are different, the relative retention times of the components differ but the elution order remains the same. Related compounds E and F, which are degradation products and are listed in the *Paricalcitol Injection* monograph, are not observed in the Capsules procedure and are therefore not included in *Table 4*.

(SM3: A. Wise, E. Gonikberg.)

Correspondence Number—C133281

Comment deadline: July 31, 2015

Add the following:

■ **Paricalcitol Capsules**

DEFINITION

Paricalcitol Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of paricalcitol ($C_{27}H_{44}O_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

[Note—Protect paricalcitol solutions from light.]

Solution A: Acetonitrile, isopropyl alcohol, and water (50:15:35)

Solution B: Acetonitrile and isopropyl alcohol (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	100	0
21	0	100
29	0	100
30	100	0
35	100	0

Internal standard solution: 0.75 µg/mL of triphenylene in acetonitrile

Standard stock solution: 10 µg/mL of paricalcitol from USP Paricalcitol Solution RS in acetonitrile

Standard solution: 0.5 µg/mL of paricalcitol from *Standard stock solution* and 0.15 µg/mL of triphenylene from *Internal standard solution* in acetonitrile

Sample solution: Nominally 0.5 µg/mL of paricalcitol from Capsules and 0.15 µg/mL of triphenylene from *Internal standard solution*. Prepare as follows. Transfer 10 Capsules to a suitable container. Transfer the volume of acetonitrile specified in *Table 2* to a separate beaker. Cut each Capsule to allow the oil to flow freely from the shell. Rinse any tools used to open the shells by pouring in portions of acetonitrile from the beaker into the sample container. When the rinsing is complete, add the remainder of the acetonitrile to the sample container and add the volume of *Internal standard solution* specified in *Table 2*. [Note—To facilitate cutting the Capsules open, soften them in a suitable microwave oven for NMT 10 s; taking care not to melt the Capsules. A microwave oven of 600–825 watts was found to be suitable for this procedure.]

Table 2

Capsule Strength (µg/Capsule)	Volume of Acetonitrile (mL)	Volume of Internal Standard Solution (mL)	Internal Standard Correction Factor (F)
1	16.0	4.0	1
2	32.0	8.0	1
4	65.0	15.0	0.9375

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 2.0 mL/min

Injection volume: 50 µL

Run time: 20 min

System suitability

Sample: *Standard solution*

[Note—The relative retention times for paricalcitol and triphenylene are 1.0 and 2.0, respectively.]

Suitability requirements**Resolution:** NLT 4.0 between paricalcitol and triphenylene**Tailing factor:** NMT 2.0 for the paricalcitol peak**Relative standard deviation:** NMT 2.0% for the peak response ratios of paricalcitol to triphenylene**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of paricalcitol ($C_{27}H_{44}O_3$) in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

 $R_{\bar{U}}$ peak response ratio of paricalcitol to triphenylene from the *Sample solution* $R_{\bar{S}}$ peak response ratio of paricalcitol to triphenylene from the *Standard solution* $C_{\bar{S}}$ concentration of paricalcitol from USP Paricalcitol Solution RS in the *Standard solution* ($\mu\text{g/mL}$) $C_{\bar{U}}$ nominal concentration of paricalcitol in the *Sample solution* ($\mu\text{g/mL}$) F = internal standard correction factor (see *Table 2*)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**

- **Disintegration** { 701 }

Apparatus: Basket-rack assembly with disks**Time:** 15 min

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

- **Organic Impurities**

[Note—Unless otherwise specified, protect paricalcitol solutions from light.]

Solution A: Water**Solution B:** Acetonitrile**Solution C:** Acetonitrile and isopropyl alcohol (50:50)**Mobile phase:** See *Table 3*.**Table 3**

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	85	15	0
55	5	95	0
56	0	0	100
60	0	0	100
61	85	15	0
70	85	15	0

Diluent: Acetonitrile and water (50:50)**Degradation stock solution:** Dilute 1 mL of USP Paricalcitol Solution RS with *Diluent* to 5

mL.

Degradation solution A: Transfer 1 mL of the *Degradation stock solution* and 0.1 mL of 30% hydrogen peroxide into a suitable container, and allow to stand at room temperature for 1 h. Dilute with *Diluent* to 10 mL, and mix. This solution contains paricalcitol and related compounds A and B.

Degradation solution B: Mix 1 mL of the *Degradation stock solution* and 1 mL of 0.1 N hydrochloric acid, and heat at 70° for 1 h. Cool to room temperature, dilute with *Diluent* to 10 mL, and mix. This solution contains paricalcitol and related compounds C, D, G, H and I.

Internal standard solution: 0.08 µg/mL of calcitriol from USP Calcitriol Solution RS in acetonitrile

Standard solution: 0.01 µg/mL of paricalcitol from USP Paricalcitol Solution RS and 0.016 µg/mL of calcitriol from *Internal standard solution* in *Diluent*

Sample solution: Nominally 1.9 µg/mL of paricalcitol from Capsules. Prepare as follows. Remove the contents of a suitable number of Capsules using a syringe or cut the Capsules open with scissors and transfer the contents to a suitable container. Transfer a portion of mixed Capsule contents containing nominally 1.9 mg of paricalcitol to a 10-mL volumetric flask. Add acetonitrile to obtain a total mass of 5 g. Transfer 2.0 mL of *Internal standard solution* to the flask and dilute with water to volume. Mix using a vortex mixer. Centrifuge for 5 min at approximately 3000 rpm. Continue centrifuging for 5 min intervals until the top layer is clear, then transfer the supernatant to a suitable chromatographic vial within 2 min.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 300 µL

Run time: 55 min

System suitability

Sample: *Standard solution*

[Note—The relative retention times for paricalcitol and calcitriol are 1.0 and 1.03, respectively. See *Table 4* for all other relative retention times.]

Suitability requirements

Relative standard deviation: NMT 7.0% for the peak response ratio of paricalcitol to calcitriol

Analysis

Samples: *Degradation solution A*, *Degradation solution B*, *Standard solution*, and *Sample solution*

Identify the impurities in the *Sample solution* on the basis of the relative retention times of the components of *Degradation solution A* and *Degradation solution B* in *Table 4*.

Table 4

Name ^a	Degradation Solution	Relative Retention Time
Related compound A	A	0.74
Related compound B	A	0.84
Related compound C	B	0.91
Related compound D	B	0.97
7Z-Paricalcitol ^b	—	1.01
Related compound G	B	1.47
Related compound H	B	1.50
Related compound I	B	1.51
Total impurities	—	—

^a Related compounds A, B, C, D, G, H, and I are specified unidentified degradation products. No information is available about chemical structures or chemical names for these impurities.

^b (7Z,22E)-19-Nor-9,10-secoergosta-5,7,22-triene-1 α ,3 β ,25-triol.

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of each impurity to calcitriol from the *Sample solution*

R_S = peak response ratio of paricalcitol to calcitriol from the *Standard solution*

C_S = concentration of paricalcitol from USP Paricalcitol Solution RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of paricalcitol in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria

Any individual impurity: NMT 2.0%

Total impurities: NMT 3.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in a tight container. Store at controlled room temperature.
- **USP Reference Standards** $\langle 11 \rangle$
 - USP Calcitriol Solution RS
 - USP Paricalcitol Solution RS

■ 1S (USP39)

BRIEFING

Penicillin G Potassium, USP 38 page 4785. The following revisions are proposed:

1. An *Identification* test based on the chromatographic retention time in the *Assay* is added to strengthen the monograph.
2. In preparation for the omission of flame tests from *Identification Tests—General* \langle

191), proposed in PF 41(2) [Mar.–Apr. 2015], it is proposed to replace the flame test for potassium with *Test a* for potassium from 2.3.1 *Identification Reactions of Ions and Functional Groups* in the *European Pharmacopoeia*. This is consistent with *Identification test D* in the *European Pharmacopoeia* monograph for *Benzylpenicillin Potassium*.

3. The *Assay* is revised to delete the column efficiency requirement. The remaining requirements are adequate to evaluate the system suitability.
4. The requirement in *Sterility Tests* is revised to make it more flexible.
5. The *USP Reference Standards* section is updated to add a Reference Standard that is used for the *Bacterial Endotoxins Test*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

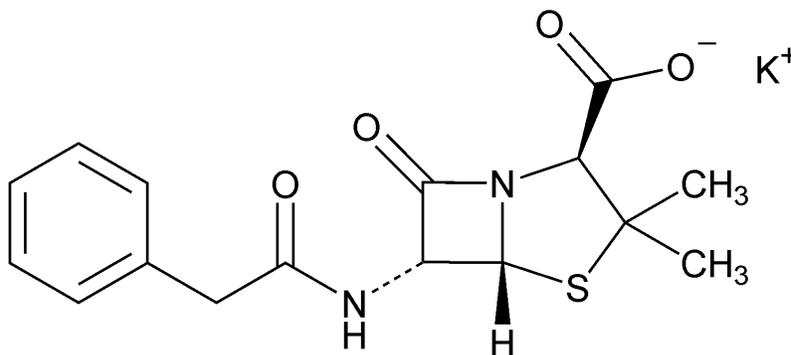
Interested parties are encouraged to submit validated procedures and approved acceptance criteria along with supporting data to further improve this monograph and other monographs containing Penicillin G Potassium.

(SM1: A. Wise.)

Correspondence Number—C157026

Comment deadline: July 31, 2015

Penicillin G Potassium



$C_{16}H_{17}KN_2O_4S$ 372.48

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-, monopotassium salt, [2*S*-(2 α ,5 α ,6 β)]-; Monopotassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [113-98-4].

DEFINITION

Penicillin G Potassium has a potency of NLT 1440 and NMT 1680 Penicillin G Units/mg.

IDENTIFICATION

- **A. Infrared Absorption** (197K)

Delete the following:

- ● **B. Identification Tests—General, Potassium** (191): Meets the requirements of the flame test ■ 1S (USP39)

Add the following:

- ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

Add the following:

- ● **C.**

Diluent: Glycerin and water (25:14)

Solution A: 106 mg/mL of sodium carbonate in water

Solution B: 120 mg/mL of sodium sulfide in *Diluent*, prepared as follows. Dissolve sodium sulfide in *Diluent*, using about 45% of the final volume and heat. Allow to cool, and dilute with *Diluent* to the final volume.

Solution C: 150 mg/mL of tartaric acid in water

Sample solution: 0.1 g of Penicillin G Potassium in 2 mL of water

Analysis

Part 1: Add 1 mL of *Solution A* to the *Sample solution* and heat.

Part 2: To the hot solution from *Part 1* add 0.05 mL of *Solution B*.

Part 3: Cool the mixture from *Part 2* in iced water and add 2 mL of *Solution C*. Allow to stand.

Acceptance criteria: Meets the requirements for *Parts 1, 2, and 3*

Part 1: No precipitate is formed.

Part 2: No precipitate is formed.

Part 3: A white precipitate is formed.

■ 1S (USP39)

ASSAY

Change to read:

- **Procedure**

Solution A: 0.01 M monobasic potassium phosphate

Mobile phase: Methanol and *Solution A* (40:60)

System suitability solution: 0.1 mg/mL each of USP Penicillin G Potassium RS and 2-phenylacetamide in water

Standard solution: 0.1 mg/mL of USP Penicillin G Potassium RS in water. This solution contains about 160 Penicillin G Units/mL. Shake as needed to dissolve.

Sample solution: 0.1 mg/mL of Penicillin G Potassium in water. Shake as needed to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability**Samples:** *System suitability solution* and *Standard solution*

[Note—The relative retention times for 2-phenylacetamide and penicillin G are about 0.8 and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 2.0 between 2-phenylacetamide and penicillin G, *System suitability solution***Column efficiency:** ~~NLT 1000 theoretical plates, *Standard solution*~~

■ ■ 1S (USP39)

Tailing factor: NMT 2.0, *Standard solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the potency of penicillin G potassium, in Penicillin G Units/mg, in the portion of Penicillin G Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

 r_U = peak response of penicillin G from the *Sample solution* r_S = peak response of penicillin G from the *Standard solution* C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL) C_U = concentration of Penicillin G Potassium in the *Sample solution* (mg/mL) P = potency of USP Penicillin G Potassium RS (Penicillin G Units/mg)**Acceptance criteria:** 1440–1680 Penicillin G Units/mg**SPECIFIC TESTS**

- **Crystallinity** 〈 695 〉: Meets the requirements
- **pH** 〈 791 〉
Sample solution: 60 mg/mL of Penicillin G Potassium in water
Acceptance criteria: 5.0–7.5
- **Loss on Drying** 〈 731 〉
Sample: 100 mg of Penicillin G Potassium
Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.
Acceptance criteria: NMT 1.5%
- **Bacterial Endotoxins Test** 〈 85 〉: Where the label states that Penicillin G Potassium is sterile or it must be subjected to further processing during the preparation of injectable dosage forms, it has NMT 0.01 USP Endotoxin Units/100 Penicillin G Units.

Change to read:

- **Sterility Tests** 〈 71 〉: Where the label states that Penicillin G Potassium is sterile, it meets the requirements. ~~when tested as directed under *Test for Sterility of the Product to Be Examined, Membrane Filtration*~~

■ ■ 1S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

Change to read:● **USP Reference Standards** 〈 11 〉

- USP Endotoxin RS ■_{1S} (USP39)

USP Penicillin G Potassium RS

BRIEFING

Penicillin V Potassium, USP 38 page 4800. The following revisions are proposed:

1. An *Identification* test based on the chromatographic retention time in the *Assay* is added to strengthen the monograph.
2. In preparation for the omission of flame tests from *Identification Tests—General* 〈 191 〉, proposed in PF 41(2) [Mar.–Apr. 2015], it is proposed to replace the flame test for potassium with *Test a* for potassium from 2.3.1 *Identification Reactions of Ions and Functional Groups* in the *European Pharmacopoeia*. This is consistent with *Identification* test *D* in the *European Pharmacopoeia* monograph for *Phenoxymethylpenicillin Potassium*.
3. The *Assay* is revised to correct the column dimensions and correct the units in the *Procedure*. The column efficiency requirement is deleted; the remaining requirements are adequate to evaluate the system suitability.
4. The calculation in the test for *Limit of Phenoxyacetic Acid* was revised to replace peak areas with peak responses.
5. The *USP Reference Standards* section was revised to include a Reference Standard that is used in the *Assay* and the test for *Limit of p-Hydroxyphenicillin V*.

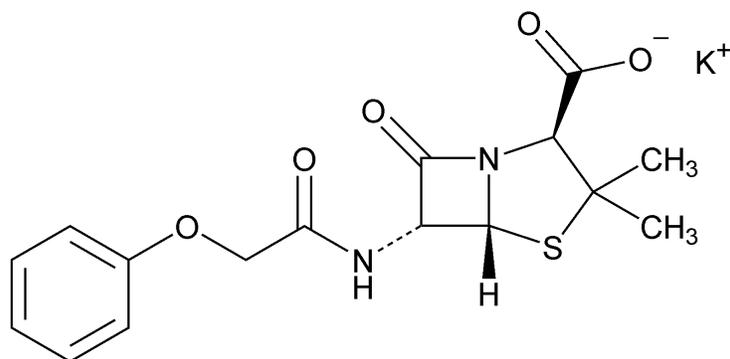
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM1: A. Wise.)

Correspondence Number—C157026

Comment deadline: July 31, 2015

Penicillin V Potassium



$C_{16}H_{17}KN_2O_5S$ 388.48

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-, monopotassium salt, [2*S*-(2 α ,5 α ,6 β)]-; Monopotassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [132-98-9].

DEFINITION

Penicillin V Potassium has a potency of NLT 1380 and NMT 1610 Penicillin V Units/mg.

IDENTIFICATION

- **A. Infrared Absorption** (197K)

Delete the following:

- **B. Identification Tests—General, Potassium** (191): Meets the requirements of the flame test ■1S (USP39)

Add the following:

- **B.** The retention time of the penicillin V peak of the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

Add the following:

- **C.**

Diluent: Glycerin and water (25:14)

Solution A: 106 mg/mL of sodium carbonate in water

Solution B: 120 mg/mL of sodium sulfide in *Diluent*, prepared as follows. Dissolve sodium sulfide in *Diluent*, using about 45% of the final volume and heat. Allow to cool, and dilute with *Diluent* to the final volume.

Solution C: 150 mg/mL of tartaric acid in water

Sample solution: 0.1 g of Penicillin V Potassium in 2 mL of water

Analysis

Part 1: Add 1 mL of *Solution A* to the *Sample solution* and heat.

Part 2: To the hot solution from *Part 1* add 0.05 mL of *Solution B*.

Part 3: Cool the mixture from *Part 2* in iced water and add 2 mL of *Solution C*. Allow to stand.

Acceptance criteria: Meets the requirements for *Parts 1, 2, and 3*

Part 1: No precipitate is formed.

Part 2: No precipitate is formed.

Part 3: A white precipitate is formed.

■ 1S (USP39)

ASSAY

Change to read:

• Procedure

Mobile phase: Acetonitrile, glacial acetic acid, and water (350: 5.75: 650)

System suitability solution: 2.5 mg/mL each of USP Penicillin G Potassium RS and USP Penicillin V Potassium RS in *Mobile phase*

Standard solution: 2.5 mg/mL of USP Penicillin V Potassium RS in *Mobile phase*

Sample solution: 2.5 mg/mL of Penicillin V Potassium in *Mobile phase*

Chromatographic system

(See *Chromatography* 〈621〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: ~~4-mm × 30-cm; packing L1~~

■ 3.9-mm × 30-cm; 10-μm packing L1 ■ 1S (USP39)

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for *p*-hydroxyphenicillin V, penicillin G, and penicillin V are about 0.4, 0.8, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between penicillin G and penicillin V, *System suitability solution*

Column efficiency: ~~NLT 1800 theoretical plates for penicillin V, *System suitability solution*~~

■ 1S (USP39)

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the potency of penicillin V potassium, in USP

■ 1S (USP39)

Penicillin V Units/mg, in the portion of Penicillin V Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = sum of the *p*-hydroxyphenicillin V and penicillin V peak responses from the *Sample solution*

r_S = sum of the *p*-hydroxyphenicillin V and penicillin V peak responses from the *Standard solution*

C_S = concentration of USP Penicillin V Potassium RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin V Potassium in the *Sample solution* (mg/mL)

P = potency of USP Penicillin V Potassium RS (USP

■ ■ 1S (USP39)

Penicillin V Units/mg)

Acceptance criteria: 1380–1610 USP

■ ■ 1S (USP39)

Penicillin V Units/mg

IMPURITIES

Change to read:

• **Limit of Phenoxyacetic Acid**

Mobile phase: Acetonitrile, glacial acetic acid, and water (35:1:65)

Diluent: pH 6.6 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)

Standard solution: 0.1 mg/mL of phenoxyacetic acid in *Diluent*

Sample solution: 20 mg/mL of Penicillin V Potassium in *Diluent*. Use this solution on the day prepared.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of phenoxyacetic acid in the portion of Penicillin V Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = phenoxyacetic acid peak area

■ response ■ 1S (USP39)

from the *Sample solution* r_S = phenoxyacetic acid peak area

■ response ■ 1S (USP39)

from the *Standard solution* C_S = concentration of phenoxyacetic acid in the *Standard solution* (mg/mL) C_U = concentration of Penicillin V Potassium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5%

• **Limit of *p*-Hydroxyphenicillin V**

Mobile phase, System suitability solution, Standard solution, Sample solution,

Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of *p*-hydroxyphenicillin V in the portion of Penicillin V Potassium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U *p*-hydroxyphenicillin V peak response from the *Sample solution*

r_T sum of the *p*-hydroxyphenicillin V and penicillin V peak responses from the *Sample solution*

Acceptance criteria: NMT 5.0%

SPECIFIC TESTS

- **Optical Rotation** 〈 781S 〉, *Specific Rotation*
Sample solution: 10 mg/mL of Penicillin V Potassium in carbon dioxide-free water
Acceptance criteria: +220° to +235°
- **Crystallinity** 〈 695 〉: Meets the requirements
- **pH** 〈 791 〉
Sample solution: 30 mg/mL of Penicillin V Potassium in water
Acceptance criteria: 4.0–7.5
- **Loss on Drying** 〈 731 〉
Sample: 100 mg of Penicillin V Potassium
Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.
Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label it to indicate that it is to be used in the manufacture of nonparenteral drugs only.

Change to read:

- **USP Reference Standards** 〈 11 〉
 ■ USP Penicillin G Potassium RS ■_{1S} (USP39)

USP Penicillin V Potassium RS

BRIEFING

Sildenafil Tablets. Because there is no existing *USP* monograph for this dosage form, a new monograph based on validated methods of analysis is being proposed. The liquid chromatographic procedure for the *Assay* and the test for *Organic Impurities* are based on analyses performed with the Waters Symmetry C18 brand of L1 column. The typical retention time for sildenafil is about 6.5 min.

(SM4: M. Koleck.)

Correspondence Number—C118327; C138606

Comment deadline: July 31, 2015

Add the following:

■ **Sildenafil Tablets**

DEFINITION

Sildenafil Tablets contain sildenafil citrate equivalent to NLT 90% and NMT 110% of the labeled amount of sildenafil ($C_{22}H_{30}N_6O_4S$).

IDENTIFICATION

● **A. Infrared Absorption** 〈 197K〉

Solution A: Ammonium hydroxide and water (10:90)

Standard solution: 1.4 mg/mL of USP Sildenafil Citrate RS in *Solution A*

Sample solution: Grind 1 Tablet and add a sufficient amount of *Solution A* to obtain nominally 1 mg/mL of sildenafil. Sonicate for 2 min, shake well, and centrifuge. Use the supernatant.

Analysis: For each of the *Standard solution* and the *Sample solution*, prewash a 6-cc C18 solid phase extraction cartridge with 10 mL of methanol followed by 10 mL of *Solution A*, discarding both washings. Apply 5 mL each of the *Standard solution* and the *Sample solution* to separate prewashed cartridges and draw each solution through the cartridge. Wash each cartridge with 10 mL of water and allow the cartridge to dry under vacuum. Elute the sildenafil from each cartridge with 5 mL of methanol, collecting the eluant in a suitable container. Add about 200 mg of potassium bromide to each container, mix well, and evaporate to dryness. To about 70 mg of each dried mixture, add about 140 mg of potassium bromide and mix.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

● **Procedure**

Buffer: Dilute 7 mL of triethylamine with water to 1 L. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: *Buffer*, methanol, and acetonitrile (58:25:17)

Diluent: Acetonitrile and water (90:10)

Standard solution: 0.028 mg/mL of USP Sildenafil Citrate RS in *Mobile phase*

Sample stock solution: Disperse 1 Tablet in 5 mL of *Diluent* with the aid of sonication. Dilute with *Mobile phase* to 250.0 mL. Sonicate, if necessary. Centrifuge and use the supernatant.

Sample solution: Nominally 0.02 mg/mL of sildenafil prepared by diluting a suitable portion of the *Sample stock solution* with *Mobile phase*

Chromatographic system

(See *Chromatography* 〈 621〉, *System Suitability*.)

Mode: LC

Detector: UV 290 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 1.5 times the retention time of sildenafil

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.3

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sildenafil ($C_{22}H_{30}N_6O_4S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of sildenafil from the *Sample solution*

r_S = peak response of sildenafil from the *Standard solution*

C_S = concentration of USP Sildenafil Citrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of sildenafil in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of sildenafil, 474.58

M_{r2} = molecular weight of sildenafil citrate, 666.70

Acceptance criteria: 90%–110%

PERFORMANCE TESTS

• Dissolution (711)

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 15 min

Standard solution: 0.03 mg/mL of USP Sildenafil Citrate RS in *Medium*

Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*, if necessary

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: Maximum at about 290 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sildenafil ($C_{22}H_{30}N_6O_4S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Sildenafil Citrate RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*, if needed

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

M_{r1} molecular weight of sildenafil, 474.58

M_{r2} molecular weight of sildenafil citrate, 666.70

Tolerances: NLT 80% (Q) of the labeled amount of sildenafil ($C_{22}H_{30}N_6O_4S$) is dissolved.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

- **Organic Impurities**

Buffer, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*, except for *Run time*.

Run time: NLT 3 times the retention time of sildenafil

System suitability solution: A mixture of sildenafil and sildenafil *N*-oxide in *Mobile phase*, prepared as follows. Dissolve 70 mg of USP Sildenafil Citrate RS in 1 mL of a solution of hydrogen peroxide and formic acid (2:1), allow to stand for NMT 10 min, and then dilute with *Mobile phase* to 250.0 mL.

Standard solution: 0.0014 mg/mL of USP Sildenafil Citrate RS in *Mobile phase*

Sensitivity solution: 0.00035 mg/mL of USP Sildenafil Citrate RS in *Mobile phase* from the *Standard solution*

Sample stock solution: Transfer 5 Tablets to a 250-mL volumetric flask and disperse in 25 mL of *Diluent* with the aid of sonication. Dilute with *Mobile phase* to volume. Sonicate, if necessary. Centrifuge and use the supernatant.

Sample solution: Nominally 0.5 mg/mL of sildenafil prepared by diluting a suitable portion of the *Sample stock solution* with *Mobile phase*

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.6 between sildenafil *N*-oxide and sildenafil, *System suitability solution*

Tailing factor: NMT 1.3, *Standard solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of any individual degradation product from the *Sample solution*

r_S = peak response of sildenafil from the *Standard solution*

C_S = concentration of USP Sildenafil Citrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of sildenafil in the *Sample solution* (mg/mL)

M_{r1} molecular weight of sildenafil, 474.58

M_r molecular weight of sildenafil citrate, 666.70

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sildenafil	1.0	—
Sildenafil <i>N</i> -oxide ^a	1.2	0.20
Any individual degradation product	—	0.20
Total degradation products	—	0.50

^a 1- {[3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl}-4-methylpiperazine *N*⁴-oxide.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP Reference Standards** { 11 }
USP Sildenafil Citrate RS
- 1S (USP39)

BRIEFING

Sodium Chloride and Dextrose Tablets, *USP 38* page 5317. It is proposed to omit this monograph for the following reasons. No drug products formulated as defined under Sodium Chloride and Dextrose Tablets are currently marketed in the United States. The drug product is currently not used in veterinary medicine in the United States.

(SM4: M. Koleck.) Correspondence Number—C157333

Comment deadline: July 31, 2015

Delete the following:

■ **Sodium Chloride and Dextrose Tablets**

» ~~Sodium Chloride and Dextrose Tablets contain not less than 92.5 percent and not more than 107.5 percent of the labeled amount of sodium chloride (NaCl) and of dextrose (C₆H₁₂O₆·H₂O).~~

~~**Packaging and storage**—Preserve in well-closed containers.~~

~~**Identification**—~~

~~**A:** A filtered solution of Tablets responds to the flame test for *Sodium* {191} and to the test for *Chloride* {191}.~~

~~**B:** Add a few drops of the filtered solution tablets to 5 mL of hot alkaline cupric tartrate TS: a copious red precipitate of cuprous oxide is formed.~~

Change to read:

Somatropin is a protein hormone consisting of 191 amino acid residues, and its structure corresponds to the major component of the growth hormone extracted from human pituitary glands. It is produced as a lyophilized powder or bulk solution by methods based on recombinant DNA technology. ~~When prepared as a lyophilized powder, it contains NLT 910 µg of somatropin/mg, calculated on the anhydrous basis. When prepared as a bulk solution, it contains NLT 910 µg of somatropin/mg of total protein.~~

■ ■ 1S (USP39)

The presence of host-cell DNA and host-cell protein impurities in Somatropin is process specific—the limits of these impurities are determined by validated methods. Manufacturers must demonstrate a correlation between the Assay and a validated and approved growth-promotion-based bioassay. It may contain excipients.

[Note—One mg of anhydrous Somatropin is equivalent to 3.0 USP Somatropin Units.]

IDENTIFICATION

- **A.** The retention time of the somatropin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Chromatographic Purity*, except that a *Standard solution* is also chromatographed and prepared by reconstituting a vial of USP Somatropin RS with *Diluent* to obtain a known concentration of about 2 mg/mL.
- **B. Peptide Mapping**

Solution A: Trifluoroacetic acid and water (1:999). Filter, and degas.

Solution B: Water, trifluoroacetic acid, and acetonitrile (100:1:899)

Solution C: 0.05 M solution of tris(hydroxymethyl)aminomethane (Tris). Adjust with hydrochloric acid to a pH of 7.5.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	80	20
40	75	25
65	50	50
70	20	80
71	100	0
86	100	0

Trypsin solution: 1 mg/mL of trypsin in *Solution C*. Store in a freezer, if necessary.

Standard stock solution: 2.0 mg/mL of USP Somatropin RS in *Solution C*

Standard solution: To 1 mL of the *Standard stock solution* add 30 µL of *Trypsin solution*.

Cap the tube, and place it in a water bath at 37° for 4 h. [Note—If this solution is not injected immediately, store it in a freezer.]

Sample stock solution: 2.0 mg/mL of Somatropin in *Solution C*

Sample solution: To 1 mL of the *Sample stock solution* add 30 µL of *Trypsin solution*. Cap the tube, and place it in a water bath at 37° for 4 h. [Note—If this solution is not injected immediately, store it in a freezer.]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 100 µL

Analysis

Samples: *Standard solution* and *Sample solution*, separately injected

[Note—Condition the chromatographic system by running a blank gradient program before injecting the digests.]

Acceptance criteria: The chromatographic profile of the *Sample solution* is similar to that of the *Standard solution*.

- **C. Somatropin Bioidentity Tests** { 126 } : Meets the requirements

[Note—The bioidentity test may be performed either on the Somatropin bulk drug substance or on the finished pharmaceutical product.]

ASSAY

- **Somatropin Content**

Buffer solution: Dissolve 5.18 g of dibasic sodium phosphate and 3.65 g of monobasic sodium phosphate in 950 mL of water. Adjust with phosphoric acid or sodium hydroxide solution to a pH of 7.0. Dilute with water to 1000 mL.

Mobile phase: Isopropyl alcohol and *Buffer solution* (3:97). Filter, and degas.

Diluent: *Buffer solution* and water (1: 1.5)

System suitability solution: Place 1 vial of USP Somatropin RS in an oven at 50° for 12–24 h. Remove from the oven, and dissolve the contents of the vial in *Diluent* to obtain a solution with a known concentration of about 1 mg/mL and a dimer content of 1%–2%.

Standard solution: Known concentration of about 1 mg/mL of USP Somatropin RS in *Diluent*

Sample solution: About 1 mg/mL of accurately weighed Somatropin in *Diluent*, or by diluting a bulk solution of Somatropin with *Diluent*. [Note—If necessary, the amount of protein in solution can be determined by the test for *Total Protein Content*.]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 7.8-mm × 30-cm; packing L33

Column temperature: Ambient

Flow rate: 0.6 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NMT 0.4 for the ratio of the valley height, between the dimer and the monomer, and the dimer peak height

Tailing factor: NMT 1.7 for the monomer (major) peak

Analysis

Samples: *Standard solution* and *Sample solution*, separately injected

Record the chromatograms for NLT twice the retention time of the somatropin monomer (major) peak, and measure the peak responses for the monomer.

Calculate the concentration, in mg/mL, of somatropin in the *Sample solution* taken:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U peak response of the monomer from the *Sample solution*

r_S peak response of the monomer from the *Standard solution*

C_S concentration of USP Somatropin RS in the *Standard solution* (mg/mL)

Acceptance criteria: NLT 910 µg of somatropin/mg on the anhydrous basis. When prepared as a bulk solution, it contains NLT 910 µg of somatropin/mg of total protein.

IMPURITIES

Change to read:

• Chromatographic Purity

Diluent: 0.05 M Tris in water. Adjust with hydrochloric acid to a pH of 7.5.

Mobile phase: *n*-Propyl alcohol and degassed *Diluent* (29:71). Filter.

System suitability solution: 2.0 mg/mL of Somatropin in *Diluent*. Pass through a filter to sterilize or add sodium azide to a final concentration of 0.01%, and allow to stand at room temperature for 24 h. [Note—Use within 48 h of preparation, or store the solution in a refrigerator until ready to use.]

Sample solution: 2.0 mg/mL of Somatropin in *Diluent*, prepared immediately before use

■ 1S (USP39)

[Note—Maintain the solutions between 2° and 8°, and use within 24 h. If an automatic injector is used, maintain the temperature between 2° and 8°.]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L26

Column temperature: 45°

Flow rate: 0.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.0 between somatropin and its adjacent peak

Tailing factor: 0.9–1.8 for the somatropin (major) peak

Analysis

Sample: *Sample solution*

Calculate the percentage of impurities in the portion of Somatropin taken:

$$\text{Result} = [A_U / (A_U + A_S)] \times 100$$

A_U sum of all the peak responses other than the somatropin (major) peak and disregarding any peak due to the solvent

A_S peak response of somatropin

Acceptance criteria: NMT 6.0% of total impurities

- **Limit of High Molecular Weight Proteins**

Buffer solution, Mobile phase, Diluent, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Samples: *System suitability solution* and *Sample solution*

Measure the areas of the main peak and of the peaks eluting before the main peak, excluding the solvent peaks.

Calculate the percentage of high molecular weight proteins in the portion of Somatropin taken:

$$\text{Result} = [A_H / (A_H + A_M)] \times 100$$

A_H sum of the areas of the high molecular weight peaks

A_M peak area of the monomer from the *Sample solution*

Acceptance criteria: NMT 4% of high molecular weight proteins

SPECIFIC TESTS

- **Total Protein Content**

(See *Ultraviolet-Visible Spectroscopy* 〈 857 〉.)

Buffer solution: 0.025 M solution of monobasic potassium phosphate in water. Adjust with sodium hydroxide to a pH of 7.0.

Sample solution: Dissolve a weighed quantity of Somatropin in *Buffer solution* to obtain a solution with an absorbance value between 0.5 and 1.0 at the wavelength of maximum absorbance at 280 nm.

Analysis: Determine the absorbance of the *Sample solution* using a spectrophotometric cell of path length 1 cm, at the wavelength of maximum absorbance around 280 nm and at 320 nm, using *Buffer solution* as the blank.

Calculate the protein content, in mg, in the portion of Somatropin taken:

$$\text{Result} = (A_{max} - A_{320}) \times (V/0.82)$$

A_{max} absorbance value of the *Sample solution* at the wavelength of maximum absorbance

A_{320} absorbance value of the *Sample solution* at 320 nm

V = volume of the *Sample solution*

- **Microbial Enumeration Tests** 〈 61 〉 and **Tests for Specified Microorganisms** 〈 62 〉: The total aerobic microbial count is NMT 3×10^2 cfu/g, the test being performed on 0.2–0.3 g of powder, accurately weighed.

- **Water Determination** 〈 921 〉, *Method 1c*: NMT 10%, when prepared as a lyophilized powder

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 10 USP Endotoxin Units/mg of Somatropin

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store between -10° and -25° .
- **Labeling:** The labeling states that the material is of recombinant DNA origin.
- **USP Reference Standards** 〈 11 〉
 - USP Endotoxin RS
 - USP Somatropin RS

BRIEFING

Somatropin for Injection, *USP 38* page 5340. It is proposed to clarify the instructions for the preparation and storage of the *Sample solution* in the test for *Chromatographic Purity*. Additionally, redundant acceptance criteria were deleted from the *Definition* since they are already present in the *Somatropin Content* test.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BIO1: M. Kibbey.)

Correspondence Number—C156562

Comment deadline: July 31, 2015

Somatropin for Injection

DEFINITION

Change to read:

Somatropin for Injection is a sterile, lyophilized mixture of Somatropin with one or more suitable buffering and stabilizing agents. ~~It contains NLT 89.0% and NMT 110.0% of the amount of somatropin stated on the label.~~

■ **1S** (*USP39*)

Manufacturers must demonstrate a correlation between the *Assay* and a validated and approved growth-promotion-based bioassay.

[Note—One mg of anhydrous Somatropin is equivalent to 3.0 USP Somatropin Units.]

IDENTIFICATION

- **A.** The retention time of the somatropin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Chromatographic Purity*, except that a *Standard solution* is also chromatographed and prepared by reconstituting a vial of USP Somatropin RS with *Diluent* to obtain a known concentration of about 2 mg/mL.
 - **B. Somatropin Bioidentity Tests** 〈 126 〉: Meets the requirements
- [Note—The bioidentity test may be performed either on the Somatropin bulk drug substance or on the finished pharmaceutical product.]

ASSAY

- **Somatropin Content**

Buffer solution: 5.18 g of dibasic sodium phosphate and 3.65 g of monobasic sodium

phosphate in 950 mL of water. Adjust with phosphoric acid or sodium hydroxide solution to a pH of 7.0. Dilute with water to 1000 mL.

Mobile phase: Isopropyl alcohol and *Buffer solution* (3:97). Filter and degas.

Diluent: *Buffer solution* and water (1: 1.5)

System suitability solution: Place 1 vial of USP Somatropin RS in an oven at 50° for 12–24 h. Remove from the oven, and dissolve the contents of the vial in *Diluent* to obtain a solution with a known concentration of about 1 mg/mL and a dimer content of 1%–2%.

Standard solution: Known concentration of about 1 mg/mL of USP Somatropin RS in *Diluent*

Sample solution: Dissolve the contents of a suitable number of containers in *Diluent* to obtain a concentration of 1 mg/mL of somatropin.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 7.8-mm × 30-cm; packing L33

Column temperature: Ambient

Flow rate: 0.6 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NMT 0.4 for the ratio of the valley height, between the dimer and the monomer, and the dimer peak height

Tailing factor: NMT 1.7 for the monomer (major) peak

Analysis

Samples: *Standard solution* and *Sample solution*, separately injected

Record the chromatograms for NLT twice the retention time of the somatropin monomer (major) peak, and measure the peak responses for the monomer.

Calculate the percentage of somatropin per container:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/N) \times 100$$

r_U = peak response of the monomer from the *Sample solution*

r_S = peak response of the monomer from the *Standard solution*

C_S = concentration of USP Somatropin RS in the *Standard solution* (mg/mL)

V = total volume of the *Sample solution* (mL)

N = number of containers used to obtain the *Sample solution*

Acceptance criteria: 89.0%–110.0%

IMPURITIES

Change to read:

• Chromatographic Purity

Diluent: 0.05 M tris(hydroxymethyl)aminomethane in water. Adjust with hydrochloric acid to a pH of 7.5.

Mobile phase: *n*-Propyl alcohol and degassed *Diluent* (29:71). Filter.

System suitability solution: 2.0 mg/mL of somatropin in *Diluent*. Pass through a filter to sterilize or add sodium azide to a final concentration of 0.01%, and allow to stand at room temperature for 24 h. [Note—Use within 48 h of preparation, or store the solution in a refrigerator until ready to use.]

Sample solution: 2.0 mg/mL of somatropin in *Diluent*. ~~Prepared immediately before use~~

■ ■ 1S (USP39)

[Note—Maintain the solutions between 2° and 8°, and use within 24 h. If an automatic injector is used, maintain the temperature between 2° and 8°.]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L26

Column temperature: 45°

Flow rate: 0.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.0 between somatropin and its adjacent peak

Tailing factor: 0.9–1.8 for the somatropin (major) peak

Analysis

Sample: *Sample solution*

Calculate the percentage of impurities in the portion of Injection taken:

$$\text{Result} = [A_U / (A_U + A_S)] \times 100$$

A_U sum of all the peak responses other than the somatropin (major) peak, disregarding any peak due to the solvent

A_S peak response of somatropin

Acceptance criteria: NMT 12% of total impurities

- **Limit of High Molecular Weight Proteins**

Buffer solution, Mobile phase, Diluent, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *System suitability solution* and *Sample solution*

Measure the areas of the main peak and of the peaks eluting before the main peak, excluding the solvent peaks.

Calculate the percentage of high molecular weight proteins in the portion of Injection taken:

$$\text{Result} = [A_H / (A_H + A_M)] \times 100$$

A_H sum of the areas of the high molecular weight peaks

$A_{\overline{m}}$ peak area of the monomer from the *Sample solution*

Acceptance criteria: NMT 6% of high molecular weight proteins

SPECIFIC TESTS

- **Bacterial Endotoxins Test** (85): NMT 20 USP Endotoxin Units/mg of Somatropin
- **Sterility Tests** (71), *Test for Sterility of the Product to Be Examined, Membrane Filtration:* Meets the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store between 2° and 8°.
- **Labeling:** The labeling states that the material is of recombinant DNA origin.
- **USP Reference Standards** (11)
 - USP Endotoxin RS
 - USP Somatropin RS

BRIEFING

Succinylcholine Chloride for Injection, *USP 38* page 5365. It is proposed to omit the monograph for the following reasons. No drug products formulated as defined under Succinylcholine Chloride for Injection are currently marketed in the United States. The drug product is not currently used in veterinary medicine in the United States.

(SM4: M. Koleck.) Correspondence Number—C127195

Comment deadline: July 31, 2015

Delete the following:

■ Succinylcholine Chloride for Injection

» ~~Succinylcholine Chloride for Injection is Succinylcholine Chloride suitable for parenteral use.~~

~~**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1):~~

~~**USP Reference standards** (11)—~~

- ~~USP Endotoxin RS~~
- ~~USP Succinylcholine Chloride RS~~
- ~~USP Succinylmonocholine Chloride RS~~

~~**Completeness of solution** (641)—A 500 mg portion dissolves in 10 mL of carbon dioxide-free water to yield a clear and colorless solution.~~

~~**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1):~~

~~**Bacterial endotoxins** (85)—It contains not more than 2.0 USP Endotoxin Units per mg of succinylcholine chloride.~~

Chromatographic purity—

Standard solution— Transfer 20 mg each of choline chloride and *USP Succinylmonocholine Chloride RS*, accurately weighed, to a 50 mL volumetric flask, dissolve in 40 mL of methanol, dilute with methanol to volume, and mix.

Test solution— Prepare, immediately prior to use, a solution of succinylcholine chloride in methanol having a concentration of about 50 mg per mL.

Procedure— Separately apply 2 μ L of the *Test solution* and 5 μ L of the *Standard solution* to a suitable high performance 10 \times 10 cm thin layer chromatographic plate (see *Chromatography* ~~(621)~~) coated with a 0.10 mm layer of chromatographic cellulose. Allow the spots to dry, and immediately place the plate, its coated surface toward the nearer wall, in the dry trough of a twin trough chromatographic chamber whose other trough contains a solvent system consisting of the upper layer of a mixture of butyl alcohol, water, and 96% formic acid (65:35:15) that has been shaken and allowed to stand for 24 hours until the phases have separated. Equilibrate the chromatographic chamber for 30 minutes, and tilt the chamber to introduce the developing solvent into the trough containing the plate. Develop the chromatogram until the solvent front has moved about three fourths of the length of the plate, remove the plate from the developing chamber, quickly and thoroughly evaporate the solvent with the aid of a current of air, and dry at 105^o for 15 minutes. [Note—During the drying, support the plate in such a manner that only the upper and lower edges of the plate, outside the chromatographic zone, are in direct contact with any heated surface.] Spray the plate with potassium iodoplatinate TS, dry at 105^o for about 2 minutes, and allow to cool to room temperature: any spots from the *Test solution* are not greater in size or intensity than the spots, occurring at the respective *R_f* values (approximately 0.6 for succinylmonocholine chloride, and 0.5 for choline chloride), produced by 5 μ L of the *Standard solution*, corresponding to 0.8% of each compound. Estimate the size and intensities of any other spots detected by comparison with the spot produced by succinylmonocholine chloride in the *Standard solution*. The total of any such spots detected is not more than 2.0%.

Other requirements— It conforms to the Definition, responds to the *Identification* tests, and meets the requirements for *Water*, *Residue on ignition*, *Chloride content*, and *Assay* under *Succinylcholine Chloride*. It meets also the requirements for *Sterility Tests* ~~(71)~~, *Uniformity of Dosage Units* ~~(905)~~, and *Labeling* under *Injections* ~~(1)~~. ■ 1S (USP39)

BRIEFING

Temozolomide, *USP 38* page 5480. On the basis of comments received, it is proposed to revise the *Definition* and make the following changes in the test for *Organic Impurities* based on the *Related substance* of *Temozolomide* in *Pharmeuropa*.

1. USP Dacarbazine Related Compound A RS is deleted. Aminoimidazolecarboxamide is given as a nickname for dacarbazine related compound A free base. Aminoimidazolecarboxamide is calculated using a relative response factor and a footnote is added to *Table 1* for its relative retention time.
2. The resolution is determined between temozolomide acid and temozolomide peaks.
3. Two peaks for aminoimidazolecarboxamide may be observed. A footnote in *Table 1* is

revised to indicate that two peaks may be observed and to use the sum of the peak areas for calculation.

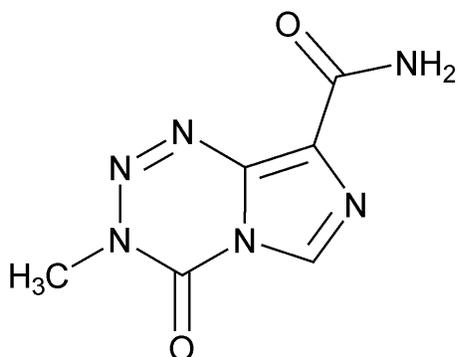
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: F. Mao.)

Correspondence Number—C125085

Comment deadline: July 31, 2015

Temozolomide



$C_6H_6N_6O_2$ 194.15

Imidazo[5,1-*d*]-1,2,3,5-tetrazine-8-carboxamide, 3,4-dihydro-3-methyl-4-oxo-;
3,4-Dihydro-3-methyl-4-oxoimidazo[5,1-*d*]-as-tetrazine-8-carboxamide [85622-93-1].

DEFINITION

Change to read:

Temozolomide contains NLT 98.0% and NMT 102.0% of temozolomide ($C_6H_6N_6O_2$), calculated on the as-is

■ anhydrous ■ 1S (*USP39*)

basis.

[**Caution**—Temozolomide is cytotoxic. Great care should be taken to prevent inhaling particles of Temozolomide and exposure to the skin.]

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

[Note—Shake the solutions containing temozolomide to aid the dissolution. Do not sonicate.]

Change to read:

- **Procedure**

Solution A: 0.5% (v/v) glacial acetic acid in water

Mobile phase: *Solution A* and methanol (96:4), containing 0.94 g/L of sodium 1-

hexanesulfonate (0.005 M)

Diluent: Dimethyl sulfoxide. [Note—Use a freshly opened bottle.]

Standard solution: 1.0 mg/mL of USP Temozolomide RS in *Diluent*

Sample solution: 1.0 mg/mL of Temozolomide in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.9

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of temozolomide ($C_6H_6N_6O_2$) in the portion of Temozolomide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak area from the *Sample solution*

r_S peak area from the *Standard solution*

C_S concentration of USP Temozolomide RS in the *Standard solution* (mg/mL)

C_U concentration of Temozolomide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the as-is

■ anhydrous ■ 1S (USP39)

basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Delete the following:

- **Heavy Metals, Method II** 〈 231 〉: NMT 30 ppm ● (Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

[Note—Shake the solutions containing temozolomide to aid the dissolution. Do not sonicate.]

Mobile phase, Diluent, and Sample solution: Proceed as directed in the *Assay*.

Standard solution: 2.0 μg/mL each of USP Temozolomide RS and USP Dacarbazine Related Compound A RS in *Diluent*

■ 1.0 μg/mL of USP Temozolomide RS in *Diluent* ■ 1S (USP39)

System suitability solution: 0.5 µg/mL each of USP Temozolomide RS and USP Dacarbazine Related Compound A RS in *Diluent* from the *Standard solution*

■ ■ 1S (USP39)

Peak identification

■ **System suitability** ■ 1S (USP39)

solution: Mix 5 mL of 0.1 N hydrochloric acid and 5 mL of 1.0 mg/mL of USP Temozolomide RS in *Diluent*. Heat the container for 1 h on a steam or boiling water bath. [Note—The preparation forms 2-azahypoxanthine, temozolomide acid, and dacarbazine-related compound A

■ aminoimidazolecarboxamide. ■ 1S (USP39)

]

Chromatographic system: Proceed as directed in the *Assay*, using a run time of NLT 3.2 times the retention time of the temozolomide peak.

System suitability

Sample: *Standard solution* and

■ ■ 1S (USP39)

System suitability solution

Suitability requirements

Resolution: NLT 2.0 between the temozolomide and dacarbazine-related compound A peaks, *Standard solution*

■ NLT 1.5 between temozolomide acid and temozolomide peaks ■ 1S (USP39)

Relative standard deviation: NMT 10% for both the dacarbazine-related compound A and temozolomide peaks, *System suitability solution*

■ ■ 1S (USP39)

Analysis

Samples: *Sample solution*, *Standard solution*, and *Peak identification solution*

■ *System suitability solution* ■ 1S (USP39)

Inject the *Peak identification solution*

■ *System suitability solution*, ■ 1S (USP39)

and identify the organic impurities according to the relative retention times given in *Table 1*.

Calculate the percentage of dacarbazine-related compound A (free base) in the portion of Temozolomide taken:

$$\text{Result} = (f_U/f_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

f_U = peak area of dacarbazine-related compound A from the *Sample solution*

f_S = peak area of dacarbazine-related compound A from the *Standard solution*

C_S = concentration of USP Dacarbazine Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Temozolomide in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of dacarbazine-related compound A (free base), 126.12

M_{r2} = molecular weight of dacarbazine-related compound A (hydrochloride salt), 162.58

■ 1S (USP39)

Calculate the percentage of each individual impurity in the portion of Temozolomide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of temozolomide from the *Standard solution*

C_S = concentration of USP Temozolomide RS in the *Standard solution* (mg/mL)

C_U = concentration of Temozolomide in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. [Note—Disregard any unspecified impurity peaks less than 0.05%.]

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Azahypoxanthine ^a	0.42	1.6	0.2
Temozolomide related compound A ^b	0.53	1.0	0.5
Temozolomide acid ^c	0.84	1.0	0.1
Temozolomide	1.0	—	—
Dacarbazine related compound A (free base)	1.37	—	
■ Aminoimidazolecarboxamide ■ 1S (USP39) ^d ■ ■ 1S (USP39) ■ 2.5 ■ 1S (USP39)			0.1
Cyanotemozolomide ^{f,g} (if present)	2.3	1.0	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.8

^a 4a,5-Dihydro-4*H*-imidazo[4,5-*d*][1,2,3]triazin-4-one.

^b 4-Diazo-4*H*-imidazole-5-carboxamide.

^c 3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carboxylic acid.

^d 5-Aminoimidazole-4-carboxamide. ~~It is a free base of dacarbazine related compound A.~~

■ Two peaks may be observed, use sum of the peak areas for calculation. ■ 1S (USP39)

■ ^eIt may vary and depend on the column. ■ 1S (USP39)

^f 3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carbonitrile.

^g If possible from the manufacturing process.

SPECIFIC TESTS

- **Water Determination** { 921 }, *Method 1c*: NMT 0.4%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, and store at room temperature.

Change to read:

- **USP Reference Standards** 〈 11 〉

~~USP Dacarbazine Related Compound A RS
5-Aminoimidazole-4-carboxamide hydrochloride.
C₄H₆N₄O·HCl 162.58~~

■ **1S (USP39)**

USP Temozolomide RS

BRIEFING

Theophylline, *USP 38* page 5524. As a part of USP monograph modernization efforts, the following revisions are proposed:

1. Replace the current HPLC procedure in the *Assay* that uses an internal standard with the same UHPLC procedure that is proposed in the test for *Organic Impurities*. The liquid chromatographic procedure is performed using the Acquity BEH C18 brand of L1 column. The typical retention time of theophylline is about 4 min.
2. Add a stability-indicating UHPLC procedure in the test for *Organic Impurities* using the same chromatographic system as the *Assay*.
3. Revise *Identification* test *B* to match the proposed *Assay* method by removing the reference to the internal standard.
4. Delete the tests for *Melting Range or Temperature* and *Acidity* because these tests are nonspecific and provide no additional value to the monograph.
5. Add five new Reference Standards used in the *Assay* and the test for *Organic Impurities* to the *USP Reference Standards* section.

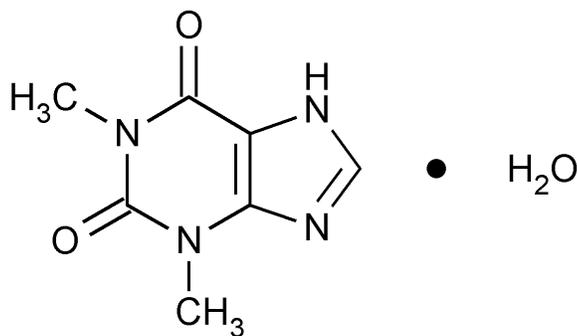
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: D.A. Porter.)

Correspondence Number—C119614

Comment deadline: July 31, 2015

Theophylline



C₇H₈N₄O₂·H₂O 198.18

C₇H₈N₄O₂ 180.17

1*H*-Purine-2,6-dione, 3,7-dihydro-1,3-dimethyl-, monohydrate;
 Theophylline monohydrate [5967-84-0].
 Anhydrous [58-55-9].

DEFINITION

Theophylline contains one molecule of water of hydration or is anhydrous. It contains NLT 97.0% and NMT 102.0% of theophylline (C₇H₈N₄O₂), calculated on the dried basis.

IDENTIFICATION

• A. Infrared Absorption (197K)

Change to read:

- **B.** The retention time of the major peak in the chromatogram of the *Sample solution* corresponds to that in the chromatogram of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.

■ The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

ASSAY

Change to read:

• Procedure

Solution A: Transfer 2.72 g of sodium acetate trihydrate to a 2000 mL volumetric flask, add 200 mL of water, and shake until dissolution is complete. Add 10.0 mL of glacial acetic acid, and dilute with water to volume.

Mobile phase: Acetonitrile and *Solution A* (7:93)

Internal standard solution: 0.5 mg/mL of theobromine prepared as follows. Transfer 50 mg of theobromine to a 100 mL volumetric flask, dissolve in 10 mL of 6 N ammonium hydroxide, and dilute with *Mobile phase* to volume.

Standard stock solution: 1 mg/mL of USP Theophylline RS, in *Mobile phase*

Standard solution: 0.1 mg/mL each of USP Theophylline RS and theobromine, from the *Standard stock solution* and *Internal standard solution*, respectively, in *Mobile phase*.

Sample stock solution: 1 mg/mL of Theophylline in *Mobile phase* prepared as follows. Transfer 100 mg of Theophylline to a 100 mL volumetric flask, add 50 mL of *Mobile phase*, shake by mechanical means until solution is complete, and dilute with *Mobile phase* to volume.

Sample solution: 0.1 mg/mL each of Theophylline and theobromine, from the *Sample stock solution* and *Internal standard solution*, respectively, in *Mobile phase*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4 mm × 30 cm; packing L1

Flow rate: 1.0 mL/min

~~**Injection volume:** 10–25 μ L~~

~~**System suitability**~~

~~**Sample:** *Standard solution*~~

~~[Note—The relative retention time of theophylline relative to that of theobromine is about 1.6.]~~

~~**Suitability requirements**~~

~~**Resolution:** NLT 2.0 between theophylline and theobromine peaks~~

~~**Tailing factor:** NMT 2.0, theophylline peak~~

~~**Relative standard deviation:** NMT 1.5%~~

~~**Analysis**~~

~~**Samples:** *Standard solution* and *Sample solution*~~

~~Calculate the percentage of theophylline ($C_7H_8N_4O_2$) in the portion of Theophylline taken:~~

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

~~R_U peak response ratio of theophylline to the internal standard from the *Sample solution*~~

~~R_S peak response ratio of theophylline to the internal standard from the *Standard solution*~~

~~C_S concentration of USP Theophylline RS in the *Standard solution* (mg/mL)~~

~~C_U concentration of Theophylline in the *Sample solution* (mg/mL)~~

■ Solution A:

10 mM ammonium acetate prepared as follows. Transfer 770.8 mg of ammonium acetate to a 1-L volumetric flask, and dissolve in 80% flask volume of water. Adjust with glacial acetic acid to a pH of 5.5 and dilute with water to volume. Pass through a suitable filter of 0.2- μ m pore size.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

Impurity stock solution: 0.2 mg/mL of USP Theophylline Related Compound F RS in water

System suitability solution: 1 mg/mL of USP Theophylline RS and 1 μ g/mL of USP

Theophylline Related Compound F RS, from *Impurity stock solution*, in water. Sonicate as needed to aid in the dissolution.

Standard solution: 0.2 mg/mL of USP Theophylline RS in water

Sample solution: 0.2 mg/mL of Theophylline in water

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 2.1-mm × 10-cm; 1.7-μm packing L1

Column temperature: 40°

Flow rate: 0.4 mL/min

Injection volume: 1 μL

System suitability

Samples: *System suitability solution* and *Standard solution*.

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between theophylline and theophylline related compound F, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of theophylline (C₇H₈N₄O₂) in the portion of Theophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of theophylline from the *Sample solution*

r_S

= peak response of theophylline from the *Standard solution*

C_S

= concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U

= concentration of Theophylline in the *Sample solution* (mg/mL)

■ 1S (USP39)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.15%

Add the following:

■ • Organic Impurities

Solution A, Solution B, Mobile phase, Impurity stock solution, and **System suitability solution:** Proceed as directed in the *Assay*.

Standard stock solution: 50 μg/mL each of USP Caffeine RS, USP Theophylline RS, USP Theophylline Related Compound B RS, USP Theophylline Related Compound C RS, USP Theophylline Related Compound D RS, and USP Theophylline Related Compound F RS

Standard solution: 1 µg/mL each of USP Caffeine RS, USP Theophylline RS, USP Theophylline Related Compound B RS, USP Theophylline Related Compound C RS, USP Theophylline Related Compound D RS, and USP Theophylline Related Compound F RS, from *Standard stock solution*, in water

Sample solution: 1.0 mg/mL of Theophylline in water

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector

For unspecified impurities with absorption maxima near 220 nm: UV 220 nm

For specified impurities and unspecified impurities with absorption maxima near 270 nm: UV 270 nm

Column: 2.1-mm × 10-cm; 1.7-µm packing L1

Column temperature: 40°

Flow rate: 0.4 mL/min

Injection volume: 1 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between theophylline and theophylline related compound F, *System suitability solution*

Relative standard deviation: NMT 3.0% for each peak present in the *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

For impurities detected at 270 nm

Calculate the percentage of caffeine, theophylline related compound B, theophylline related compound C, and theophylline related compound D in the portion of Theophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of caffeine, theophylline related compound B, theophylline related compound C, or theophylline related compound D from the *Sample solution*

r_S peak response of the corresponding Reference Standard from the *Standard solution*

C_S concentration of USP Caffeine RS, USP Theophylline Related Compound B RS, USP Theophylline Related Compound C RS, or USP Theophylline Related Compound D RS in the *Standard solution* (mg/mL)

C_U concentration of Theophylline in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity with absorption maxima near 270 in the portion of Theophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any other individual unspecified impurity from the *Sample solution*

r_S peak response of theophylline from the *Standard solution*

C_S concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U concentration of Theophylline in the *Sample solution* (mg/mL)

For impurities detected at 220 nm

Calculate the percentage of any other individual unspecified impurity with absorption maxima near 220 in the portion of Theophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any other individual unspecified impurity from the *Sample solution*

r_S peak response of theophylline from the *Standard solution*

C_S concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U concentration of Theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound C	0.36	0.10
Theophylline related compound B	0.63	0.10
Theophylline related compound D	0.69	0.10
Theophylline	1.0	—
Theophylline related compound Fa	1.09	—
Caffeine	1.20	0.10
Any other individual unspecified impurity	—	0.10
Total impurities	—	0.5
a Included for establishing system suitability only.		

■ 1S (USP39)

SPECIFIC TESTS

Delete the following:

- ~~Melting Range or Temperature < 741 >: 270°–274°, but the range between beginning and end of melting does not exceed 3°~~ ■ 1S (USP39)

Delete the following:

- ~~Acidity~~

~~**Sample solution:** Dissolve 500 mg in 75 mL of water, and add 1 drop of methyl red TS.~~

~~**Acceptance criteria:** NMT 1.0 mL of 0.020 N sodium hydroxide is required to change the red color to yellow.~~ ■ 1S (USP39)

- ~~Loss on Drying < 731 >~~

~~**Analysis:** Dry at 105° for 4 h.~~

Acceptance criteria: The hydrous form loses 7.5%–9.5% of its weight, and the anhydrous form loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **Labeling:** Label it to indicate whether it is hydrous or anhydrous.

Change to read:

- **USP Reference Standards** 〈 11 〉

- USP Caffeine RS ■ 1S (USP39)

USP Theophylline RS

- USP Theophylline Related Compound B RS

3-Methyl-1*H*-purine-2,6-dione.

C₆H₆N₄O₂ 166.14

USP Theophylline Related Compound C RS

N-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide.

C₇H₁₀N₄O₃ 198.18

USP Theophylline Related Compound D RS

Theophyllidine;

N-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide.

C₆H₁₀N₄O 154.17

USP Theophylline Related Compound F RS

Etophylline;

7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

C₉H₁₂N₄O₃ 224.22

- 1S (USP39)

BRIEFING

Sterile Purified Water, *USP 38* page 5807. In *PF 38(3)* [May–June 2012], four sterile water monographs, including *Sterile Purified Water*, were proposed to be updated. At the time, an *Oxidizable Substances* test was required. The change was to allow a *Total Organic Carbon* test as an alternative to the *Oxidizable Substances* test. The change became official in the *First Supplement to USP 36*. In the briefing of the same *PF* article, it was proposed to delete the *Oxidizable Substances* test in 18–24 months. This revision proposal deletes the option of the *Oxidizable Substances* test, and retains the *Total Organic Carbon* test that is specific to sterile waters.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(GCCA: A. Hernandez-Cardoso.)

Correspondence Number—C157681

Comment deadline: July 31, 2015

Sterile Purified Water

[Note—For microbiological guidance, see *Water for Pharmaceutical Purposes* 〈 1231 〉.]

H₂O 18.02**DEFINITION**

Sterile Purified Water is Purified Water sterilized and suitably packaged. It contains no antimicrobial agents. [Note—Do not use Sterile Purified Water in preparations intended for parenteral administration. For such purposes use Water for Injection, Bacteriostatic Water for Injection, or Sterile Water for Injection.]

SPECIFIC TESTS**Delete the following:**

- **Oxidizable Substances**

Sample: 100 mL

Analysis: Add 10 mL of 2 N sulfuric acid, and heat to a boil. For Sterile Purified Water in containers having a fill volume of less than 50 mL, add 0.4 mL of 0.02 M potassium permanganate, and boil for 5 min; where the fill volume is 50 mL or more, add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min. If a precipitate forms, cool in an ice bath to room temperature, and pass through a sintered glass filter.

Acceptance criteria: The pink color does not completely disappear. Alternatively, perform the test for *Total Organic Carbon* $\langle 643 \rangle$, *Sterile Water*. ■ 1S (USP39)

Change to read:

- **Total Organic Carbon** $\langle 643 \rangle$, *Sterile Water*: Meets the requirements. Alternatively, perform the test for *Oxidizable Substances*.

■ 1S (USP39)

- **Water Conductivity** $\langle 645 \rangle$, *Sterile Water*: Meets the requirements
- **Sterility Tests** $\langle 71 \rangle$: Meets the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in suitable tight containers.
- **Labeling:** Label it to indicate the method for preparation and to indicate that it is not for parenteral administration.

Add the following:

- **USP Reference Standards** $\langle 11 \rangle$

USP 1,4-Benzoquinone RS

USP Sucrose RS

*(ERR 1-Oct-2014)

BRIEFING

Sterile Water for Inhalation, USP 38 page 5806. In PF 38(3) [May–June 2012], four sterile water monographs, including *Sterile Water for Inhalation*, were proposed to be updated. At the time, an *Oxidizable Substances* test was required. The change was to allow a *Total*

Organic Carbon test as an alternative to the *Oxidizable Substances* test. The change became official in the *First Supplement to USP 36*. In the briefing of the same *PF* article, it was proposed to delete the *Oxidizable Substances* test in 18–24 months. This revision proposal deletes the option of the *Oxidizable Substances* test, and retains the *Total Organic Carbon* test that is specific to sterile waters.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(GCCA: A. Hernandez-Cardoso.)
Correspondence Number—C157681

Comment deadline: July 31, 2015

Sterile Water for Inhalation

[Note—For microbiological guidance, see *Water for Pharmaceutical Purposes* 〈1231〉.]

Change to read:

•H₂O 18.02•(ERR 1-Oct-2014)

DEFINITION

Sterile Water for Inhalation is prepared from Water for Injection that is sterilized and suitably packaged. It contains no added antimicrobial agents. [Note—Do not use Sterile Water for Inhalation for parenteral administration or for other sterile compendial dosage forms.]

SPECIFIC TESTS

Delete the following:

■● **Oxidizable Substances**

Sample: 100 mL

Analysis: Add 10 mL of 2 N sulfuric acid, and heat to boiling. For Sterile Water for Inhalation in containers having a fill volume of less than 50 mL, add 0.4 mL of 0.02 M potassium permanganate, and boil for 5 min; where the fill volume is 50 mL or more, add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min. If a precipitate forms, cool in an ice bath to room temperature, and pass through a sintered-glass filter.

Acceptance criteria: The pink color does not completely disappear. Alternatively, perform the test for *Total Organic Carbon* 〈643〉, *Sterile Water*. ■1S (USP39)

Change to read:

● **Total Organic Carbon** 〈643〉, *Sterile Water*: Meets the requirements. Alternatively, perform the test for *Oxidizable Substances*.

■1S (USP39)

- **Water Conductivity** 〈645〉, *Sterile Water*: Meets the requirements
- **Sterility Tests** 〈71〉: Meets the requirements

- **Bacterial Endotoxins Test** 〈 85 〉: Less than 0.5 USP Endotoxin Units/mL

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **Labeling:** Label it to indicate that it is for inhalation therapy only and that it is not for parenteral administration.

Change to read:

- **USP Reference Standards** 〈 11 〉

● USP 1,4- Benzoquinone RS ● (ERR 1-Oct-2014)

USP Endotoxin RS

● USP Sucrose RS ● (ERR 1-Oct-2014)

BRIEFING

Sterile Water for Injection, *USP 38* page 5806. In *PF 38(3)* [May–June 2012], four sterile water monographs, including *Sterile Water for Injection*, were proposed to be updated. At the time, an *Oxidizable Substances* test was required. The change was to allow a *Total Organic Carbon* test as an alternative to the *Oxidizable Substances* test. The change became official in the *First Supplement to USP 36*. In the briefing of the same *PF* article, it was proposed to delete the *Oxidizable Substances* test in 18–24 months. This revision proposal deletes the option of the *Oxidizable Substances* test, and retains the *Total Organic Carbon* test that is specific to sterile waters.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(GCCA: A. Hernandez-Cardoso.)

Correspondence Number—C157681

Comment deadline: July 31, 2015

Sterile Water for Injection

[Note—For microbiological guidance, see *Water for Pharmaceutical Purposes* 〈 1231 〉.]

Change to read:

● H₂O 18.02 ● (ERR 1-Oct-2014)

DEFINITION

Sterile Water for Injection is prepared from Water for Injection that is sterilized and suitably packaged. It contains no antimicrobial agent or other added substance.

SPECIFIC TESTS

Delete the following:

- ● ~~Oxidizable Substances~~

Sample: 100 mL

Analysis: Add 10 mL of 2 N sulfuric acid, and heat to a boil. For Sterile Water for Injection in containers having a fill volume less than 50 mL, add 0.4 mL of 0.02 M potassium permanganate, and boil for 5 min; where the fill volume is 50 mL or more, add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min. If a precipitate forms, cool in an ice bath to room temperature, and pass through a sintered-glass filter.

Acceptance criteria: The pink color does not completely disappear. Alternatively, perform the test for *Total Organic Carbon* $\langle 643 \rangle$, *Sterile Water*. ■ 1S (USP39)

Change to read:

- **Total Organic Carbon** $\langle 643 \rangle$, *Sterile Water*: Meets the requirements. Alternatively, perform the test for *Oxidizable Substances*.

■ 1S (USP39)

- **Water Conductivity** $\langle 645 \rangle$, *Sterile Water*: Meets the requirements
- **Particulate Matter in Injections** $\langle 788 \rangle$: Meets the requirements
- **Sterility Tests** $\langle 71 \rangle$: Meets the requirements
- **Bacterial Endotoxins Test** $\langle 85 \rangle$: Less than 0.25 USP Endotoxin Units/mL

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers of not larger than 1-L size. Glass containers are preferably of Type I or Type II glass.
- **Labeling:** Label it to indicate that no antimicrobial agent or other substance has been added, and that it is not suitable for intravascular injection without first having been made approximately isotonic by the addition of a suitable solute.

Change to read:

- **USP Reference Standards** $\langle 11 \rangle$

● USP 1,4-Benzoquinone RS ● (ERR 1-Oct-2014)

USP Endotoxin RS

● USP Sucrose RS ● (ERR 1-Oct-2014)

BRIEFING

Sterile Water for Irrigation, USP 38 page 5807. In PF 38(3) [May–June 2012], four sterile water monographs, including *Sterile Water for Irrigation*, were proposed to be updated. At the time, an *Oxidizable Substances* test was required. The change was to allow a *Total Organic Carbon* test as an alternative to the *Oxidizable Substances* test. The change became official in the *First Supplement to USP 36*. In the briefing of the same PF article, it was proposed to delete the *Oxidizable Substances* test in 18–24 months. This revision proposal deletes the option of the *Oxidizable Substances* test, and retains the *Total Organic Carbon* test that is specific to sterile waters.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(GCCA: A. Hernandez-Cardoso.)
Correspondence Number—C157681

Comment deadline: July 31, 2015

Sterile Water for Irrigation

[Note—For microbiological guidance, see *Water for Pharmaceutical Purposes* 〈 1231 〉.]

Change to read:

•H₂O 18.02•(ERR 1-Oct-2014)

DEFINITION

Sterile Water for Irrigation is prepared from Water for Injection that is sterilized and suitably packaged. It contains no antimicrobial agent or other added substance.

SPECIFIC TESTS

Delete the following:

■ • **Oxidizable Substances**

Sample: 100 mL

Analysis: Add 10 mL of 2 N sulfuric acid, and heat to a boil. For Sterile Water for Irrigation in containers having a fill volume less than 50 mL, add 0.4 mL of 0.02 M potassium permanganate, and boil for 5 min; where the fill volume is 50 mL or more, add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min. If a precipitate forms, cool in an ice bath to room temperature, and pass through a sintered glass filter.

Acceptance criteria: The pink color does not completely disappear. Alternatively, perform the test for *Total Organic Carbon* 〈 643 〉, *Sterile Water*. ■ 1S (USP39)

Change to read:

- **Total Organic Carbon** 〈 643 〉, *Sterile Water*: Meets the requirements. Alternatively, perform the test for *Oxidizable Substances*.

■ ■ 1S (USP39)

- **Water Conductivity** 〈 645 〉, *Sterile Water*: Meets the requirements
- **Sterility Tests** 〈 71 〉: Meets the requirements
- **Bacterial Endotoxins Test** 〈 85 〉: Less than 0.25 USP Endotoxin Units/mL

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass. The container may contain a volume of more than 1 L and may be designed to empty rapidly.
- **Labeling:** Label it to indicate that no antimicrobial agent or other substance has been added. The designations "For irrigation only" and "Not for injection" appear prominently on the label.

Change to read:

● **USP Reference Standards** 〈 11 〉

● USP 1,4-Benzoquinone RS ● (ERR 1-Oct-2014)

USP Endotoxin RS

● USP Sucrose RS ● (ERR 1-Oct-2014)

Stage 4 Harmonization

This section contains monographs or chapters undergoing harmonization by the Pharmacopoeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (**Stages 1** through **7**, as defined below). Stage 4 drafts are available for comments. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF*, this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

STIMULI TO THE REVISION PROCESS

Stimuli articles do not necessarily reflect the policies of the USPC or the USP Council of Experts

STIMULI TO THE REVISION PROCESS

This section may contain the following:

- reports or statements of Expert Committees
- original research reports
- evaluations of new and existing pharmacopeial methods
- commentaries
- articles relevant to compendial issues.

These items are published to stimulate discussion and continual review of Pharmacopeial standards. Generally, if an Expert Committee publishes an article on which they are specifically seeking comment, this will be clearly stated in the article. Readers may submit comments on issues raised in this section, but comment is not as critical as that for the *In-Process Revision* section. Readers interested in submitting comments should see *Instructions to Authors*.

Instructions to Authors

Contributions in the form of original research reports, evaluations of new and existing compendial methods, and other commentaries and articles relevant to drug standards or to *USP-NF* revision will be considered for publication in *Pharmacopeial Forum* under the section *Stimuli to the Revision Process*. Manuscripts are received with the explicit understanding that they have not been published previously in any language or medium and that they are not simultaneously under consideration by any other publication. All manuscripts are subject to review by USP headquarters staff, Committee members, or qualified outside referees, and if accepted for publication they will be subject to editing by USP staff. Accepted manuscripts become the property of the USP Convention (USPC) and may not be subsequently published elsewhere without written permission from the USPC. Authors are also responsible for obtaining permission for reprinting any illustrations that have been published elsewhere.

Abstract—Include an abstract of not more than 250 words stating the purpose and the results or conclusions of the article.

Style and Usage—*Stimuli* articles generally follow the current *Chicago Manual of Style* except in scientific usage (numbers, abbreviations, etc.). For the latter, authors should use the current *AMA Manual of Style* or the current *ACS Style Guide*. Authors may usefully consult a current edition of *Pharmacopeial Forum*.

References—Consult the current *AMA Manual of Style*, which is generally consistent with the National Library of Medicine's *Recommended Formats for Bibliographic Citation*. A current edition of *Pharmacopeial Forum* will offer examples of reference formats.

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Determination of Thermodynamic Solubility of Active Pharmaceutical Ingredients for Veterinary Species: A New USP General Chapter

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ABSTRACT This *Stimuli* article discusses the approach for the development of a new general chapter on solubility determination for veterinary drug products. Possible procedures are discussed, with emphasis on the shake-flask method. Recommendations are included on the test conditions for products to treat dogs and cattle. The Expert Panel welcomes comments from the public and stakeholders.

INTRODUCTION

The United States Pharmacopeia (USP) held the workshop "Solubility Criteria for Veterinary Products" on November 7–8, 2012, at USP headquarters (1). One of the workshop outcomes was a decision by participants that the content of the new general chapter would focus on species-appropriate descriptions of conditions for testing in vitro drug solubility. Determining the solubility of drug candidates is important in pharmaceutical research, both for the discovery phase and the development phase (2,3). In the early stages of drug research, solubility—together with other physicochemical parameters (lipophilicity, ionization, and permeability)—is used to screen drug candidates. This information is needed to support formulation development, including product optimization and salt selection. During the later phases of product development, solubility considerations are useful in planning for challenges that may be encountered when addressing formulation modifications and the associated bioequivalence issues.

BACKGROUND

In the pharmaceutical literature, two commonly used solubility terms are: 1) kinetic solubility, the concentration of a compound at the time when an induced precipitate first appears in the solution; and 2) equilibrium (or thermodynamic) solubility, the concentration of a compound in a saturated solution when an excess of solid is present, and the solution and solid are at equilibrium. A third term, intrinsic solubility, refers to the equilibrium solubility of the free acid or base form of an ionizable compound at a pH where it is fully un-ionized (2). Aqueous equilibrium solubility is the partitioning of a compound between its pure phase and water. At equilibrium, there is a dynamic, balanced process of dissolution of the solid into solution and recrystallization of the drug from solution. The molar aqueous solubility (S_W) can be empirically correlated to activity and crystal lattice energy via the general solubility equation (GSE) proposed by Yalkowsky (4,5):

$$\log S_W = -0.01(MP-25) - \log K_{OW} + 0.5$$

MP = melting point

K_{OW} = octanol-water partition coefficient

There are multiple computational tools for prediction of aqueous solubility including ACD Labs, COSMO-RS, QikPro, and others (6)

As a physicochemical property, solubility is influenced by: temperature; pressure; purity of materials; composition of buffer solutions and properties of the compound, such as polymorphism; aggregation; and the formation of supersaturated solutions. The effect of pH on the solubility of ionizable compounds is a well-known and an extensively examined phenomenon (2,7). For poorly soluble compounds, determining the solubility in the presence of various solubilizing agents presents a special set of challenges (7).

When solubility is measured, the value obtained is also influenced by several experimental factors, including: stirring time, sedimentation time, composition of the aqueous buffer, temperature, amount of solid excess, and the technique used for phase-separation (2).

EXPERIMENTAL METHODS

Saturation Shake-Flask Method

The shake-flask method is based on the phase solubility technique that was developed 40 years ago and is still considered by most to be the most reliable and widely used method for solubility measurement today (2,3,8-10).

The sample is typically prepared by adding an excess of solid to the solubility medium, which is in a stoppered flask or vial. The amount of medium in the flask or vial does not need to be measured accurately. Although it is important to ensure that the amount of added material is sufficient to produce a suspension, it is also important to avoid adding an amount of material that will significantly alter the properties of the solubility medium, including its pH (7,8). The pH of the suspension should be verified after the addition of the compound and at the end of the experiment (3). It is generally agreed that saturation or equilibrium has been reached when multiple samples, assayed after different equilibration time periods, yield the same result for apparent solubility.

The time to reach equilibrium can vary as a function of the type of agitation used, the active ingredient properties, the amount of material used, and the equilibration method used. With an agitation rate that is adequate to prevent particle agglomeration and to ensure particle contact with the diluent, samples generally reach equilibrium quickly (often within 24 h). However, for poorly soluble compounds, the equilibrium time may be prolonged, well beyond 24 h, because of a poor dissolution rate that is further depressed as the equilibrium process advances and the concentration in solution gets closer to the limit of drug solubility. One way to expedite the process is to increase the effective surface area for dissolution. This can be achieved by either vortexing or sonicating the samples prior to equilibrium evaluation.

Other challenges in determining the solubility of poorly soluble compounds are their poor wettability and their tendency to float. Potential remedies include the use of small glass microspheres to de-aggregate the particles with agitation or sonication, or adding a quantity of surfactant that is below the critical micelle concentration to serve as a wetting agent. If the addition of surfactant causes too much foaming, a small amount (just 1 or 2 drops) of an appropriate defoaming agent, such as *n*-octanol, can be added.

Filtration and centrifugation are both commonly used to separate the saturated solution from the solute phase. However, filter sorption can be a significant source of error. Generally, filter sorption is most critical for hydrophobic and poorly soluble compounds, and as expected filter

sorption is directly proportional to filter surface area (7).

High performance liquid chromatography (HPLC) is the most commonly used analytical tool for the analysis of saturated solutions. The advantage of HPLC over the spectrophotometric method is that HPLC can detect impurities and any instability (7,8).

pH–Solubility Profile

For drugs with ionizable functional groups, determining solubility as a function of pH is very important. Typically, there are two ways to control pH. One approach is to use buffers. Because multiple buffer systems are needed to control the entire pH range, the solubility results may be complicated by salt formation with the buffer species. This can be detected by examining the residual solid from solubility determinations.

Another way to control pH is through the use of a pH-stat, where pH is controlled by titrating with acidic and/or basic solutions. Ionic equilibrium can be monitored continuously by measuring the solution pH. Equilibrium has been reached, it is generally assumed, when the pH no longer changes over a period of time.

Solubility of Salts

Use of the equilibrium method for determining the solubility of pharmaceutical salts may be challenging for certain compounds, such as those with poor intrinsic solubility. Theoretically, after an excess amount of solid salt is equilibrated in water, the solution concentration at equilibrium should represent the solubility of the salt. However, this is only true if the pH of the saturated solution is below pH of maximum solubility (11). For compounds with low intrinsic solubility and weak basicity or acidity, their salts may convert to the un-ionized form in the solubility medium. In such cases, the measured solubility is only the solubility of the un-ionized form at those particular pH values.

When determining the solubility of salts in simulated gastric fluid, or in pH 1 or pH 2 hydrochloric acid solutions, the salt may convert to the hydrochloride salt, depending on the relative solubility of the salts. If the simulated gastric fluid contains sodium chloride, the common ion effect of the chloride ion may significantly depress the solubility of the hydrochloride salt (7).

Potentiometric Titration

The potentiometric acid–base titration for the solubility measurements is based on a characteristic shift in the middle of the titration curve that is caused by precipitation. For the titration, accurate volumes of a standardized acid or base are added to a solution containing an ionizable substance and 0.15 M potassium chloride (KCl), which is included to increase the accuracy of the measurements. Sparging (a technique that involves bubbling a chemically inert gas, such as nitrogen, argon, or helium, through a liquid) with argon prevents CO₂ from the atmosphere from influencing the pH value. A glass electrode is used to monitor the pH value continuously. The potentiometric titration curve is obtained by plotting the pH value against the consumed volume of acid/base (8).

Turbidimetry

Turbidimetry involves the dissolution of a compound in an organic solvent, typically dimethyl sulfoxide (DMSO). The resulting solution is added to a pH 7 buffered solution in 1-min intervals. Further aliquots of the solution are added after the first detection of turbidity by light

scattering. Subsequently, the volume added can be plotted against the turbidity. The solubility is then estimated by back-extrapolation to the point where precipitation began. This method can be used to measure as many as 50–300 samples/day. The drawbacks include the use of DMSO, which can increase the solubility to an unknown and unpredictable extent, and the short duration of the experiment, which leads to a kinetic rather than thermodynamic estimate of the solubility. Because DMSO dilutions readily form a supersaturated solution, the kinetic measurement can easily overestimate the equilibrium (thermodynamic) solubility (8).

Miniaturization, High-Throughput, and Automation in Solubility Measurement

Solubility is not only important in pre-formulation studies. It is also important in lead selection and optimization during drug development. Thus, it is desirable to have methods that can determine equilibrium solubility with as little compound as possible and with the necessary high-throughput characteristics that will enable the method to be used in support of lead optimization (7).

To this end, Glomme et al. (8) developed a miniaturized version of the shake-flask method. The procedure is almost identical to the traditional shake-flask method but Glomme's small scaled version allows for the use of very small solvent volumes (e.g., 0.5–2 mL, depending upon the equipment used).

There are also several computer programs that will support the numerical estimation of compound solubility based upon the chemical structure of the compound. Although some error in the estimations may exist, the accuracy of the calculated values is generally adequate to support further synthesis plans (8).

Systems that automate all the steps to measure equilibrium solubility have also been developed and are commercially available (7).

TEST CONDITIONS

The test conditions (media composition, temperature, and others) will be defined by animal species. Initially, the *USP* general chapter will address the conditions used for dogs and cattle, and later it will be expanded by adding other animal species through future revisions of the chapter.

Dogs

Media composition: Buffer solutions prepared according to USP–NF instructions in the section *Reagents, Indicators and Solutions; Buffer Solutions*.

pH: 1.2, 4.6, and 6.8

Temperature: 39°

Time: Time to equilibrium (or saturation) or at least 24 h

Cattle

Media composition and pH: Hydrochloric acid, pH 2.5; acetate buffer, pH 3.5; phosphate

buffer containing 40 mM acetic acid, 35 mM propionic acid, and 15 mM butyric acid, pH 5.0; phosphate buffer with no surfactant, pH 6.8; phosphate buffer containing 70 mM acetic acid, 15 mM propionic acid, and 10 mM butyric acid, pH 6.8

Temperature: 39^o

Time: Time to equilibrium (or saturation) or at least 24 h

The following conditions are suggested when preparing the bovine solubility medium (12):

A. Preparation of short chain fatty acid (SCFA) stock solution (see *Table 1*).

Table 1

SCFA	mL/L	Density (g/mL)	MW ^a (g/mole)	Concentration (mM)
Acetic acid (Ac)	6	1.0491	60.05	104.8
Propionic acid (Pr)	4	0.9920	74.08	53.6
Butyric acid (Bu)	2	0.9640	88.10	21.9

^a MW, molecular weight.

B. Dilution of stock solution used to obtain SCFA concentration for specific pH (see *Table 2*)

Table 2

	DF ^a	Concentration SCFA (mM)
pH 6.8	—	—
Ac ^b	1.5	70
Pr	3.6	15
Bu	2.19	10
pH 5.0	—	—
Ac	2.62	40
Pr	1.53	35
Bu	1.46	15

^a DF: Dilution factor of the stock solution used to obtain the desired SCFA concentration.

^b Ac, Acetic acid; Pr, Propionic acid; Bu, Butyric acid.

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Performance Test for Parenteral Dosage Forms

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ABSTRACT

Procedures and acceptance criteria for testing parenteral drug products are divided into two categories: 1) those that assess product quality attributes, e.g., identification, sterility, and particulate matter, and are contained in general chapter *Injections and Implanted Drug Products* (1); and 2) those that assess product performance, e.g., in vitro release of the drug substance from the drug product. A product performance test, i.e., drug release test, should be carried out using appropriate test parameters and procedures. This *Stimuli* article addresses performance tests for parenteral drug products and the various methodologies.

INTRODUCTION

Parenteral drug products include solutions, suspensions, emulsions, sterile powders for solutions or suspensions (including liposomes), implants (including microparticles), and products that consist of both a drug and a device, such as drug-eluting stents. Two categories of tests, product quality and product performance, are performed on such drug products. These tests provide assurances of batch-to-batch quality, reproducibility, reliability, and performance. Product quality tests are performed to assess attributes, such as assay, identification, and content uniformity, and are part of the compendial requirements (see general chapter *Injections and Implanted Drug Products* (1)). Product performance tests are conducted to assess drug release from the dosage form.

Parenteral drug products such as solutions, suspensions, and emulsions are relatively simple in comparison to microsphere and liposome preparations. For the less complex products, a simple drug-release test performed with Apparatus 2 (paddle method) can be used. However, the use of Apparatus 4 (the flow-through cell) may be a preferred method for coarse suspensions or emulsions. Dialysis sac and reversed dialysis sac methods can also be used for emulsions. Parenteral products, such as microspheres and liposomes, are complex in nature, which complicates the development and validation of assays for in vitro drug release. Methodologies can be developed, validated, and standardized, but because of the complexity of the various types of parenteral drug products, a single method for determining drug release is not currently feasible, although a significant amount of research is being conducted in this regard. An American Association of Pharmaceutical Scientists workshop report recommended the use of Apparatus 4 for evaluating the release profile of novel systems, such as nanoparticles and liposomes (1).

In the development of dissolution/drug-release tests or the selection of the drug-release medium, apparatus, procedure, or analytical method, consult general information chapter *The Dissolution Procedure: Development and Validation* (1092). In developing the performance test specifications, one should consider the mechanism of drug release as well as the drug's mechanism of action (2). In vitro release (IVR) is a key performance attribute used to evaluate and demonstrate product performance; IVR is used for process and product control. Long-

acting drug products are typically designed to release the drug over an extended period of time. Accelerated methods are necessary to assist in the evaluation of these drug products, without affecting the drug-release mechanism (3,4).

PERFORMANCE TEST METHODS FOR PARENTERAL DOSAGE FORMS

For most parenteral drug products, the performance test includes drug release from the formulation. The performance test may include the dialysis sac, reverse dialysis sac, manual shaker bath (sample and separate), paddle method (Apparatus 2), flow-through system (Apparatus 4), or other methods for testing in vitro release. Standardized methods such as Apparatus 2 and 4 are preferred to allow data comparison between laboratories.

In Vitro Drug-Release Testing Using the Dialysis Sac Method

The dialysis sac method involves placing the formulation (e.g., liposomes) into a dialysis sac or tube. This dialysis sac is then placed in a large volume of release medium (receiver chamber). The receiver chamber is stirred to ensure mixing of the released drug. However, the inside of the dialysis sac is not stirred, and this can result in aggregation problems as well as data that cannot be reproduced. The drug released from the formulation diffuses through the dialysis sac membrane, and the drug concentration is determined by taking samples from the release medium at appropriate time intervals. The temperature of the entire chamber is maintained at $37 \pm 2^{\circ}$ using a water bath (5).

SELECTION OF MEMBRANE

Cellulose ester, regenerated cellulose, and polyvinylidene difluoride are the most commonly used membranes for the dialysis sac. The membrane should have appropriate molecular weight so that drug diffusion through the membrane is not a rate-limiting step.

CONDITIONING OF MEMBRANE

The dialysis membrane should be conditioned through soaking in the release medium for an appropriate length of time. This helps remove any extractable substance from the membrane that could potentially interfere with the analysis.

SAMPLE INTRODUCTION

The formulation can be introduced to the dialysis sac using a syringe or fine-tip pipet. The formulation can be placed in the dialysis sac directly or can be mixed with a small volume of release medium.

AGITATION

Agitation can be achieved using a shaker water bath or a magnetic stirrer. Appropriate control of hydrodynamics is important for reproducible results.

TEMPERATURE

The temperature of the entire chamber is usually maintained at 37° . The temperature should be calibrated using a certified thermometer and held at $37 \pm 0.5^{\circ}$ throughout the test.

VOLUME FOR DRUG-RELEASE TESTING

When selecting the volume of the medium outside of the dialysis sac, the analyst should take into account the solubility of the active pharmaceutical ingredient (API) and sink conditions. Replenishing the medium at various stages of the test may be necessary to achieve sink conditions. When drug release is rapid, it may be impossible to maintain sink conditions within the dialysis sac, and as a result, the dialysis sac becomes a rate-limiting membrane (6,7).

SAMPLING PARAMETERS

Sampling time points should be chosen to adequately describe any initial burst release and provide an adequate number of data points to create a drug release profile. Sampling should occur within ± 2 min for time points up to 12 h; within ± 5 min for time points between 12 and 24 h; and within ± 30 min for later time points. It is important that the medium is mixed adequately during sampling so that a representative sample is obtained. The medium may be replenished after sampling.

MEDIUM FOR DRUG-RELEASE TESTING

The medium should be chosen with consideration of the physical and chemical characteristics of the drug substance and dosage unit. See chapter (1092) for further guidance. The medium should be degassed using an appropriate technique unless the use of surfactants is necessary. A specified volume of medium should be transferred accurately to the receiver chamber. Media should be stirred throughout the test.

ANALYSIS AND CALCULATIONS

The assay for a dissolution sample is HPLC, although other methods, such as spectrophotometry, may be used if feasible. On-line fiber optic UV probes are useful for fast-releasing formulations because complete characterization of the release profile is possible (more data points can be collected in short time intervals).

A cumulative release profile can be obtained by analyzing samples at appropriate time points; the concentration ($\mu\text{g/mL}$ or mg/mL) of the analyte is determined at each time point using appropriate analytical methods. The concentration is then multiplied by the total volume of the dissolution medium, thereby determining the total amount released during different time intervals. This allows determination of the cumulative amount released. Calculations should account for all dissolution medium adjustment and replenishments.

In Vitro Drug Release Using the Reverse Dialysis Sac Method

The reverse dialysis sac method involves placing the formulation directly into an appropriate volume of release medium in a suitable chamber. Two or more dialysis sacs are placed in the release medium. At appropriate time intervals, one dialysis sac is removed, and the drug concentration is analyzed to calculate the percentage of release. This approach helps to maintain sink conditions because the formulation is diluted in the large volume of release medium outside the dialysis sacs (5,6,8). In addition, there are no problems of aggregation of the delivery system due to lack of direct stirring within the dialysis sacs [as can occur in standard (i.e., not reverse) dialysis methods].

In Vitro Drug Release Using Apparatus 2

The use of Apparatus 2 may be an effective way to perform in vitro drug-release testing for some parenteral formulations. Depending on the hydrophobic/hydrophilic nature of the drug product and its molecular weight, floating and/or aggregation of the formulation may occur. When aggregation occurs, it is justified to use another apparatus to perform the in vitro release testing. Modifications to the medium during a run are more challenging with Apparatus 2 than with Apparatus 4, and for this reason Apparatus 2 may not be a viable method. Typically, standard 1-L dissolution vessels are used with standard medium volumes (500–1000 mL). The temperature of each vessel is maintained at $37 \pm 2^{\circ}$. A set of six vessels is used for each test.

SAMPLE INTRODUCTION

The dosage form is introduced into the medium using typical sample handling tools such as pipets, spatulas, or weighing boats. Other means of introduction should be well specified, repeatable, and reproducible.

AGITATION

Rotation speeds of 50–100 rpm should be used, although the use of other rotation speeds may be warranted.

SINK CONDITIONS

Replenishing medium at various stages of the test may be required to achieve sink conditions. Changing the medium volume at various stages may also be necessary. The exact manner for doing these should be specified.

SAMPLING PARAMETERS

Sampling time points should be chosen to adequately describe any initial burst release and provide an adequate number of data points for the drug-release profile. Sampling should occur within ± 2 min for time points up to 12 h; it should occur within ± 5 min for time points between 12 and 24 h; and it should occur within ± 30 min for later time points. An accurately determined volume is withdrawn from each vessel, preferably with the paddles still rotating. The medium may be replenished after sampling.

DISSOLUTION MEDIUM

The medium is chosen by considering the physical and chemical data for the drug substance and dosage unit. See chapter [1092](#) for further guidance. The effect of deaeration should be determined. If deaeration affects the dissolution rate, the medium should be degassed using an appropriate technique. Evaporative loss of medium should be minimized. Replenishment of evaporated medium may be necessary for tests of long duration.

ANALYSIS AND CALCULATIONS

The usual assay for a dissolution sample is HPLC, although other methods, such as spectrophotometry, may be used if feasible. When performing calculations, the analyst should take into consideration all of the dissolution medium adjustments and replenishments.

In Vitro Drug Release Using Apparatus 4

The use of the flow-through cell, Apparatus 4, may be an effective procedure for performing in vitro drug-release testing of some parenteral formulations that are not clear solutions. Typically, the standard tablet cells, 12.0 and 22.6 mm, are used without the tablet clip present, and the unit is operated in the closed-system configuration. The use of other cell designs is allowable. Dissolution medium composition should be selected on the basis of the physical and chemical properties of the drug substance or its API. The flow rate through the cell can also depend on the application. The temperature of the cells is usually maintained at $37 \pm 2^{\circ}$. A set of six cells is used for each test.

SAMPLE PREPARATION IN THE CELLS

The standard cell is initially fitted with a 5-mm bead to stop back-flow. One approach can be to add glass beads (typically 1-mm borosilicate glass) to fill the cell up to the intersection of the conical part and the cylindrical part of the cell. Then, an accurately weighed portion of pharmaceutical product to be investigated (e.g., suspension) is added to the cell. Depending on the product and the possibility of aggregation, it may be necessary to disperse the dosage form, typically with more glass beads. The use of additional glass beads to disperse the product also helps to prevent channeling of the fluid through areas of the product (as can occur in a suspension bed), because this can result in non-wetting of portions of the product and a subsequent lack of reproducibility of the data. For nanoparticulate systems, the use of a dialysis sac adapter may be considered. This allows dispersion of the nanoparticulates within the dialysis sac, together with a volume of media as in the "normal" dialysis method discussed above. The media flows around the dialysis sac, which is a preferred dialysis method for nanoparticulates, because it uses a standardized apparatus. Nanoparticulates cannot be suspended directly in the USP Apparatus 4 flow-through cell, because they would either pass through or block the filter material, resulting in sample loss from the flow-through cells and/or system back-pressure and hence failure (7).

FILTER MATERIAL

The filter head for the flow-through cell should be fitted with an appropriate filter material. The filter material should be durable enough so as to not physically degrade over the duration of the test. Typical filter materials, such as glass fiber filters, polyvinylidene fluoride, polytetrafluoroethylene, and regenerated cellulose can be considered. Filter studies for drug adsorption should be performed before testing. If the filter pore size is too small, unacceptable back-pressure can result in the system.

FLOW RATE

The flow rate may influence the drug-release results (for diffusion controlled systems). For this reason, the effect of flow rate on drug release should be examined. Typical flow rates include 4, 8, and 16 mL/min, although other flow rates may be used.

VOLUME FOR DRUG-RELEASE TESTING

The volume of medium used in a closed-loop configuration can vary from as low as 20 mL to several liters. When operating the system in a closed-loop setting, the volume of the medium

should be selected with consideration of the solubility of the API and sink conditions. The volume of medium can be adjusted during the same test to achieve a proper concentration. Replacement of medium at various stages of the test may be required to achieve sink conditions. Media replacement may also be conducted as part of the test; an example is changing the media to mimic environmental changes that can occur in vivo (e.g., cellular uptake of liposomes or other nanoparticles) (8).

SAMPLING PARAMETERS

Sampling time points should be chosen to adequately describe any initial burst release and provide an adequate number of data points for creating the drug-release profile. Sampling should occur within ± 2 min for time points up to 12 h; within ± 5 min for time points between 12 and 24 h; and within ± 30 min for later time points. An accurately determined volume is withdrawn from each media bottle, preferably with the pump still running. It is important that the medium is mixed adequately during sampling so that a representative sample can be obtained. The medium may be replenished after sampling.

MEDIUM FOR DRUG-RELEASE TESTING

When choosing the medium, the analyst should consider the physical and chemical characteristics of the drug substance and dosage unit. See chapter [1092](#) for further guidance. The medium should be degassed using an appropriate technique, unless the use of surfactants is necessary. A specified volume of medium should be accurately transferred to each of the six bottles. Media should be stirred throughout the test. Typically, the medium is not heated before or during the test.

ANALYSIS AND CALCULATIONS

The assay for a dissolution sample is HPLC, although other methods (such as spectrophotometry) may be used if appropriate. Fiber optic UV probes can be used with USP Apparatus 4. On-line fiber optic UV probes enable complete characterization of the initial burst release phase, because more data points can be collected in short time intervals. The release profile can be monitored using fiber optic UV analysis without any interference from the formulation (e.g., microspheres), because the formulation is isolated from the media in USP Apparatus 4.

Typically, release testing of microsphere formulations is performed in a closed-system configuration with USP Apparatus 4. An open system is not practical when long-term testing is required, as with some microsphere products. A cumulative release profile can be obtained in a closed-system configuration by analyzing samples at appropriate time intervals. The change in concentration ($\mu\text{g/mL}$ or mg/mL) determined for each time interval (using an appropriate analytical method), multiplied by the total volume of the dissolution medium, gives the total amount released at different time intervals. The cumulative amount released can be determined in this manner. Calculations should take into account all dissolution medium adjustments and replenishments.

PERFORMANCE TEST FOR SPECIFIC TYPES OF DOSAGE FORMS

A. Emulsions and Suspensions

For emulsions or suspensions, use either the paddle method (Apparatus 2) or the flow-thru

method (Apparatus 4). See chapter < 1092 > for further guidance.

B. Microspheres

The in vitro drug-release test should characterize the mechanism of release and should differentiate between the different release phases of the product.

Sample, separate, and dialysis sac methods have been used conventionally for performance testing of microspheres. However, these methods have the disadvantages of microsphere loss during sampling and aggregation because of improper agitation.

The flow-through cell (USP Apparatus 4) may be used for release testing of microsphere formulations. The microspheres dispersed with the glass beads are packed in the flow-through cells. This modification of the flow-through cell is useful in minimizing aggregation of microspheres and separating them from the release media (9,10).

The standard tablet cells, 12.0 and 22.6 mm, may be used without the tablet clip, and the unit is operated in the closed-system configuration or the open configuration. Appropriate flow rates can be selected, depending on the formulation and application. The temperature of the cells is usually maintained at $37 \pm 2^\circ\text{C}$. For accelerated testing, higher temperatures can be used. A set of six cells is used for each test.

C. Nanoparticles/Nanocrystals

Dissolution of nanoparticle suspensions can be measured by using sampling-separation technique or by monitoring changes in the light-scattering device (11).

D. Liposomes

Membrane dialysis methods, such as dialysis sac and reverse dialysis sac, are conventionally used for performance testing of liposomes. These methods are needed for separating liposomes from the release media. However, these methods are not based on compendial dissolution/release apparatuses. The procedure and apparatus used for dialysis sac and reverse dialysis sac methods vary among laboratories, and results from different laboratories may not be comparable. Therefore, a dialysis method based on a compendial dissolution/release apparatus may be more appropriate for performance testing of liposomes.

USP Apparatus 4, using a flow-through cell fitted with a dialysis adapter, may be used for performance testing of liposome formulations. An adapter has been designed for the 22.6-mm diameter flow-through cell. A dialysis membrane is placed over the adapter, and this assembly is then placed over the conical part of the flow-through cell. The method has been shown to be superior to dialysis and reverse dialysis sac methods for liposomes containing the hydrophobic drug dexamethasone in terms of reproducibility and discriminatory ability (7).

The flow-through cell (USP Apparatus 4), fitted with a dialysis adapter, may be used for performance testing of liposome formulations. However, if placed directly in the flow-through cell, the liposomes (nanometer size range) may either block the filter or pass through it. The dialysis adapter may be used with 12- or 22.6-mm diameter flow-through cells. Flow-through cell size may be selected based upon the drug concentration in the formulation and the volume of formulation to be used for release testing. Higher volumes can be used with the 22.6-mm diameter flow-through cell.

The dialysis adapter framework consists of a circular top and bottom supported by three wires of a suitable material (such as peek, metal, or others). The circular top has an opening for

sample introduction, and this opening can be closed with a leak-proof screw. A dialysis membrane/bag is placed over this adapter frame and sealed with "O" rings. This assembly is then placed over the conical part of the flow-through cell. This method offers several advantages: 1) uniform and adequate agitation outside the dialysis bag; 2) uniform temperature in the sample cell per USP requirements; and 3) low variation among the replicates (7). The standard tablet cells, 12.0 and 22.6 mm, may be used without the tablet clip present, and the unit may be operated in the open- or closed-system configuration. Appropriate flow rates can be selected, depending on the formulation and application. The temperature of the cells is usually maintained at $37 \pm 2^\circ$. A set of six cells may be used for each test.

E. Implants

The flow-through cell (USP Apparatus 4) may be used for release testing of implant formulations (12). The implants may be held in the flow-through cell with a special holder. The standard tablet cells, 12.0 and 22.6 mm, may be used without the tablet clip when the unit is operated in the closed-system configuration or in the open configuration. Appropriate flow rates can be selected, depending on the formulation and application. The temperature of the cells is usually maintained at $37 \pm 2^\circ$. For accelerated testing, higher temperatures can be used. A set of six cells is used for each test.

F. Drug-Eluting Stents

USP Apparatus 4 and 7 with stent holders may be used for performance testing of drug-eluting stents. Small volumes of release media can be used with both of these systems (about 15 and 4 mL for USP Apparatus 4 and 7, respectively).

Small-volume dissolution apparatuses, with volumes of 1–4 mL, have been used to measure drug release from medical devices, such as drug-eluting stents. The instrument features a magnetically driven reciprocation mechanism and heater jackets to determine the rate of drug release from stents.

G. In Situ Forming Gels

In situ forming gels are very challenging because of problems associated with gelling in vitro; a suitable depot must be formed without emulsification or sample dispersion occurring. USP Apparatus 2 and 4 have been used. Most in vitro release tests have been performed using variants of the sample-and-separate methodology. In addition, the dialysis method has also been used to evaluate in situ depot-forming formulations. When performing an in vitro release test, the pregelled formulation is usually held in a special retainer to achieve a defined geometry or area of formulation–buffer interface. Alternatively, the already formed formulation can be placed into the release medium using a syringe (13).

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Pharmacopeial Forum is covered in *Current Contents/Life Sciences* and in the *Science Citation Index (SCI)*, in *International Pharmaceutical Abstracts*, and in *Current Awareness in Biological Sciences*.

The United States Pharmacopeial Convention comprises representatives from colleges and national and state organizations of medicine and pharmacy. It publishes the *U.S. Pharmacopeia* and *National Formulary*, the legally recognized compendia of standards for drugs and products of other health care technologies. The *USP* and *NF* include assays and tests for the determination of strength, quality, and purity and requirements for packaging and labeling. **Pharmacopeial Forum's Public Review and Comment Process**

This section presents an overview of the public review and comment process, conducted through *Pharmacopeial Forum (PF)*, for the development of official standards in the *United States Pharmacopeia* and the *National Formulary (USP-NF)*.

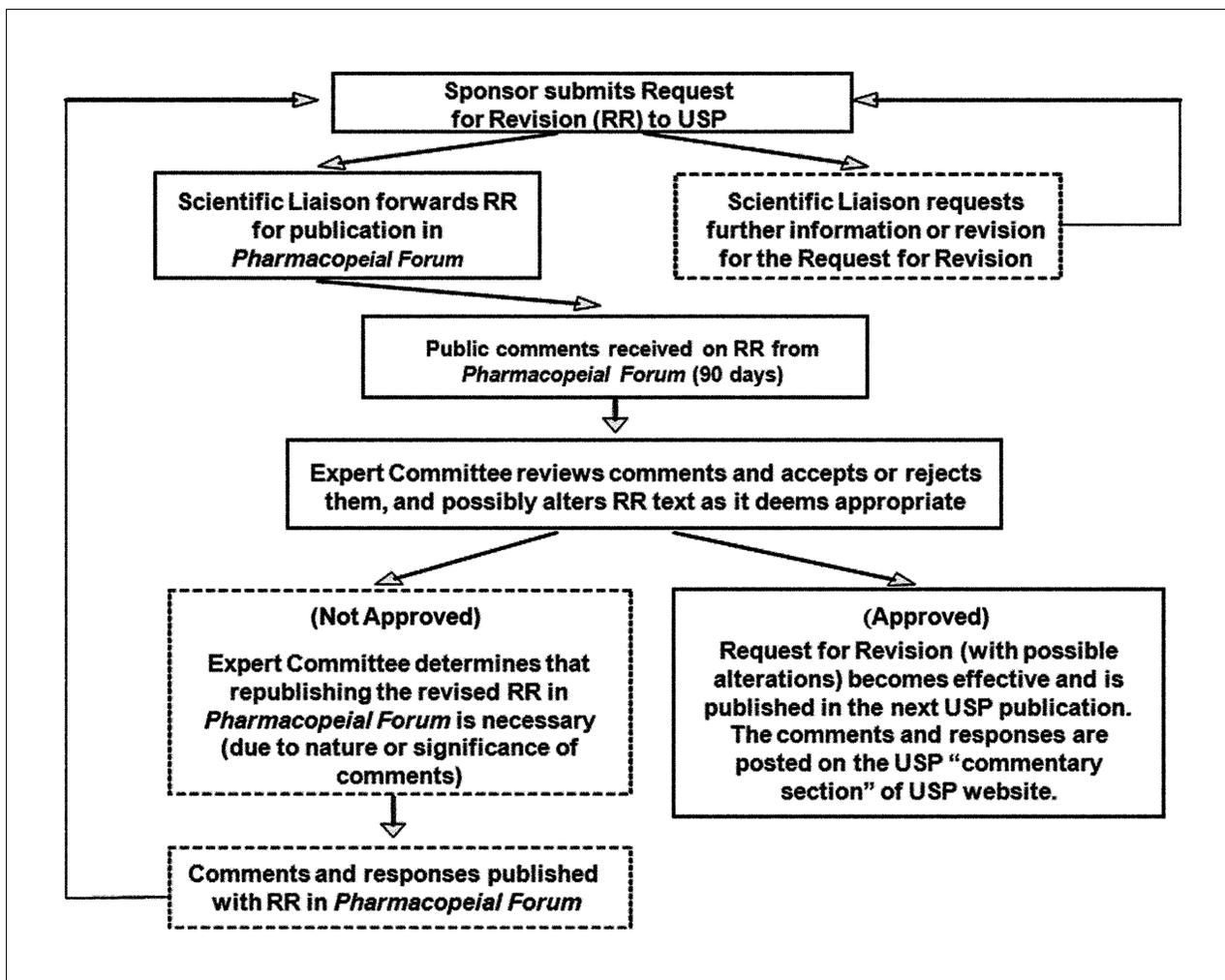
USP publishes *PF* on a bimonthly basis to provide an opportunity to review and comment on new or revised standards.

There are two types of proposed revisions in *PF*:

1. **Proposed Revisions**—New or revised standards targeted for adoption through USP's Standard Revision Process. USP's Revision Process calls for publication of a proposed revision in *PF* for a 90-day notice and comment period. After the comment period and subsequent review of comments and approval by the relevant USP Expert Committee, the official standard is published in the next available *USP-NF* or *Supplement*. If comments received are significant, or if the Expert Committee's consideration of comments results in significant additional changes, the Expert Committee may determine that republishing in *PF* is necessary prior to the revision becoming official. See the *In-Process Revision* section for current proposed revisions.
2. **Proposed Interim Revision Announcements**—New or revised standards that become official through an accelerated process in accordance with USP's Guideline on Accelerated Revisions (available on the USP Web site). *Interim Revision Announcements (IRAs)* allow for a revision to become official prior to the next *USP-NF* or *Supplement*. *IRAs* are first presented for a 90-day public comment period in the *Proposed Interim Revision Announcement* section of the *PF*. Note that final *IRAs*, as well as *Errata*, and *Revision Bulletins*, which also are defined in the Accelerated Revision Guideline, appear only on the USP Web site.

USP welcomes comments and data on proposed revisions. A summary of comments received, along with USP's responses, will be published in the *Revisions and Commentary* section of the USP website (<http://www.usp.org/USPNF/revisions/>).

The chart below shows the public review and comment process and its relationship to standards development.



Questions on the process should be addressed to the USP Executive Secretariat, United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852 (e-mail: execsec@usp.org).

HOW TO USE PF

The various sections of *PF* are briefly described below. A more detailed description of each section is provided at the beginning of that section. A general description of the types and amount of information expected in a *Request for Revision* is available in the *Guideline for Submitting Requests for Revision to the USP–NF* on the USP Web site (www.usp.org/USPNF/submitMonograph/subGuide.html). Note that the Expert Committee listing and the Scientific Staff Directory also are located on the USP Web site (see below for links).

Proposed and Adopted Revisions to the USP–NF

Section	Content	How Readers Can Respond
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Section	Content	How Readers Can Respond
Proposed Interim Revision Announcements	<p>Proposals for <i>Interim Revision Announcements (IRAs)</i> that will be published as official <i>USP</i> or <i>NF</i> standards</p> <p>BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Expert Committee and Scientific Liaisons who handled the monograph or general chapter.</p>	<p>Review material and send comments within 90 days of the <i>PF</i> publication in which the standard was proposed (or per the comment deadline listed in the Briefing section). Direct comments to the USP Scientific Liaison using the contact information provided at the end of each <i>Proposed IRA</i></p>
In-Process Revision	<p>Proposals for standards that will be published as official in a future <i>USP-NF</i> book or <i>Supplement</i>.</p> <p>BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Expert Committee and USP Scientific Liaisons who handle the monograph or general chapter.</p>	<p>Review material and send comments within 90 days of the <i>PF</i> publication in which the revision was proposed (or per the comment deadline listed in the Briefing section). Direct comments to the USP Scientific Liaison using the contact information provided at the end of each proposed revision. For general inquiries or in cases where a Scientific Liaison is not identified, use the general USP telephone number 301-881-0666 or fax number 301-998-6839 or stdsmonographs@usp.org.</p>

Section	Content	How Readers Can Respond
Stage 4 Harmonization	Revision proposals from the Pharmacopoeial Discussion Group (PDG), which comprises the European Pharmacopoeia, the Japanese Pharmacopoeia, and USP. The Stage 4 draft and the briefing are published in the forum of each pharmacopoeia for public comment. The draft is published in its entirety. BRIEFING: Scientific rationale for the potential inclusion or change or for the proposed change.	Review material and provide comments to the USP Scientific Liaison using the contact information provided at the end of each Stage 4 Harmonization. Individuals who wish to correspond with the European and Japanese Pharmacopoeias concerning monographs in the Stage 4 period of international harmonization should address their comments to the coordinating pharmacopoeia, with a copy to USP. PhEur Secretariat Ms. Lynn Kelso-Eleuterio Central Secretariat European Pharmacopoeia Department European Directorate for the Health Care Council of Europe 7, Allée Kastner CS 30026 67081 Strasbourg France Tel: +33 (3) 88 41 31 48 Fax: +33 (3) 88 41 27 71 lynn.kelso@edqm.eu JP Secretariat Mr. Issei Takayama Technical Officer Pharmaceuticals and Medical Devices Agency (PMDA) Shin-kasumigaseki Building 3-3-2, Kasumigaseki, Chiyoda-ku Tokyo, 100-0013 Japan Phone: +81-3-3506-9431 Fax: +81-3-3506-9440 takayama-issei@pmda.go.jp
Stimuli to the Revision Process	Articles on standards development topics authored by the USP Council of Experts, USP staff, or other interested parties on which USP desires public input prior to further development.	Review material and provide comments to the recipient indicated (usually footnoted in each <i>Stimuli</i> article).

Other Sections

Expert Committees

A listing of the 2010–2015 Expert Committees that work on the development of USP compendial standards

(<http://www.usp.org/aboutUSP/governance/councilOfExperts/expertCommittees.html>)

Staff Directory

Names and contact information of key USP scientific staff members who work on the development of USP compendial standards

(<http://www.usp.org/USPNF/devProcess/contactScientists.html>)

Proposed Interim Revision Announcements

This section includes proposals for *Interim Revision Announcements (IRAs)* that will be published as official *USP* or *NF* standards. There is a 90-day comment period for these proposals (see <http://www.usp.org/USPNF/pf/pfRedesign.html> for the *PF* comment schedule). Readers should review material in this section and provide comments to the Scientific Liaison using the contact information appearing at the end of each proposed *IRA*. Information on how to comment can be found under the "Participation" section of www.usp.org. It is important to send comments promptly so that the Expert Committee members can consider reader's input as they are deciding whether to advance standards to official status. The approved official text will be published under *IRAs* in the "New Official Text" section of USP's Web site.

Each proposal is preceded by a Briefing that indicates the proposed revisions.

Symbols—New text is enclosed in symbols and set off from the current official text as shown in the following example:

◆new text◆

Where the symbols appear together with no enclosed text, such as

◆◆

, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the date the proposal, if approved, will become official as an *IRA*. For example, ◆(IRA 1-Apr-2011)

BRIEFING

Methocarbamol, *USP 38* page 4313 and the *Revision Bulletin* posted on the USP website with an official date of May 1, 2015. The monograph does not have individual limits for the specified impurities although they are listed with specific relative retention times. It is interpreted that these specified impurities are controlled at any individual unspecified impurity limit. This interpretation results in the total impurities in the drug substance being more than the total degradation products limit in the *Methocarbamol Tablets* monograph. Per International Conference on Harmonization guidelines the impurities in the drug substance should be either equal to or less than the limits in the dosage form but never more than the dosage form. USP has received a request from a manufacturer to revise the monograph with their approved limits. To address these inconsistencies, it is proposed to revise the monograph as follows:

1. Specify the acceptance criteria for the limit of guaifenesin to NMT 0.15%.
2. Specify the acceptance criteria for the limit of methocarbamol isomer and methocarbamol dioxolone to NMT 0.05%.
3. Revise the acceptance criteria for the total impurities from NMT 2.0% to NMT 1.0%.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

The comment period for this revision ends September 30, 2015. In the absence of significant adverse comments, it is proposed to implement this revision via an *Interim Revision Announcement* to the *Second Supplement to USP 39–NF 34*, with an official date of January 1, 2016.

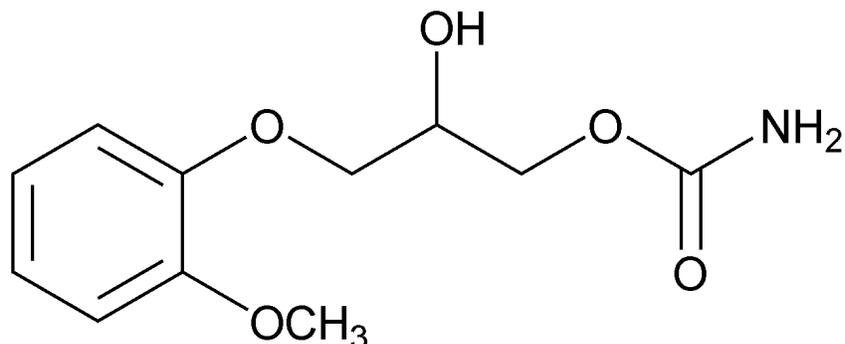
Interested parties are encouraged to submit comments to Ravi Ravichandran, Ph.D., Principal Scientific Liaison to the Monograph Development committee (301-816-8330 or rr@usp.org).

(SM4: R. Ravichandran.)

Correspondence Number—C157411

Comment deadline: September 30, 2015

Methocarbamol



$C_{11}H_{15}NO_5$ 241.24

1,2-Propanediol, 3-(2-methoxyphenoxy)-, 1-carbamate, (±)-;
(±)-3-(*o*-Methoxyphenoxy)-1,2-propanediol 1-carbamate [532-03-6].

DEFINITION

Methocarbamol contains NLT 98.5% and NMT 101.5% of methocarbamol ($C_{11}H_{15}NO_5$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)

Change to read:

- **B.**

▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲*USP38*

ASSAY

Change to read:

- **Procedure**

▲ **Buffer:** 6.8 g/L of *monobasic potassium phosphate* in water. Adjust with *phosphoric acid* or *sodium hydroxide* to a pH of 4.5.

Mobile phase: Methanol and *Buffer* (30:70)

System suitability solution: 1.0 mg/mL of USP Methocarbamol RS and 0.005 mg/mL of USP Guaifenesin RS in *Mobile phase*

Standard solution: 0.1 mg/mL of USP Methocarbamol RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Methocarbamol in *Mobile phase*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 274 nm

Column: 4.6-mm × 15-cm; 3-μm packing L1

Column temperature: 30°

Flow rate: 0.8 mL/min

Injection volume: 20 μL

Run time: 1.5 times the retention time of methocarbamol

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 3.5 between methocarbamol and guaifenesin, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methocarbamol ($C_{11}H_{15}NO_5$) in the portion of Methocarbamol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of methocarbamol from the *Sample solution*

r_S peak response of methocarbamol from the *Standard solution*

C_S concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)

C_U concentration of Methocarbamol in the *Sample solution* (mg/mL)

▲USP38

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Delete the following:

- **Heavy Metals, Method I** 〈 231 〉

Sample solution: 1.0 g in a 10-mL mixture of methanol and 1 N acetic acid (7:3), diluted with water to 25 mL

Acceptance criteria: NMT 20 ppm* (Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

▲ **Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.005 mg/mL of USP Methocarbamol RS in *Mobile phase*

Sample solution: 1 mg/mL of Methocarbamol in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 3.5 between methocarbamol and guaifenesin, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Methocarbamol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of methocarbamol from the *Standard solution*

C_S = concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)

C_U = concentration of Methocarbamol in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Guaifenesin	0.84	1.2	— *0.15*(IRA 1-Jan-2016)
Methocarbamol isomer ^a	0.90	1.0	— *0.05*(IRA 1-Jan-2016)
Methocarbamol	1.0	—	—
Methocarbamol dioxolone ^b	1.3	1.0	— *0.05*(IRA 1-Jan-2016)
Any individual unspecified impurity	—	—	0.05
Total impurities	—	—	2.0 *1.0*(IRA 1-Jan-2016)
^a 1-Hydroxy-3-(2-methoxyphenoxy)propan-2-yl carbamate.			
^b 4-[(2-Methoxyphenoxy)methyl]-1,3-dioxolan-2-one.			

SPECIFIC TESTS

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 60° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in tight containers.

▲Store at controlled room temperature. ▲*USP38*

- **USP Reference Standards** 〈 11 〉

USP Guaifenesin RS

USP Methocarbamol RS

BRIEFING

Protamine Sulfate Injection, *USP 38* page 5071 and the *Revision Bulletin* posted on the USP website with an official date of June 1, 2015. The Monographs—Biologics and Biotechnology 1 Expert Committee intends to revise this monograph based on comments received. The specification for the pH test was omitted via *Revision Bulletin*. A new pH specification is being proposed to allow public comment.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

The comment period for this revision ends on September 30, 2015. In the absence of significant adverse comments, it is proposed to implement this revision through an *Interim Revision Announcement*, with an official date of January 1, 2016.

(BIO1: A. Szajek.)

Correspondence Number—C160569

Comment deadline: September 30, 2015

Protamine Sulfate Injection**DEFINITION****Change to read:**

Protamine Sulfate Injection is a sterile, isotonic solution of Protamine Sulfate.

▲Protamine Sulfate used in the manufacture of Protamine Sulfate Injection complies with the compendial requirements stated in the *Protamine Sulfate* monograph. ▲*USP38*

Each mg of Protamine Sulfate, used in the manufacture of the Injection, neutralizes NLT 100 USP Heparin Units, calculated on the dried basis. It contains NLT 90.0% and NMT 120.0% of the labeled amount of protamine sulfate.

IDENTIFICATION**Add the following:**

- ▲● **A.** The retention times of the four major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*. ▲*USP38*

Change to read:

- ▲● **B.** ▲*USP38*

Identification Tests—General 〈 191 〉, *Sulfate*: Meets the requirements

ASSAY

Change to read:

- **Procedure**

▲ **Solution A:** 0.3 M sodium phosphate, pH 1.8. Pass the solution through a membrane filter of 0.45- μ m pore size, and degas before use.

Solution B: *Solution A* and acetonitrile (93.5: 6.5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
15	55	45
25	55	45
30	85	15

[Note—Initial gradient composition may be adjusted as appropriate to obtain sufficient resolution. The end of the gradient can be increased to re-equilibrate the column for the next injection.]

Standard solution: 0.5 mg/mL of USP Protamine Sulfate RS in 0.01 M hydrochloric acid

Sample solution: 0.5 mg/mL of protamine sulfate in 0.01 M hydrochloric acid

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 55 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 100 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Retention time: The chromatogram of the *Standard solution* must show four major peaks (in increasing elution order: protamine peptides 1, 2, 3, and 4), with protamine peptide 4 eluting no later than 15 min. [Note—See the standard chromatogram provided with USP Protamine Sulfate RS.]

Resolution: The resolution between protamine peptides 1 and 2, calculated by the tangent method, is NLT 2.0.

Relative standard deviation: NMT 2.0% for the total integrated areas of the six

chromatograms of the *Standard solution*, using vertical drop-down integration

Analysis

Samples: *Standard solution* and *Sample solution*

Separately inject equal volumes (about 100 µL) of the *Standard solution* (at least six injections) and the *Sample solution* into the chromatograph. Record the chromatograms for approximately 30 min, and measure the responses for all the peaks observed using a full scale comparable to the height of the largest peak and using vertical drop-down integration.

Calculate the percentage of the labeled amount of protamine sulfate in the portion of Injection taken:

$$\text{Result} = \Sigma[(r_U/r_S) \times (C_S/C_U)] \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Protamine Sulfate RS in the *Standard solution* (mg/mL)

C_U nominal concentration of protamine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–120.0%▲*USP38*

SPECIFIC TESTS

- **Bacterial Endotoxins Test** 〈 85 〉: It contains NMT 7.0 USP Endotoxin Units/mg of protamine sulfate.

Add the following:

- **pH** 〈 791 〉: 6.0–7.0 •(IRA 1-Jan-2016)

Add the following:

- ▲● **Particulate Matter in Injections** 〈 788 〉: Meets the requirements for small-volume injections ▲*USP38*

- **Other Requirements:** It meets the requirements in *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I glass. Store at controlled room temperature

▲or at 2°–8°.▲*USP38*

- **Labeling:** Label it to indicate the approximate neutralization capacity in USP Heparin Units.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Endotoxin RS

USP Heparin Sodium for Assays RS



USP Protamine Sulfate RS

▲USP38

In-Process Revision

This section contains proposals for adoption as official *USP* or *NF* standards (either proposed *new* standards or proposed *revisions* of current *USP* or *NF* standards). These may be any of the following: (1) proposed revisions placed directly under *In-Process Revision*, or (2) modifications of revisions previously proposed under *In-Process Revision*. Readers should review material in this section and provide comments to the Scientific Liaison using the contact information appearing at the end of each proposal. Information on how to comment can be found under the "Participation" section of www.usp.org. It is important to send comments promptly, using the comment deadline listed after each title.

Briefings Each Proposal is preceded by a Briefing in the following format:

Briefing
<p>Name of Item, citations of the most recent <i>USP</i> publications in which this item appeared. Rationale for the revision. Other relevant information. (For example, if a chromatographic method is being proposed, column specifications and retention times for compounds of interest.) Finally, the Expert Committee acronym (the name of the Scientific Liaison who handled the particular monograph or general chapter, and the <i>USP</i> tracking correspondence number, as shown in the example below:</p>
<p>(Expert Committee Acronym: Liaison Name.)</p>
<p>Correspondence Number—CXXXXX</p>

Symbols Proposed revisions are shown with language proposed for deletion or replacement crossed out. All *USP-NF* revisions use the following symbols that indicate the final destination of the official text:

◆ new text ◆

if slated for an *IRA*;

▲ new text ▲

if slated for *USP-NF*;

■ new text ■

if slated for a *Supplement* to *USP-NF*. The same symbols indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as

◆ ◆

, or

■ ■

, or

▲ ▲

, it means that text has been deleted and no new text was proposed to replace it.

In all revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA*, *Supplement*, or the *USP* or *NF* as the publication where the revision will appear if approved. For example, ■

2S (USP 34) indicates that the proposed revision is slated for the *Second Supplement to USP 34*, and ▲
 USP35 and ▲NF30 indicates that the revisions are proposed for *USP 35* and *NF 30*, respectively.

Official Title Changes Where the specification "**Monograph title change**" is found, it indicates that the official title stated after that specification will be substituted for the former title in the appropriate places throughout that monograph once this revision becomes official.

BRIEFING

⟨ 1130 ⟩ **Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)**, *USP 38* page 1267 and *PF 40(2)* [Mar.–Apr. 2014]. During manufacture of biopharmaceuticals produced by cells, it is important to reduce, as much as possible, the quantity of residual host cell DNA, thereby maximizing product purity, safety, and quality. This revision proposal modernizes the official chapter to reflect current methodology and expectations for residual DNA procedures, and also addresses public comments received during a previous revision proposal published in *PF 40(2)* and canceled. Specifically, additional options for polymerase chain reaction-based methods are introduced in addition to hybridization and methods based on the use of DNA binding proteins. The chapter also proposes a wider acceptance range of 50%–150% for sample recovery, based on current methods approved by regulators. The chapter is also referenced by the following official *USP–NF* chapters:

- *Gene Therapy Products* ⟨ 1047 ⟩
- *Growth Factors and Cytokines Used in Cell Therapy Manufacturing* ⟨ 92 ⟩
- *Nucleic Acid-Based Techniques—General* ⟨ 1125 ⟩
- *Nucleic Acid-Based Techniques—Extraction, Detection, and Sequencing* ⟨ 1126 ⟩
- *Nucleic Acid-Based Techniques—Microarray* ⟨ 1128 ⟩
- *Vaccines for Human Use—Polysaccharide and Glycoconjugate Vaccines* ⟨ 1234 ⟩
- *Vaccines for Human Use—General Considerations* ⟨ 1235 ⟩
- *Vaccines for Human Use—Bacterial Vaccines* ⟨ 1238 ⟩

These links will remain as written when this chapter becomes official.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCBA: M. Kibbey.)

Correspondence Number—C154181

Comment deadline: September 30, 2015

⟨ 1130 ⟩ NUCLEIC ACID-BASED TECHNIQUES—APPROACHES FOR DETECTING TRACE NUCLEIC ACIDS (RESIDUAL DNA TESTING)

Change to read:

INTRODUCTION

The basic principles of nucleic acid amplification technologies (NAT) and definitions of the various techniques are covered in *Nucleic Acid-Based Techniques—General* ⟨ 1125 ⟩. This

chapter covers the analytical procedure used to quantify residual DNA in biopharmaceuticals.

Quantification of residual DNA impurities in biopharmaceuticals is based on safety concerns. The cells used to produce biopharmaceuticals can be sources of a range of complex, heterogeneous, and potentially unsafe impurities, and host cell DNA is among these. Much of the safety concern associated with residual DNA in biopharmaceuticals lies in the possibility that host cell DNA, particularly continuous cell-line DNA, may result in tumors or adverse reactions. Cells used to produce biopharmaceuticals may possibly carry viruses or harbor harmful nucleic acid, and the residual DNA in a given biopharmaceutical product may be infectious. Although animal testing has shown that extraneous DNA can cause tumors or infections, no reports to date have demonstrated this risk in humans. Therefore, some regulatory agencies have allowed a target of 100 pg or less of residual DNA per dose in biopharmaceuticals, and levels up to 10 ng of residual DNA per dose may be considered, depending on the source of the residual DNA and the product's route of administration.

One can address residual DNA in biopharmaceutical processes in two ways: by validating clearance during process validation or by monitoring residual DNA levels by routine testing of the drug substance. The level of concern regarding residual DNA can be tied to the potential source of the residual DNA (e.g., infectious viral DNA) and the route of administration, so the residual DNA specification and procedure for monitoring DNA clearance for a given product should be developed in consultation with regulatory agencies. Regardless of whether routine testing of a drug product is used to determine residual DNA content or whether DNA clearance is demonstrated by process validation, analytical procedures for the quantification of residual DNA are required. The analytical procedures used to determine the residual DNA content of biopharmaceuticals can include hybridization, instrumentation based on DNA-binding protein, quantitative PCR (q-PCR), or other DNA amplification methods. The expectation is that the analytical procedure used to quantify residual DNA in biopharmaceuticals has a detection limit approximating 10 pg per dose. The assays based on hybridization, DNA-binding protein, and q-PCR are typically the techniques of choice because they can meet the sensitivity expectation.

■ Process characterization and the theoretical safety concerns with process-related impurities highlight the need for residual DNA testing in biopharmaceutical products. The ability of a manufacturing process to remove residual DNA from a biopharmaceutical product is an indicator of the quality and consistency of the process. Additionally, the cells used to produce a biopharmaceutical can be sources of a range of complex, heterogeneous, and potentially unsafe impurities, with host cell DNA among these impurities. Historically, the safety concern associated with residual DNA in biopharmaceuticals lies in the possibility that host cell DNA, particularly continuous cell-line DNA, may result in tumors or adverse reactions. Cells used to produce biopharmaceuticals may also carry viruses or harbor harmful nucleic acid. Although animal testing has shown that extraneous DNA can cause tumors or infections, no reports to date have demonstrated this risk in humans. Residual DNA content, up to 10 ng of residual DNA per parental dose, may be considered for DNA originating from mammalian cell cultures, but the residual DNA content may vary depending on the source of the residual DNA and the route of administration of the product. One can address residual DNA in biopharmaceutical processes in two ways: 1) by validating clearance during process validation; and/or 2) by monitoring residual DNA levels through routine testing of the drug substance. The level of concern regarding residual DNA can be tied to its source and the route of administration, so the residual DNA specification and procedure for monitoring DNA clearance for a given product should be developed in consultation with regulatory agencies. Regardless of whether routine testing of a

drug substance is used to determine residual DNA content or whether DNA clearance is demonstrated by process validation, analytical procedures for the quantification of residual DNA are required. DNA amplification techniques, such as quantitative PCR (qPCR), are used most often for residual DNA testing because of their superior sensitivity and unique advantages (e.g., high specificity). The expectation is that the analytical procedure used to quantify residual DNA in biopharmaceuticals has a detection limit well below the DNA level allowed by regulators for biopharmaceuticals (often 10 ng/dose). Assays based on hybridization, DNA-binding protein, and qPCR are typically the techniques of choice because they can meet the sensitivity expectation.

■ 2S (USP39)

Change to read:

SAMPLE PRETREATMENT

Analysis of residual DNA requires accurate quantification of pg levels of DNA in mg (or larger) quantities of product. The sample itself, whether it is a protein or other chemical entity, can create sample matrix effects that must be overcome in order to yield a useful assay. Protein samples may require only digestion with proteinase (e.g., Proteinase K, Pronase) to allow the analytical method to quantitatively recover the residual DNA. Treating the sample with a detergent may be required to dissociate the residual DNA from the sample matrix. Traditionally, extraction methods based on phenol and chloroform, followed by ethanol precipitation, have been applied to the purification of DNA in molecular biology research. The phenol/chloroform extraction technique may be a useful pretreatment for residual DNA samples prior to analysis. Because of the typically low levels of residual DNA present in samples, quantitative DNA recovery with ethanol precipitation may be difficult. For this reason, a carrier molecule (e.g., glycogen) may be necessary to aid in DNA recovery if this technique is used.

A commercial kit is available¹ and has been used successfully for pretreatment of residual DNA samples. The commercial kit uses a chaotrope (sodium iodide) and a detergent (sodium *N*-lauroyl sarcosinate) to disrupt the association of the DNA with the sample. The DNA is then co-precipitated using glycogen as the carrier molecule in the presence of isopropanol.

Each of these pretreatment techniques may yield acceptable results, or analysts may combine the techniques to obtain acceptable recovery of the residual DNA from the sample. Sample extraction is an extra handling step that may cause the incomplete recovery of the residual DNA or may introduce environmental DNA into the sample, so great care must be taken during any sample manipulations. Addition of DNA spiked samples in the residual DNA assay is a common practice. A recovery of 80% to 120% of the spiked DNA is an acceptance criterion often applied to residual DNA assays to ensure that the assay yields acceptable results. When sample characteristics (e.g., matrix effects, sample preparation method) make achieving a recovery acceptance criterion of 80–120% impractical, then correcting the observed DNA concentration by the load recovery percentage is also an acceptable approach. During the qualification of a residual DNA assay, some scientists treat the samples with DNase I to degrade the DNA in the sample in order to demonstrate that the assay response was due to DNA and not some other sample component.

■ Analysis of residual DNA requires accurate quantification of picogram levels of DNA in mg (or

larger) quantities of product, which may be in a variety of matrices. In certain circumstances, the sample can be analyzed neat in the analytical procedure with acceptable recovery and precision. When the product or other sample components interfere with the assay sample, dilution may be all that is required to overcome the interference, so long as the specified DNA content of the sample remains within the useful range of the analytical procedure. When sample dilution is not effective in reducing assay interference, it may be necessary to use more extensive sample pretreatment procedures, such as proteolytic digestion, chemical dissociation, or extractions. It may be necessary to use a combination of different pretreatment steps to remove interference to an acceptable level. Extensive sample manipulation can lead to losses of DNA or introduction of environmental DNA, and should be a consideration when using one or more sample pretreatment steps. Contamination with environmental DNA may only be a concern when using a residual DNA procedure that is not sequence specific.

Protein samples may only require digestion with proteinase (e.g., Proteinase K, Pronase) to allow the analytical method to quantitatively recover the residual DNA. It may also be possible that the DNA is bound to the sample components, and chemical dissociation (e.g., detergents) may disrupt the binding, allowing sufficient recovery in the residual DNA assay. Residual DNA test procedures often use protein reagents and the use of a chemical dissociation reagent. These materials must be used at a sufficiently low level or removed so that the analysis is not compromised.

It may be necessary to extract the DNA from the sample to remove the inhibitory substances that are causing the reduced DNA recovery. Extraction procedures are typically based on precipitating the DNA from the sample or DNA-specific binding to a matrix (e.g., magnetic beads). Historically, extraction methods based on phenol and chloroform, followed by ethanol precipitation, have been applied to the purification of DNA in molecular biology research. The phenol/chloroform extraction technique may be a useful pretreatment for residual DNA samples before analysis, but the phenol/chloroform extraction technique might not be the best choice for the low levels of DNA typically found in biopharmaceutical samples. Because of these low levels, quantitative DNA recovery with ethanol precipitation may be difficult. For this reason, a carrier molecule (e.g., glycogen) may be necessary to aid in DNA recovery if the phenol/chloroform extraction technique is used. Commercial kits are available and have been used successfully for pretreatment of residual DNA samples for improved recovery in the residual DNA assay. For example, some kits use a chaotrope (sodium iodide) and a detergent (sodium *N*-lauroyl sarcosinate) to disrupt the association of the DNA with the sample. The DNA is then co-precipitated using glycogen as the carrier molecule in the presence of isopropanol. Extraction of DNA from the sample, based on binding to a solid matrix, can be found in various formats. One of the most popular formats uses magnetic beads, where the beads are added to a sample with a binding solution to capture the sample DNA on the beads. The beads are then captured and held in the sample tube using a magnetic stand while the supernatant containing the interfering substances is removed and discarded. The beads are washed repeatedly using a magnetic stand and a wash solution. Finally, the DNA is eluted from the beads for the assay using an elution buffer, with the beads being removed from the sample preparation using the magnetic stand.

The sample manipulation involved with pretreatment may reduce the recovery of the residual DNA or introduce environmental DNA into the sample. Great care must be taken during any sample manipulations to avoid DNA losses or contamination. The addition of target DNA-spiked samples in the residual DNA assay is a common practice. The target DNA-spiked sample should not be confused with the internal positive control (IPC), which is typically a non-target DNA

added after the sample pretreatment step to detect the presence of PCR inhibitors and to evaluate DNA amplification during the analysis. The IPC may also be introduced before the extraction to improve the control of this step. A recovery of 50%–150% of the spiked target DNA is often applied to residual DNA assays to ensure that the assay yields acceptable results. When sample characteristics (e.g., matrix effects or sample preparation method) make achieving a recovery acceptance criterion of 50%–150% impractical, then correcting the observed DNA concentration by using the load recovery percentage is also an acceptable approach. ■2S (USP39)

HYBRIDIZATION-BASED RESIDUAL DNA ASSAY

The first residual DNA assays were based on DNA hybridization, wherein a DNA probe created from host cell DNA detects and quantifies the amount of complementary DNA present in the product under assay. Double-stranded host cell DNA consists of two complementary strands of DNA that are held together by hydrogen bonding. The double-stranded DNA in the test sample is denatured to single strands and immobilized to a membrane, typically a nitrocellulose or nylon membrane. The sample is probed using host cell DNA that has been denatured and labeled. The host cell DNA probe is not a specific sequence but is prepared by a random labeling procedure during which a radioactive or fluorescent label is introduced into the host cell DNA to produce the probe. When the denatured, labeled DNA probe is brought into contact with the membrane-immobilized DNA, the probe will bind to complementary sequences of the host cell DNA. If the probe is radioactive, the membrane is placed against autoradiography film for a sufficient length of time, the film is developed, and a dark spot will be observed where the test DNA was immobilized. If the probe has a fluorescent label, the intensity of the spots is determined using a phosphor- or fluorescence-imaging system. The intensity of the spot is proportional to the amount of probe that was hybridized to the test DNA and therefore is proportional to the amount of residual DNA in the sample. The intensity of the spot can be compared visually with the intensity of spots that correspond to a standard curve yielding semiquantitative results (i.e., visual quantitation), or the intensity can be determined using an instrument (e.g., densitometer) to create a quantitative value that is compared with the values obtained from the standard curve.

DNA-BINDING PROTEIN-BASED RESIDUAL DNA ASSAY

Instrumentation is commercially available for the quantitation of residual DNA in biopharmaceuticals. The instrumentation requires reagents that use DNA-binding protein and antibodies targeted for DNA in a four-step analytical procedure.

1. The first step requires that the DNA be denatured into single-stranded DNA by sample heating. The denatured DNA is mixed with a single reagent that contains DNA-binding protein that is conjugated with streptavidin and a monoclonal anti-DNA antibody that is conjugated to urease. The DNA-binding protein and the monoclonal antibody are specific for single-stranded DNA but do not have any sequence specificity. This liquid phase facilitates the formation of reaction complexes that contain DNA, streptavidin, and urease.

2. During the second step, the sample is filtered through a biotinylated membrane that binds to the streptavidin and captures the complexes on the membrane, which is washed to remove any reagents that are not bound to the membrane.
3. During the third step, the membrane is inserted into a sensor on the instrument, where the urease in the DNA complex reacts with a urea solution in the sensor, producing ammonia and a change in pH that is detected using a light-addressable potentiometric sensor (LAPS). The change in pH directly correlates with the amount of DNA in the sample.
4. In the fourth step, the raw data from the instrument are analyzed using the appropriate software to determine the residual DNA content of the sample.

Change to read:

~~QUANTITATIVE PCR-BASED RESIDUAL DNA ASSAY~~

~~POLYMERASE CHAIN REACTION TECHNIQUES~~ ■ 2S (USP39)

~~Real-time q-PCR~~

~~qPCR~~ ■ 2S (USP39)

~~is a procedure that is well adapted to fast sample throughput and has applications in many areas of biopharmaceutical manufacture (e.g., copy number detection, virus detection). The technique can quantify the amount of a nucleic acid target sequence in DNA from a variety of samples. The DNA probe~~

~~and primers~~ ■ 2S (USP39)

~~used in the analysis is the~~

~~are~~ ■ 2S (USP39)

~~key to the procedure. The~~

~~most common qPCR method for detection of this amplification is referred to as the 5' nuclease assay. In this format, the~~ ■ 2S (USP39)

~~probe has a reporter dye attached to one end and a quencher dye attached to the other end.~~

~~A DNA primer~~

~~pair of DNA primers~~ ■ 2S (USP39)

~~is also added to the reaction. During the amplification reaction, DNA polymerase I attaches where the DNA primer binds to the single-stranded sample (template) DNA and moves along the sample DNA, synthesizing new complementary DNA. While following the template DNA, DNA polymerase I cleaves any complementary DNA in the path. If DNA polymerase I encounters the labeled DNA probe it will cleave the reporter dye from the probe. The reporter dye is released into solution and, in the absence of the quencher dye, can be quantitated as a fluorescent measurement. Repeating the reaction cycle results in an amplification of the fluorescent signal. The number of cycles required for the fluorescent measurement to exceed a threshold value correlates to the amount of starting residual DNA in the sample. By comparing with a standard curve the fluorescence obtained from a sample, analysts can quantify the residual DNA in the sample.~~

~~If DNA polymerase I encounters the labeled DNA probe, DNA polymerase I will cleave the probe. The reporter dye is released into solution and, in the absence of the quencher dye, the resulting fluorescence is measured. Repeating the reaction cycle results in an amplification of~~

the fluorescence signal. The number of cycles required for the fluorescence measurement to exceed a threshold value correlates to the amount of starting residual DNA in the sample. By comparing the fluorescence obtained from a sample to a standard curve, analysts can quantify the residual DNA in the sample.

Alternative Detection Strategies

A number of innovative detection strategies have been developed and commercialized beyond that described above. A few of the most common are as follows:

1. Intercalating cyanine dyes fluoresce after binding to double-stranded DNA. The amount of dye incorporated is proportional to the amount of target amplicon generated. These dyes are inexpensive and easy to use. The disadvantage of this technique is the lack of a specific probe to confer sequence specificity beyond that afforded by the primers, and the dye will also bind somewhat to single-stranded DNA and RNA molecules. Consequently, primer dimers or nonspecific products may affect the quantification. However, it is possible to check for the specificity of the system by running a melting curve at the end of the PCR run, based on the principle that every product has a different dissociation temperature and depending on the size and base content.
2. Other sensitive probes exist that contain a stem-loop structure with a fluorophore and a quencher at their 5' and 3' ends, respectively. The stem is usually six bases long, mainly consisting of cytosines and guanines, and holds the probe in the hairpin configuration. The "stem" sequence keeps the fluorophore and the quencher in close proximity, but only in the absence of a sequence complementary to the "loop" sequence. In the presence of a complementary sequence, the probe unfolds and hybridizes to the target, leading to separation between the fluorophore and the quencher, and the probe fluoresces. The amount of signal is proportional to the amount of target amplicon sequence. The increase in fluorescence that occurs is reversible, because there is no cleavage of the probe. It is also possible to design the stem structure to add specificity to this type of probe. However, these probes are often expensive, and the signal can be weak due to the limited possible physical separation between the fluorophore and the quencher.
3. Another variety of the second example above uses a single-stranded nucleic acid sequence containing the specific PCR primers, the specific probe with a stem-loop tail separating a fluorophore and a quencher, and a blocking group. The stem-loop tail is separated from the PCR primer sequence by a "PCR blocker", a chemical modification that prevents the polymerase from copying the stem-loop sequence of the primer. After extension of the primer during PCR amplification, the specific probe sequence is able to bind to its complement within the same strand of DNA. This hybridization event opens the hairpin loop so that fluorescence is no longer quenched, and an increase in signal is observed. Covalent attachment of the probe to the target amplicon ensures that each probe has a target in close proximity. Enzymatic cleavage is not required, nor a separate probe hybridization step, thereby reducing the time needed for signaling. This can result in stronger signals and lower background with faster cycling; however, these probes can be quite expensive and complicated to design.
4. Fluorogenic minor groove binder probes are short linear probes that have a minor groove binder with a non-fluorescent quencher on the 5' end and a fluorophore on the 3' end. The minor groove binder prevents the exonuclease activity of the DNA polymerase from cleaving the probe. Quenching occurs when the random coiling of the probe in the free

form brings the quencher and the fluorophore close together. The probe is elongated when bound to its target and quenching is decreased, leading to an increase in fluorescent signal proportional to the amount of accumulated amplicon. These probes are also expensive and can produce a low signal-to-noise ratio.

Quantitative Multiplex PCR-Based Residual DNA Assay

An evolution of qPCR is quantitative multiplex PCR, where several pairs of primers and the corresponding probes are introduced in the reaction medium to simultaneously detect multiple targets. Benefits include higher throughput and better control of false-negative results, whereas disadvantages come from amplification and detection interferences, as outlined in *Nucleic Acid-based Techniques—Amplification* (1127). One of the applications of this technique is a duplex qPCR, where the introduction of an exogenous DNA, called IPC, enhances confidence in the accuracy of the analysis when appropriately amplified. Multiplex qPCR is not used as often as single-target qPCR to assay host cell residual DNA in biopharmaceuticals.

■ 2S (USP39)

Delete the following:

■

PRACTICAL APPLICATIONS OF RESIDUAL DNA TESTING

Analysts choosing hybridization, DNA-binding protein, or q-PCR techniques for residual DNA analysis should consider how the assay will be used, the structure of the DNA available (e.g., fragment length), and regulatory issues. The cost of analysis can be significant and should be considered when evaluating an assay format. Traditionally, hybridization assays were performed using ^{32}P -labeled DNA and autoradiography. Because ^{32}P decays quickly, probes prepared with ^{32}P have a limited shelf life, and the precautions necessary for handling radioactive material can be cumbersome.

These issues with ^{32}P labeling may make fluorescence labeling of the hybridization probe a more desirable option. If the hybridization assay is assessed visually, this represents a semiquantitative assay, but if the intensity of the spots is determined using a densitometer or other image system, the results can be quantitative. DNA-binding protein assays and q-PCR give quantitative results. Quantitative assays are typically preferred instead of semiquantitative assays because the results are considered more accurate and precise, which allows better process monitoring and control.

Due to sample interference, a sample pretreatment step is often required to obtain accurate and reproducible results. Pretreatment steps can influence the recovery of DNA, so it is often necessary to design the assay with a spike-recovery control and an acceptance criterion to ensure assay performance. Commercial sources of host cell and vector DNA are typically not available to prepare in-house controls. In-house controls are usually prepared in the laboratory and quantified by UV spectroscopy, using standard techniques employed in molecular biology, to determine the DNA content and purity. Additionally, it is a good practice to evaluate in-house residual DNA controls by agarose gel electrophoresis to demonstrate that the DNA is of a proper size for the assay employed and has not degraded.

The hybridization assay uses genomic and/or vector DNA, labeled randomly throughout the

DNA, as the hybridization probe reagent. For this reason the hybridization assay is specific for the source of DNA but is not specific for a given sequence. A synthesized probe, specific for a specific sequence, can be prepared and used in the hybridization assay if this level of specificity is desirable. The DNA-binding protein residual DNA assay is not sequence-specific and hence not specific for the host DNA. Therefore, laboratory personnel should avoid contaminating samples for this assay with environmental DNA before denaturing the DNA; otherwise the DNA result may be falsely elevated. The q-PCR probe is sequence-specific, which creates some special challenges for development of a q-PCR residual DNA assay. The q-PCR-specific sequence must be a stable sequence within a highly conserved region of DNA. The recovery of the probe target sequence must consistently represent the recovery of all the residual DNA. As a guideline, for a DNA fragment to be detected by hybridization, q-PCR, and DNA-binding protein assays, it must have no fewer than 50, 150, and 600 base pairs, respectively. A bioprocess typically may have operations that shear DNA into smaller fragments, and this must be taken into consideration when selecting an assay. Procedures exist to determine whether the DNA fragments in a sample are too small for adequate residual DNA recovery with a given assay. As noted, residual DNA assays are extremely sensitive. Detection limits as low as <1, 3, and 6 pg of DNA per sample have been reported for q-PCR, DNA-binding protein, and hybridization assays, respectively.

Although safety concerns regarding residual DNA impurities are not as prominent as they once were, the levels of residual DNA in any bioprocess remain a key quality attribute and help define the process. ■ 2S (USP39)

Add the following:

■

RESIDUAL DNA TESTING POINTS TO CONSIDER

When developing a residual DNA assay, one should consider how the assay will be used, the structure of the DNA available (e.g., fragment length), and regulatory issues. The cost of analysis can be significant and should be considered when evaluating an assay format. In addition, environmental, health, and safety aspects should be considered. Traditionally, hybridization assays were performed using phosphorus (^{32}P)-labeled DNA and autoradiography. Because ^{32}P decays quickly, probes prepared with ^{32}P have a limited shelf life, and the precautions necessary for handling radioactive material can be cumbersome. These issues with ^{32}P labeling may make fluorescence labeling of the hybridization probe a more desirable option. If the hybridization assay is assessed visually, this process represents a semiquantitative assay, but if the intensity of the spots is determined using a densitometer or other imaging system, the results may be quantitative. DNA-binding protein assays and qPCR give quantitative results. Quantitative assays are typically preferred instead of semiquantitative assays (e.g., older hybridization-based methods), because the results are considered more accurate and precise, which allows better process monitoring and control. Because of sample matrix interference, a sample pretreatment step is often required to obtain accurate and reproducible results. Pretreatment steps can influence the recovery of DNA, so it is usually necessary to design the assay with a spike-recovery control and an acceptance criterion to ensure assay performance.

In-house controls are usually prepared in the laboratory and qualified by ultraviolet spectroscopy, using standard techniques employed in molecular biology, to determine the DNA content and purity. The hybridization assay uses genomic and/or vector DNA, labeled randomly throughout the DNA, as the hybridization probe reagent. For this reason, the hybridization assay is specific for the source of DNA but is not specific for a given sequence. A synthesized probe, specific for a specific sequence, can be prepared and used in the hybridization assay if this level of specificity is desirable. The DNA-binding protein residual DNA assay is not sequence specific and hence not specific for the host DNA. Therefore, laboratory personnel should avoid contaminating samples for this assay with environmental DNA before denaturing the DNA; otherwise, the DNA result may be falsely elevated. The qPCR probe has the advantage of being sequence specific, but this creates some special challenges for development of a qPCR residual DNA assay. The qPCR-specific sequence must be a stable sequence within a suitable region of DNA. The recovery of the target sequence must consistently represent the recovery of all the residual DNA. Biopharmaceutical manufacturing processes typically may have operations that shear DNA into smaller fragments, and this must be taken into consideration when selecting an assay. Procedures exist to determine whether the DNA fragments in a sample are too small for adequate residual DNA recovery with a given assay. When bridging from one DNA assay technique to another, a thorough understanding of the DNA analyte is critical. Some assays can detect both single-stranded and double-stranded DNA, whereas some can only detect double-stranded DNA (e.g., some fluorescent dye-binding assays). There are assays that are not sequence specific, and those assays that are sequence specific can be influenced by the copy number of the target sequence present in the DNA. There are assays that require two or more antibody molecules to bind to the DNA fragment (e.g., DNA-binding protein-based residual DNA assay), and if the DNA fragments are too small and present in sufficient quantity, they can saturate the reagents and inhibit the assay (hook effect). Although safety concerns regarding residual DNA impurities are not as prominent as they once were, the levels of residual DNA in any bioprocess remain a key quality attribute and help define the process. ■2S (USP39)

‡

■ DNA Extractor Kit, Wako Chemicals. ■2S (USP39)

BRIEFING

Carbon Tetrachloride, *USP 38* page 1827. It is proposed to update the specification of this reagent.

Additionally, minor editorial changes have been made to update the reagent to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C159251

Comment deadline: September 30, 2015

Change to read:

Carbon Tetrachloride, CCl_4 —**153.82** [56-23-5]—~~Use a grade meeting the specifications of *ACS Reagent Chemicals*, 8th Edition.~~

■ Use a suitable grade with a content of NLT 99.9% by gas chromatography. ■2S (USP39)

BRIEFING

4-Hydroxy-4-**phenylpiperidine**. It is proposed to add this new reagent used to prepare the *Internal standard solution* in the *Limit of Almotriptan Related Compound D and Almotriptan N-Dimer* test in the monograph for *Almotriptan Malate*.

(HDQ: M. Marques.)

Correspondence Number—C159297

Comment deadline: September 30, 2015

Add the following:

■ 4-Hydroxy-4-**phenylpiperidine** (*4-Phenyl-4-piperidinol*), $C_{11}H_{15}NO$ —**177.24** [40807-61-2]
—Use a suitable grade with a content of NLT 99%. ■2S (*USP39*)

BRIEFING

Monosodium Glutamate. It is proposed to add this new reagent used in the *System suitability solution* in the *Assay* and the test for *Related Compounds* in the monograph for *Gamma-Aminobutyric Acid*, published in *PF* 41(3) [May–June 2015].

(HDQ: M. Marques.)

Correspondence Number—C135182

Comment deadline: September 30, 2015

Add the following:

■ Monosodium Glutamate (*L-Glutamic Acid Monosodium Salt Hydrate, (S)-2-Aminopentanedioic Acid*), $C_5H_8NNaO_4 \cdot xH_2O$ —**169.11** (anhydrous basis) [142-47-2]—Use a suitable grade with a content of NLT 99%. ■2S (*USP39*)

BRIEFING

6Z-Retinoic Acid. It is proposed to add this new reagent used as a resolution marker in the tests for *Organic Impurities* in the monographs for *Tretinoin* and *Tretinoin Cream*, published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C143819

Comment deadline: September 30, 2015

Add the following:

■ 6Z-Retinoic Acid (*9-cis-Tretinoin, 9-cis Retinoic Acid, Acidalitretinoin*), $C_{20}H_{28}O_2$ —**300.44** [5300-03-8]—Use a suitable grade with a content of NLT 98%. Store at -20° .
[Note—A suitable grade is available as catalog number R4643 from www.sigma-aldrich.com.]
■2S (*USP39*)

BRIEFING

Sulfaguanidine. It is proposed to add this new reagent used in the *Organic Impurities* test in

the monograph for *Silver Sulfadiazine*, published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C138908

Comment deadline: September 30, 2015

Add the following:

■ Sulfaguanidine (*4-Amino-N-(aminoiminomethyl)benzenesulfonamide, 4-Amino-N-guanylbenzenesulfonamide*), $C_7H_{10}N_4O_2S$ —**214.24** [57-67-0]—Use a suitable grade.

■ 2S (USP39)

BRIEFING

L## (Tretinoin, Cosmosil Cholesterol). It is proposed to add this new column packing used in the *Assays* and the *Organic Impurities* tests in the monographs for *Tretinoin* and *Tretinoin Cream*, published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C143819

Comment deadline: September 30, 2015

Add the following:

■ L## (Tretinoin, Cosmosil Cholesterol)—Cholesteryl groups chemically bonded to porous or nonporous silica or ceramic micro-particles, 1.5–10 μ m in diameter, or a monolithic rod.

■ 2S (USP39)

CONTAINERS FOR DISPENSING CAPSULES AND TABLETS

BRIEFING

Container Specifications for Capsules and Tablets, *First Supplement to USP 38*, page 7697.

(HDQ.)

Correspondence Number—C116068; C127216; C127699; C135176; C135188; C157588

The following table is provided as a reminder for the pharmacist engaged in the typical dispensing situation who already is acquainted with the *Packaging and Storage* requirements set forth in the individual monographs. It lists the capsules and tablets that are official in the *United States Pharmacopeia* and indicates the relevant tight (T), well-closed (W), and light-resistant (LR) specifications applicable to containers in which the drug that is repackaged should be dispensed.

This table is not intended to replace, nor should it be interpreted as replacing, the definitive requirements stated in the individual monographs.

Container Specifications for Capsules and Tablets

Monograph Title	Container Specification
Add the following: ■ Gamma-Aminobutyric Acid Capsules	T, LR, ■ 2S (USP39)

Monograph Title	Container Specification
Delete the following: ■ Iopanoic Acid Tablets	T, LR ■ 2S (USP39)
Delete the following: ■ Ipodate Sodium Capsules	T ■ 2S (USP39)
Add the following: ■ Methylcobalamin Tablets	T, LR ■ 2S (USP39)
Add the following: ■ Oxymorphone Hydrochloride Extended-Release Tablets	T ■ 2S (USP39)
Add the following: ■ Oxymorphone Hydrochloride Tablets	T ■ 2S (USP39)

BRIEFING

Description and Relative Solubility of USP and NF Articles, page 7219 of the *First Supplement to USP 38*.

(HDQ.)

Correspondence Number—C132963; C158409; C159993

Change to read:

Cupric Sulfate

■ (CuSO₄·5H₂O): ■ 2S (USP39)

Deep blue, triclinic crystals or blue, crystalline granules or powder. It effloresces slowly in dry air. Its solutions are acid to litmus. Very soluble in boiling water; freely soluble in water and in glycerin; slightly soluble in alcohol.

Add the following:

■ **Cupric Sulfate (anhydrous):** A white or grayish-white powder. Soluble in water; insoluble in alcohol. ■ 2S (USP39)

Add the following:

■ **Febantel:** White or almost white crystalline powder. Soluble in acetone; slightly soluble in dehydrated alcohol; practically insoluble in water. ■ 2S (USP39)

Change to read:

Methylene Blue: Dark green

■ or blue ■ 2S (USP39)

crystals or crystalline powder having a bronze-like luster. Is odorless or practically so, and is stable in air. Its solutions in water and in alcohol are deep blue in color.

■ Slightly ■ 2S (USP39)

soluble in water and in chloroform; sparingly soluble

■ ■ 2S (USP39)

in alcohol. *NF category*: Free radical scavenger.

BRIEFING

Methylcobalamin Tablets. Because there is no existing *USP* monograph for this dosage form, a new monograph is proposed. The liquid chromatographic procedure for *Strength* is based on analyses performed with the Supelco Supelcosil brand of L1 column. The typical retention time observed for methylcobalamin is 11.3 min.

(DS: N. Davydova.)

Correspondence Number—C127699

Comment deadline: September 30, 2015

Add the following:

■ Methylcobalamin Tablets

DEFINITION

Methylcobalamin Tablets contain NLT 90.0% and NMT 125.0% of the labeled amount of methylcobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Strength*.

STRENGTH

• Procedure

Buffer solution: 3.1 g/L of sodium dihydrogen phosphate dihydrate in water. Adjust with phosphoric acid (1 in 100) to a pH of 3.5.

Mobile phase: Transfer 200 mL of acetonitrile to a 1-L volumetric flask and dilute with *Buffer solution* to volume. Then add 3.76 g of sodium 1-hexane sulfonate, and mix to dissolve.

[Note—Use low-actinic glassware, and keep the following solutions from exposure to light.]

System suitability solution: 0.05 mg/mL of cyanocobalamin from USP Cyanocobalamin RS and 0.05 mg/mL of USP Hydroxocobalamin Acetate RS in *Mobile phase*. [Note—USP Cyanocobalamin RS is a mixture of cyanocobalamin and mannitol.]

Standard solution: 100 µg/mL of USP Methylcobalamin RS in *Mobile phase*

Sample solution: Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 5 mg of methylcobalamin, to a 50-mL volumetric flask, add a suitable amount of *Mobile phase*, swirl gently, and dilute with *Mobile phase* to volume. Shake vigorously for 10 min and immediately pass through a nylon membrane filter of 0.2-µm pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 266 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 0.6 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for cyanocobalamin and hydroxocobalamin are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3 between the cyanocobalamin and hydroxocobalamin peaks, *System suitability solution*

Column efficiency: NLT 6000 theoretical plates, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylcobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Methylcobalamin RS in the *Standard solution* (µg/mL)

C_U nominal concentration of methylcobalamin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–125.0%

PERFORMANCE TESTS

- **Disintegration and Dissolution** 〈 2040 〉: Meet the requirements for *Disintegration*
- **Weight Variation** 〈 2091 〉: Meet the requirements

CONTAMINANTS

- **Microbial Enumeration Tests** 〈 2021 〉: The total aerobic microbial count does not exceed 3×10^3 cfu/g, and the total combined molds and yeasts count does not exceed 3×10^2 cfu/g.
- **Absence of Specified Microorganisms** 〈 2022 〉: Meet the requirements of the tests for absence of *Escherichia coli*

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** 〈 11 〉
 - USP Cyanocobalamin RS
 - USP Hydroxocobalamin Acetate RS
 - USP Methylcobalamin RS
- 2S (USP39)

BRIEFING

Northern Schisandra Fruit Dry Extract. Because there is no existing *USP* monograph for

this dietary supplement, a new monograph is being proposed. The liquid chromatographic procedure in the test for *Content of Lignans* is based on analyses performed with the Acquity UPLC® HSS T3 brand of L1 column with 1.8- μ m packing. The typical retention times observed for schisandrin, schisandrol B, deoxyschisandrin, and γ -schisandrin are about 1.00, 1.29, 2.90, and 3.31 min, respectively.

(DS: C. Okunji.)

Correspondence Number—C158696

Comment deadline: September 30, 2015

Add the following:

■ Northern Schisandra Fruit Dry Extract

DEFINITION

Northern Schisandra Fruit Dry Extract consists of dried ripe fruits of *Schisandra chinensis* (Turcz.) Baill. (Fam. Schisandraceae) collected in the fall, by extraction with hydroalcoholic mixtures. It contains NLT 90.0% and NMT 110.0% of the labeled amount of schisandrin on the dried basis; NLT 90.0% and NMT 110.0% of the labeled amount of total lignans, calculated as the sum of schisandrin, schisandrol B, deoxyschisandrin (schisandrin A), and γ -schisandrin (schisandrin B).

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Standard solution A: 0.5 mg/mL of USP Schisandrin RS in methanol

Standard solution B: Sonicate 100 mg/mL of USP *Schisandra chinensis* Fruit Dry Extract RS in methanol for 10 min. Centrifuge and use the supernatant.

Sample solution: Sonicate about 250 mg (adjust the amount properly, if necessary) of Northern Schisandra Fruit Dry Extract in 5 mL of alcohol for 10 min. Centrifuge, and use the supernatant.

Chromatographic system

(See *HPTLC for Articles of Botanical Origin* (203).)

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 μ m (HPTLC plates)

Application volume: 3 μ L, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Developing solvent system: Toluene, ethyl acetate, and glacial acetic acid (23:6:1)

Developing distance: 6 cm

Derivatization reagent: 10% Sulfuric acid in ethanol. [Note—Slowly add sulfuric acid to ice-cold ethanol.]

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate, and dry in air. Develop the chromatograms in a saturated chamber, remove the plate from the chamber, air-dry, and examine under UV light at 254 nm. Then treat the plate with *Derivatization reagent*, heat

at 120° for 7 min, and examine under UV light at 366 nm.

System suitability: Under UV light at 254 nm, *Standard solution B* exhibits an intense band corresponding in R_F to the band of schisandrin in *Standard solution A*. *Standard solution B* also exhibits a band due to schisandrin A in the middle of the chromatogram, and four or five bands between the positions of the bands of schisandrin and schisandrin A. In the upper-half section, *Standard solution B* exhibits an intense band corresponding to schisandrin B.

Acceptance criteria: Under UV light at 254 nm, the *Sample solution* exhibits an intense band at an R_F corresponding to the band due to schisandrin [distinction from Southern Schisandra (*Schisandra sphenanthera*) fruit] in *Standard solution A*. The *Sample solution* exhibits additional bands corresponding in R_F to similar bands in *Standard solution B*, including a band due to schisandrin A in the middle of the chromatogram; four or five bands between the positions of the bands of schisandrin A and schisandrin; and two or three bands in the upper-half section, the most intense band at an R_F corresponding to the band of schisandrin B. Under UV light at 366 nm after derivatization, the chromatogram of the *Sample solution* does not exhibit an intense blue fluorescent band (distinction from *Schisandra sphenanthera* fruit) in the upper-third of the chromatogram.

• B. HPLC

Analysis: Proceed as directed in *Content of Lignans*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits the most intense peak with a retention time corresponding to schisandrin in *Standard solution A*, and the peaks for schisandrol B, deoxyschisandrin, and γ -schisandrin corresponding to the retention times for the same lignans in *Standard solution B*. There is no principal peak due to schisantherin A at a relative retention time of about 2.1 relative to schisandrin (distinction from *Schisandra sphenanthera* fruit).

COMPOSITION

• Content of Lignans

Solution A: Water

Solution B: Acetonitrile and methanol (1:1) (v/v)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	47	53
30	20	80

Standard solution A: 0.06 mg/mL of USP Schisandrin RS in methanol

Standard solution B: 10 mg/mL of USP *Schisandra chinensis* Fruit Dry Extract RS in methanol. Sonicate and pass through a polytetrafluoroethylene filter of 0.2- μ m pore size, and discard the first portion of the filtrate before injection.

Sample solution: Accurately transfer an amount, equivalent to 4 mg of total lignans according to the labeled content, of Northern Schisandra Fruit Dry Extract, to a 50-mL centrifuge tube. Add 10.0 mL of methanol, and sonicate for 10 min (140 W, 42 kHz). Centrifuge, and transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction one more time. Combine the extracts in the 25-mL volumetric flask and dilute with methanol to volume. Mix, pass through a polytetrafluoroethylene filter of 0.2- μ m pore

size before injection, and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: UPLC

Detector: UV 251 nm

Column: 2.1-mm × 15-cm; 1.8-μm packing L1

Column temperature: 35°

Flow rate: 0.3 mL/min

Injection volume: 3 μL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of USP *Schisandra chinensis* Fruit Dry Extract RS being used.

Resolution: NLT 1.5 between the schisandrol B peak and its following peak, *Standard solution B*

Tailing factor: NMT 2.0 for the schisandrin peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the schisandrin peak, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatogram of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of USP *Schisandra chinensis* Fruit Dry Extract RS being used, identify the retention times of the peaks corresponding to schisandrin, schisandrol B, schisandrin A, and schisandrin B in the *Sample solution*.

[Note—The approximate relative retention times of the analytes are provided in *Table 2*.]

Table 2

Analyte	Approximate Relative Retention Time	Conversion Factor
Schisandrin	1.00	1.00
Schisandrol B	1.29	1.21
Schisandrin A	2.90	1.00
Schisandrin B	3.31	1.23

Separately calculate the percentages of schisandrin, schisandrol B, schisandrin A, and schisandrin B in the portion of Northern Schisandra Fruit Dry Extract taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of schisandrin from *Standard solution A*

C_S = concentration of USP Schisandrin RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Northern Schisandra Fruit Dry Extract taken to prepare the *Sample solution* (mg)

F = conversion factor for analytes (see *Table 2*)

Calculate the percentage of the labeled amount of schisandrin in the portion of Northern Schisandra Fruit Dry Extract taken:

$$\text{Result} = (P/L) \times 100$$

P = content of schisandrin as determined above (%)

L = labeled amount of schisandrin (%)

Acceptance criteria: 90.0%–110.0% on the dried basis of schisandrin

Calculate the percentage of the labeled amount of total lignans as the sum of schisandrin, schisandrol B, schisandrin A, and schisandrin B in the portion of Northern Schisandra Fruit Dry Extract taken:

$$\text{Result} = (P/L) \times 100$$

P = content of total lignans as determined above (%)

L = labeled amount of total lignans (%)

Acceptance criteria: 90.0%–110.0% on the dried basis of total lignans

CONTAMINANTS

- **Articles of Botanical Origin** 〈 561 〉, *Pesticide Residue Analysis*: Meets the requirements
- **Microbial Enumeration Tests** 〈 2021 〉: The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria does not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** 〈 2022 〉, *Test Procedures, Test for Absence of Salmonella Species* and *Test Procedures, Test for Absence of Escherichia coli*: Meets the requirements
- **Articles of Botanical Origin** 〈 561 〉, *Test for Aflatoxins*: Meets the requirements

SPECIFIC TESTS

- **Loss on Drying** 〈 731 〉
Sample: 2 g of Northern Schisandra Fruit Dry Extract
Analysis: Dry the *Sample* at 105° for 5 h.
Acceptance criteria: NMT 8%
- **Articles of Botanical Origin** 〈 561 〉, *Total Ash*
Sample: 2 g of Northern Schisandra Fruit Dry Extract
Acceptance criteria: NMT 5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at controlled room temperature.
- **Labeling:** The label states the Latin binomial following the official name.
- **USP Reference Standards** 〈 11 〉
 USP *Schisandra chinensis* Fruit Dry Extract RS
 USP Schisandrin RS

■ 2S (USP39)

BRIEFING

Chlorobutanol, *NF 33* page 6610. As part of the USP monograph modernization effort and on the basis of a request to accommodate certain grades of chlorobutanol used in injectable dosage forms that are not currently included in the monograph, it is proposed to make the following revisions:

1. Add a *Bacterial Endotoxins Test* to the *Specific Tests* section.
2. In the *Labeling* section, add a statement to accommodate a special chlorobutanol grade that is used in injectable dosage forms.
3. Add USP Endotoxin RS to the *USP Reference Standards* section.

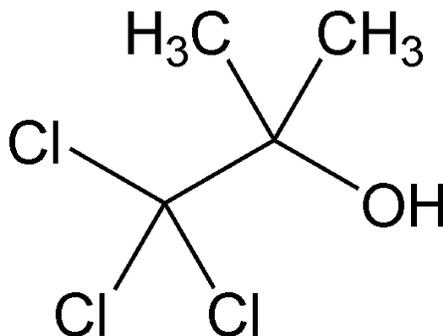
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(EXC: G. Holloway.)

Correspondence Number—C154562

Comment deadline: September 30, 2015

Chlorobutanol



$C_4H_7Cl_3O$ 177.46

$C_4H_7Cl_3O \cdot \frac{1}{2}H_2O$ 186.46

2-Propanol, 1,1,1-trichloro-2-methyl-;
 1,1,1-Trichloro-2-methyl-2-propanol [57-15-8].
 Hemihydrate [6001-64-5].

DEFINITION

Chlorobutanol is anhydrous or contains NMT one-half molecule of water of hydration. It contains NLT 98.0% and NMT 100.5% of chlorobutanol ($C_4H_7Cl_3O$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈197K〉

Change to read:

- **B.**

▲The retention time of the chlorobutanol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲NF33

ASSAY

Change to read:

• Procedure

▲ **Standard solution:** 10.0 mg/mL of USP Chlorobutanol RS and 15.0 mg/mL of 2,2,2-trichloroethanol (internal standard) in *n*-hexane

Sample solution: 10.0 mg/mL of Chlorobutanol and 15.0 mg/mL of 2,2,2-trichloroethanol (internal standard) in *n*-hexane

Chromatographic system

(See *Chromatography* 〈621〉, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 30-m fused silica; coated with a 0.25-μm layer of stationary phase G16

Temperatures

Injection port: 260°

Detector: 280°

Column: 135°

Carrier gas: Helium

Flow rate: 1.0 mL/min

Injection volume: 1 μL

Injection type: Split injection, split ratio 10:1

Run time: 12 min

System suitability

Sample: *Standard solution*

[Note—The relative retention times for chlorobutanol and 2,2,2-trichloroethanol are 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 5 between the chlorobutanol and 2,2,2-trichloroethanol peaks

Tailing factor: NMT 1.5 for the chlorobutanol peak

Relative standard deviation: NMT 0.3% for peak area ratio of chlorobutanol to the internal standard

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of chlorobutanol (C₄H₇Cl₃O) in the portion of Chlorobutanol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P \times 100$$

R_U peak area ratio of chlorobutanol to the internal standard from the *Sample solution*

R_S peak area ratio of chlorobutanol to the internal standard from the *Standard solution*

C_S concentration of USP Chlorobutanol RS in the *Standard solution* (mg/mL)

$C_{\bar{c}}$ concentration of Chlorobutanol in the *Sample solution* (mg/mL)

P = labeled purity of USP Chlorobutanol RS

▲NF33

Acceptance criteria: 98.0%–100.5% on the anhydrous basis

IMPURITIES

● Chloride

Control solution: 0.50 mL of 0.020 N hydrochloric acid in a mixture of 25 mL of diluted alcohol and 1 mL of nitric acid

Sample solution: 0.50 g of Chlorobutanol in a mixture of 25 mL of diluted alcohol and 1 mL of nitric acid

Analysis: To the *Control solution* and *Sample solution* add 2 mL of silver nitrate TS.

Acceptance criteria: 0.07%; any turbidity produced in the *Sample solution* is NMT that produced in the *Control solution*.

SPECIFIC TESTS

● **Water Determination** 〈 921 〉, *Method I*: NMT 1.0% (anhydrous form) and NMT 6.0% (hydrous form)

● Reaction

Sample: 0.5 g

Analysis: Shake the *Sample* thoroughly with 25 mL of water.

Acceptance criteria: The water remains neutral to litmus.

Add the following:

● **Bacterial Endotoxins Test** 〈 85 〉: If labeled for use in preparing parenteral dosage forms, it also meets the following requirements. The level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Chlorobutanol is used can be met. Where the label states that Chlorobutanol must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Chlorobutanol is used can be met. ■2S (NF34)

ADDITIONAL REQUIREMENTS

● **Packaging and Storage:** Preserve in tight containers.

Change to read:

● **Labeling:** Label it to indicate whether it is anhydrous or hydrous.

■ Where Chlorobutanol is intended for use in the manufacture of injectable dosage forms, it is so labeled. Where Chlorobutanol must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled. ■2S (NF34)

Change to read:

● **USP Reference Standards** 〈 11 〉

USP Chlorobutanol RS

■ USP Endotoxin RS

■ 2S (NF34)

BRIEFING

Eucalyptus Oil, PF 39(4) [Jul.–Aug. 2013]. A new monograph proposal was presented in PF 39(4). The test for *Content of Aroma Substances* is based on a GC method of analysis performed using the Supelco Wax 10 brand of G16 column.

On the basis of comments and data received, it is proposed to make the following revisions to the PF proposal in PF 39(4):

1. Add the title for *Identification* test B.
2. In the test for *Content of Aroma Substances*, the typical retention times for (+)- α -pinene, β -pinene, sabinene, (R)-(-)- α -phellandrene, (R)-(+)-limonene, eucalyptol, and camphor are 10.21, 12.75, 13.10, 14.46, 15.51, 15.85, and 24.99 min, respectively. In addition to the seven previously proposed aroma substances that were specified in PF 39(4), five additional aroma substances have been identified and are included: β -myrcene, γ -terpinene, *p*-cymene, terpinen-4-ol, and α -terpineol. β -Myrcene elutes before (R)-(-)- α -phellandrene and after sabinene; γ -terpinene and *p*-cymene elute between eucalyptol and camphor; terpinen-4-ol and α -terpineol elute after camphor. The typical retention times for β -myrcene, γ -terpinene, *p*-cymene, terpinen-4-ol, and α -terpineol are 14.27, 16.96, 17.75, 26.63, and 28.85 min, respectively. Subsequently, replace the previous *Table 2* with one providing the updated relative retention times and add a new *Table 3* to provide the relative retention times for the five additional aroma substances. Correspondingly, add the calculation for the five additional aroma substances.
3. In the *Acceptance criteria* for *Content of Aroma Substances*, change the previous *Table 3* to *Table 4*, increase the lower limits of the *Acceptance criteria* for (+)- α -pinene and (R)-(+)-limonene, and decrease the upper limit of the *Acceptance criteria* for eucalyptol. Additionally, add the *Acceptance criteria* for γ -terpinene, *p*-cymene, and new total percentage of the 12 identified aroma substances.
4. Due to the proposed change for the upper limit of the *Acceptance criteria* for eucalyptol, update the *Definition*.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(EXC: H. Wang.)

Correspondence Number—C138780; C88333

Comment deadline: September 30, 2015

Add the following:■ **Eucalyptus Oil**

[8000-48-4].

DEFINITION

Change to read:

Eucalyptus Oil is obtained by steam distillation and rectification from the fresh leaves or the fresh terminal branchlets of various species of *Eucalyptus* rich in 1,8-cineole. The species mainly used are *Eucalyptus globulus* Labill., *Eucalyptus polybractea* R.T. Baker, and *Eucalyptus smithii* R.T. Baker. It contains NLT 70.0% and NMT 97.9%

■95.0% ■2S (NF34)

of eucalyptol (1,8-cineole, C₁₀H₁₈O).

IDENTIFICATION

- **A. Infrared Absorption** 〈 197F 〉

Change to read:

- **B.**

■Identity by Aroma Substance Profile: ■2S (NF34)

The retention times of the (+)- α -pinene, β -pinene, (R)-(-)- α -phellandrene, (R)-(+)-limonene, and eucalyptol peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the test for *Content of Aroma Substances* in the *Assay*.

ASSAY**Change to read:**

- **Content of Aroma Substances**

Standard solution A: 1.0 μ L/mL of (+)- α -pinene, 0.5 μ L/mL of β -pinene, 0.5 μ L/mL of sabinene, 0.5 μ L/mL of (R)-(-)- α -phellandrene, 1.0 μ L/mL of (R)-(+)-limonene, 5 μ L/mL of USP Eucalyptol RS, and 5 mg/mL of USP Camphor RS in heptane

Standard solution B: 0.01 μ L/mL of (R)-(+)-limonene in heptane

Sample solution: 20 μ L/mL of Eucalyptus Oil in heptane

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm \times 60-m fused-silica capillary; 0.25- μ m layer of phase G16

Temperatures

Injection port: 220 $^{\circ}$

Detector: 220 $^{\circ}$

Column: See *Table 1*.

Table 1

Initial Temperature ($^{\circ}$)	Temperature Ramp ($^{\circ}$ /min)	Final Temperature ($^{\circ}$)	Hold Time at Final Temperature (min)
60	—	60	5
60	5	200	5

Carrier gas: Helium
Flow rate: 1.5 mL/min
Injection type: Split ratio, 1:50
Injection volume: 1 μ L

System suitability

Sample: *Standard solution A*

[Note—The relative retention times are listed in *Table 2*.]

Table 2

Name	Relative Retention Time
(+)- α -Pinene	0.6
β -Pinene	0.75
Sabinene	0.8
(R)-(-)- α -Phellandrene	0.9
(R)-(+)-Limonene	0.95
Eucalyptol	1.0
Camphor	1.7

Table 2

Name	Relative Retention Time
(+)- α -Pinene	0.64
β -Pinene	0.80
Sabinene	0.83
(R)-(-)- α -Phellandrene	0.91
(R)-(+)-Limonene	0.98
Eucalyptol	1.00
Camphor	1.58

■ 2S (NF34)

Suitability requirements

Resolution:

NLT 1.5 between (R)-(+)-limonene and eucalyptol

Analysis

Samples:

Standard solution A, Standard solution B, and Sample solution

Identify the peaks in the *Sample solution* based on those in *Standard solution A* as well as in *Table 3*.

Table 3

Name	Relative Retention Time
β -Myrcene	0.86–0.90
γ -Terpinene	1.05–1.07
<i>p</i> -Cymene	1.12–1.13
Terpinen-4-ol	1.68
α -Terpineol	1.82

β -Myrcene elutes before (*R*)-(-)- α -phellandrene and after sabinene, γ -terpinene and *p*-cymene elute between eucalyptol and camphor, and terpinen-4-ol and α -terpineol elute after camphor.

■ 2S (NF34)

Calculate the percentage of (+)- α -pinene (β -pinene, sabinene, (*R*)-(-)- α -phellandrene, (*R*)-(+)-limonene, or eucalyptol) in the portion of Eucalyptus Oil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of (+)- α -pinene (β -pinene, sabinene, (*R*)-(-)- α -phellandrene, (*R*)-(+)-limonene, or eucalyptol) from the *Sample solution*

r_S = peak response of (+)- α -pinene (β -pinene, sabinene, (*R*)-(-)- α -phellandrene, (*R*)-(+)-limonene, or eucalyptol) from the *Standard solution*

C_S = concentration of (+)- α -pinene (β -pinene, sabinene, (*R*)-(-)- α -phellandrene, (*R*)-(+)-limonene, or USP Eucalyptol RS) in the *Standard solution* ($\mu\text{L}/\text{mL}$)

C_U = concentration of Eucalyptus Oil in the *Sample solution* ($\mu\text{L}/\text{mL}$)

Calculate the percentage of camphor in the portion of Eucalyptus Oil taken:

$$\text{Result} = (r_U/r_S) \times [C_S/(C_U \times D)] \times 100$$

r_U = peak response of camphor from the *Sample solution*

r_S = peak response of camphor from the *Standard solution*

C_S = concentration of USP Camphor RS in the *Standard solution* (mg/mL)

C_U = concentration of Eucalyptus Oil in the *Sample solution* ($\mu\text{L}/\text{mL}$)

D = density of Eucalyptus Oil (mg/ μL)

■ Calculate the percentage of β -myrcene (γ -terpinene, *p*-cymene, terpinen-4-ol, or α -terpineol) in the portion of Eucalyptus Oil taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U

= peak response of β -myrcene (γ -terpinene, *p*-cymene, terpinen-4-ol, or α -terpineol) from the *Sample solution*

r_T

= sum of the total peak responses, except for the peaks due to solvent, from the *Sample*

solution

■ 2S (NF34)

Acceptance criteria:

See *Table 3*

■ *Table 4*. ■ 2S (NF34)

Disregard any peak with an area less than the major peak area from *Standard solution B*, corresponding to 0.05%.

Table 3

■

Table 4

■ 2S (NF34)

Name	Acceptance Criteria, NMT (%)
	0.05 ■ 0.2 ■ 2S (NF34)
(+)- α -Pinene	-10.0
β -Pinene	0.05–1.5
Sabinene	0.3
(R)-(-)- α -Phellandrene	0.05–1.5
	0.05 ■ 2.0 ■ 2S (NF34)
(R)-(+)-Limonene	-15.0
	70.0– 97.9 ■ 95.0 ■ 2S (NF34)
Eucalyptol	
Camphor	0.1

■ Percentage of γ -terpinene: 0.1%–6.0%

Percentage of *p*-cymene: 0.5%–15.0%

Total percentage of all identified aroma substances ((+)- α -pinene, β -pinene, sabinene, (R)-(-)- α -phellandrene, (R)-(+)-limonene, eucalyptol, camphor, β -myrcene, γ -terpinene, *p*-cymene, terpinen-4-ol, and α -terpineol): NLT 98.0%

■ 2S (NF34)

IMPURITIES

• Test for Aldehyde

Alcoholic hydroxylamine solution: Dissolve 3.5 g of hydroxylamine hydrochloride in 95 mL of 60% alcohol (v/v) and add 0.5 mL of a 2-mg/mL solution of methyl orange in 60%

alcohol (v/v) and sufficient 0.5 M potassium hydroxide in 60% alcohol (v/v) to give a pure yellow color. Dilute with 60% alcohol (v/v) to 100 mL.

Analysis: In a ground-glass-stoppered tube 25-mm in diameter and 150-mm long, add 10 mL of Eucalyptus Oil. Then add 5 mL of toluene and 4 mL of *Alcoholic hydroxylamine solution*. Shake vigorously, and titrate immediately with 0.5 M potassium hydroxide in 60% alcohol (v/v) until the red color changes to yellow. Continue the titration with shaking; the endpoint is reached when the pure yellow color of the indicator is permanent in the lower layer after shaking vigorously for 2 min and allowing separation to take place. The reaction is complete in about 15 min.

Repeat the titration using a further 10 mL of Eucalyptus Oil and, as a reference solution for the endpoint, the titrated liquid from the first determination, to which has been added 0.5 mL of 0.5 M potassium hydroxide in 60% alcohol (v/v).

Acceptance criteria: NMT 2.0 mL of 0.5 M potassium hydroxide in 60% alcohol (v/v) is required in the second titration.

SPECIFIC TESTS

- **Specific Gravity** $\langle 841 \rangle$: 0.906–0.927, at 20°
- **Refractive Index** $\langle 831 \rangle$: 1.450–1.470, at 20°
- **Optical Rotation** $\langle 781 \rangle$: 0°–10°, at 20°

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Do not store above 25°.
- **USP Reference Standards** $\langle 11 \rangle$
 - USP Camphor RS
 - USP Eucalyptol RS
 - USP Eucalyptus Oil RS

■ 2S (NF34)

BRIEFING

Albuterol Sulfate, *USP 38* page 2071. The FDA has requested this monograph be moved up in prioritization for modernization. The impurities specifications and analytical procedures from multiple sponsors and the corresponding monograph from the *European Pharmacopoeia* were considered, but these procedures were not specific enough to separate and to quantify all of the identified impurities. Thus, USP laboratories developed and validated suitable procedures that could be proposed for use as the test for *Organic Impurities* and the *Assay*. It is proposed to revise the monograph as follows:

1. Remove *Identification* test *B* based on the UV spectral agreement and reletter the remaining *Identification* tests, as needed, which are sufficient to identify albuterol sulfate.
2. Replace the nonspecific TLC procedure in the test for *Organic Impurities* with a specific LC procedure that was validated using the Waters Acquity UPLC BEH-C18 brand of L1 column. The typical retention time of albuterol is about 3 min. The gradient was established on an LC system with a dwell volume of 350–400 μ L.
3. The *Acceptance criteria* proposed in the test for *Organic Impurities* reflect the limits provided by several manufacturers who have approved applications for drug products

containing Albuterol Sulfate or the corresponding monograph published in the *European Pharmacopoeia*. Manufacturers are strongly encouraged to contact USP during the public comment period and provide supporting documentation if their approved specifications are different from those proposed in this revision.

4. Replace the current *Assay* with an LC procedure, which is similar to the proposed test for *Organic Impurities*. This procedure was validated using the Waters Acquity UPLC BEH-C18 brand of L1 column. The typical retention time for albuterol is about 3 min.
5. Revise the *Definition* to support the proposed *Assay*.
6. Add USP Albuterol Related Compound B RS, USP Albuterol Related Compound C RS, USP Levalbuterol Related Compound C RS, USP Levalbuterol Related Compound E RS, and USP Levalbuterol Related Compound H RS to the *USP Reference Standards* section to support the proposed revisions to the *Assay* and test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

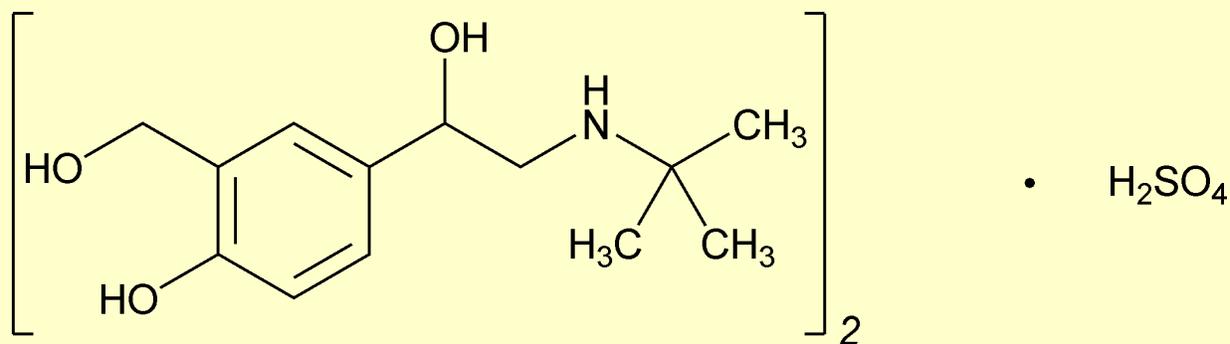
(SM4: H. Joyce, R. Ravichandran.)

Correspondence Number—C109903; C110866; C110872

Comment deadline: September 30, 2015

Albuterol Sulfate

Change to read:



■ 2S (USP39)

$(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$ 576.70

1,3-Benzenedimethanol, α^1 -[[[(1,1-dimethylethyl)amino]methyl]-4-hydroxy-, sulfate (2:1) (salt);

α^1 -[(*tert*-Butylamino)methyl]-4-hydroxy-*m*-xylene- α, α' -diol sulfate (2:1) (salt) [51022-70-9].

DEFINITION

Change to read:

Albuterol Sulfate contains ~~NLT 98.5% and NMT 101.0%~~

■ NLT 98.0% and NMT 102.0% ■ 2S (USP39)

of albuterol sulfate ($C_{13}H_{21}NO_3$)₂·H₂SO₄, calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** ~~(197K)~~

Delete the following:

- **B. Ultraviolet Absorption** ~~(197U)~~

Sample solution: 80 µg/mL in 0.1 N hydrochloric acid

Medium: 0.1 N hydrochloric acid

Acceptance criteria: Meets the requirements ~~■ 2S (USP39)~~

Change to read:

- ~~Є:~~

- **B. ■ 2S (USP39)**

Identification Tests—General ~~(191)~~, *Sulfate*

Sample solution: Shake a quantity of Albuterol Sulfate equivalent to 4 mg of albuterol with 10 mL of water, pass through a suitable filter, and use the filtrate.

Acceptance criteria: Meets the requirements

Change to read:

- ~~Đ:~~

- **C. ■ 2S (USP39)**

The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

Solution A: 3.85 g/L of ammonium acetate in water

Mobile phase: ~~Isopropanol, Solution A, and water [(5 ± 1):30:65], adjusted dropwise with acetic acid to a pH of 4.5 ± 0.3.~~

System suitability solution: ~~0.140 mg/mL of USP Albuterol Sulfate RS and 0.030 mg/mL of USP Albuterol Related Compound A RS prepared as follows. Transfer suitable quantities of USP Albuterol Sulfate RS and USP Albuterol Related Compound A RS to an appropriate volumetric flask. Dissolve in water and dilute with Mobile phase to volume.~~

Standard solution: 0.6 mg/mL of USP Albuterol Sulfate RS in water

Sample solution: 0.6 mg/mL of Albuterol Sulfate in water

Chromatographic system

~~(See Chromatography ~~(621)~~, System Suitability.)~~

Mode: ~~LC~~

Detector: ~~UV 276 nm~~

Column: ~~4.6 mm × 20 cm; packing L10~~

Flow rate: ~~2.0 mL/min~~

Injection volume: ~~10 µL~~

System suitability**Sample:** ~~System suitability solution~~**Suitability requirements****Resolution:** ~~NLT 1.5 between albuterol and albuterol-related compound A~~**Relative standard deviation:** ~~NMT 1.5%~~**Analysis****Samples:** ~~Standard solution and Sample solution~~

Calculate the percentage of albuterol sulfate [$(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$] in the portion of Albuterol Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 ~~r_U = peak response from the Sample solution~~ ~~r_S = peak response from the Standard solution~~ ~~C_S = concentration of USP Albuterol Sulfate RS in the Standard solution (mg/mL)~~ ~~C_U = concentration of the Sample solution (mg/mL)~~**Acceptance criteria:** ~~98.5%–101.0% on the anhydrous basis~~

■ **Solution A:** 9.5 g/L of sodium borate in water adjusted with a solution of sodium hydroxide in water to a pH of 10.1

Solution B: Chromatographic acetonitrile**Mobile phase:** See Table 1.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	95	5
1	95	5
9	47	53
9.1	95	5
11	95	5

[Note—The gradient was established on an LC system with a dwell volume of 350–400 μ L.]

Standard solution: 0.02 mg/mL of USP Albuterol Sulfate RS in water**Sample solution:** 0.02 mg/mL of Albuterol Sulfate in water**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 231 nm**Column:** 2.1-mm \times 10-cm; 1.7- μ m packing L1**Temperatures****Autosampler:** 4 $^{\circ}$ **Column:** 30 $^{\circ}$ **Flow rate:** 0.37 mL/min**Injection volume:** 2.5 μ L**System suitability**

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of albuterol sulfate $[(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4]$ in the portion of Albuterol Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Albuterol Sulfate RS in the *Standard solution* (mg/mL)

C_U

= concentration of Albuterol Sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis ■ 2S (USP39)

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

Change to read:

- **Organic Impurities**

Standard solution: 0.10 mg/mL of USP Albuterol Sulfate RS in water

Sample solution: 20 mg/mL of Albuterol Sulfate in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: TLC

Adsorbent: 0.25 mm layer of chromatographic silica gel

Application volume: 10 μ L

Developing solvent system: Methyl isobutyl ketone, isopropyl alcohol, ethyl acetate, ammonium hydroxide, and water (50:45:35:3:18)

Visualization: Iodine vapor

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter, applying aliquots of the *Standard solution* and the

~~Sample solution.~~ Develop in the ~~Developing solvent system~~ until the solvent front has moved three-fourths the length of the plate. Remove the plate from the developing chamber, air-dry, and expose it to iodine vapor.

Acceptance criteria: Any spot, other than the principal spot, obtained from the ~~Sample solution~~ is not greater in size and intensity than the spot produced by the ~~Standard solution~~ (0.5%), and the sum of the impurities is not greater than 2.0%.

■ **Solution A:** 9.5 g/L of sodium borate in water adjusted with a solution of sodium hydroxide in water to a pH of 10.1

Solution B: Chromatographic acetonitrile

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	95	5
1	95	5
9	47	53
9.5	47	53
9.6	95	5
11	95	5

[Note—The gradient was established on an LC system with a dwell volume of 350–400 μ L.]

System suitability solution: 20 μ g/mL of USP Albuterol Sulfate RS and 2 μ g/mL each of USP Albuterol Related Compound A RS, USP Albuterol Related Compound B RS, USP Albuterol Related Compound C RS, USP Levalbuterol Related Compound C RS, USP Levalbuterol Related Compound E RS, and USP Levalbuterol Related Compound H RS in water

Standard solution: 0.001 mg/mL of USP Albuterol Sulfate RS in water

Sample solution: 1 mg/mL of Albuterol Sulfate in water

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 231 nm

Column: 2.1-mm \times 10-cm; 1.7- μ m packing L1

Autosampler temperature: 4 $^{\circ}$

Flow rate: 0.37 mL/min

Injection volume: 6 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See Table 3 for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between levalbuterol related compound H and albuterol related compound B; NLT 1.5 between levalbuterol related compound C and albuterol related compound C; NLT 1.5 between albuterol related compound A and levalbuterol related compound E, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Albuterol Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_S = peak response of albuterol from the *Standard solution* C_S = concentration of USP Albuterol Sulfate RS in the *Standard solution* (mg/mL) C_U = concentration of Albuterol Sulfate in the *Sample solution* (mg/mL) F = relative response factor (see *Table 3*)**Acceptance criteria:** See *Table 3*.**Table 3**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Albuterol	1.00	—	—
Levalbuterol related compound D ^a	1.40	2.5	0.1
Albuterol related compound D ^b	1.45	0.68	0.1
Levalbuterol related compound H	1.50	1.0	0.10
Albuterol related compound B	1.53	0.58	0.1
Albuterol dealkyl analog ^c	1.59	0.94	0.20
Levalbuterol related compound C	1.77	0.78	0.15
Albuterol related compound C	1.84	0.57	0.1
Albuterol related compound A	1.99	0.68	0.20
Levalbuterol related compound E	2.04	0.74	0.2
Albuterol related compound E ^d	2.17	0.67	0.3
<i>N</i> -Benzyl albuterone ^e	2.73	0.64	0.10
<i>N</i> -Benzyl albuterol ^f	2.80	0.79	0.05

^a 5-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-hydroxybenzaldehyde; also known as 5-[(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxybenzaldehyde.

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levalbuterol related compound F ^g	3.19	1.1	0.10
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

a 5-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-hydroxybenzaldehyde; also known as 5-[(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxybenzaldehyde.

b 4-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-chloro-6-(hydroxymethyl)phenol; also known as (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-[3-chloro-4-hydroxy-5-(hydroxymethyl)phenyl]ethanol.

c 4-[2-(*tert*-Butylamino)-1-hydroxyethyl]phenol; also known as (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-(4-hydroxyphenyl)-ethanol.

d 2,2'-Oxybis(methylene)bis{4-[2-(*tert*-butylamino)-1-hydroxyethyl]phenol}; also known as 1,1'[oxybis[methylen(4-hydroxy-1,3phenylene)]]bis[2-[(1,1-dimethylethyl)amino]ethanol].

e 2-[Benzyl(*tert*-butyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone; also known as 2-[benzyl(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone.

f 4-{2-[Benzyl(*tert*-butyl)amino]-1-hydroxyethyl}-2-(hydroxymethyl)phenol; also known as (1*RS*)-2-[benzyl(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanol.

g 1-[4-(Benzyloxy)-3-(hydroxymethyl)phenyl]-2-(*tert*-butylamino)ethanol; also known as α -[[(1,1-dimethylethyl)amino]methyl]-4-(phenylmethoxy)-1,3-benzenedimethanol and (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-[4-(benzyloxy)-3-(hydroxymethyl)phenyl]ethanol.

■ 2S (USP39)

SPECIFIC TESTS

- **Water Determination** { 921 }, Method I: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers.

Change to read:

- **USP Reference Standards** { 11 }

USP Albuterol Sulfate RS

USP Albuterol Related Compound A RS

4-{2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl}-2-methylphenol sulfate.

■

(C₁₃H₂₁NO₂)₂·H₂SO₄ 544.70

USP Albuterol Related Compound B RS

2-(*tert*-Butylamino)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone.

$C_{13}H_{19}NO_3$	237.29
USP Albuterol Related Compound C RS	
2-(<i>tert</i> -Butylamino)-1-[3-chloro-4-hydroxy-5-(hydroxymethyl)phenyl]ethanone.	
$C_{13}H_{18}ClNO_3$	271.74
USP Levalbuterol Related Compound C RS <input type="text"/>	
4-[2-(<i>tert</i> -Butylamino)-1-hydroxyethyl]-2-(methoxymethyl)phenol;	
Also known as α -[$\{(1,1$ -Dimethylethyl)amino}methyl]-4-hydroxy-3-(methoxymethyl)-benzenemethanol.	
$C_{14}H_{23}NO_3$	253.34
USP Levalbuterol Related Compound E RS <input type="text"/>	
4-[2-(<i>tert</i> -Butylamino)-1-hydroxyethyl]-2-(ethoxymethyl)phenol;	
Also known as α -[$\{(1,1$ -Dimethylethyl)amino}methyl]-3-(ethoxymethyl)-4-hydroxy-benzenemethanol.	
$C_{15}H_{25}NO_3$	267.36
USP Levalbuterol Related Compound H RS	
4-[2-(<i>tert</i> -Butylamino)-1-methoxyethyl]-2-(hydroxymethyl)phenol.	
$C_{14}H_{23}NO_3$	253.34 \blacksquare 2S (USP39)

BRIEFING

Alprazolam Extended-Release Tablets, *USP 38* page 2100 and *PF 41(2)* [Mar.–Apr. 2015]. The revision proposal for this monograph, which appeared in *PF 41(2)*, is being further modernized as part of the USP monograph modernization efforts; it is proposed to revise the monograph as follows:

1. Add *Identification* test *B* based on the UV spectral agreement of the major peak from the *Assay*.
2. Revise the *Assay* to add the use of a diode array detector to support the proposed *Identification* test *B*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: D. Min, H. Joyce.)

Correspondence Number—C153798; C155151

Comment deadline: September 30, 2015

Alprazolam Extended-Release Tablets

DEFINITION

Alprazolam Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

C_U = nominal concentration of alprazolam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** 〈 711 〉

Test 1

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water; adjust with phosphoric acid or potassium hydroxide to a pH of 6.0 ± 0.1); 500 mL

Apparatus 1: 100 rpm

Times: 1, 4, 8, and 12 h

Mobile phase: Acetonitrile, tetrahydrofuran, and *Medium* (7:1:12)

Standard stock solution: 0.5 mg/mL of USP Alprazolam RS in acetonitrile

Standard solution: ($L/500$) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 10-cm; 5- μ m packing L7

Flow rate: 1 mL/min

Injection volume: 100 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 3000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 500 mL

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved		
	0.5-mg Tablet	2-mg Tablet	3-mg Tablet
1	NMT 25%	NMT 20%	NMT 20%
4	40%–60%	30%–55%	30%–55%
8	70%–90%	65%–90%	65%–90%
12	NLT 85%	NLT 85%	NLT 85%

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Dissolution* $\langle 711 \rangle$, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water; adjust with phosphoric acid or potassium hydroxide to a pH of 6.0 ± 0.1); 500 mL

Apparatus 1: 100 rpm

Times: 1, 4, 8, and 16 h

Mobile phase: Acetonitrile, tetrahydrofuran, and *Medium* (35:5:60)

Standard stock solution: 0.05 mg/mL of USP Alprazolam RS in methanol

Standard solution: ($L/500$) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 7.5-cm; 5- μ m packing L7

Flow rate: 1.3 mL/min

Injection volume: 80 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of alprazolam ($C_{17}H_{13}ClN_4$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U peak response of alprazolam from the *Sample solution* at each time point

r_S peak response of alprazolam from the *Standard solution*

C_S concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved at

each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_T = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (<i>i</i>)	Time (h)	Amount Dissolved			
		0.5-mg Tablet	1-mg Tablet	2-mg Tablet	3-mg Tablet
1	1	NMT 25%	NMT 25%	NMT 20%	NMT 20%
2	4	45%–60%	40%–55%	30%–50%	25%–45%
3	8	70%–90%	65%–85%	55%–75%	50%–70%
4	16	NLT 85%	NLT 85%	NLT 85%	NLT 80%

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Dissolution* { 711 }, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water; adjust with phosphoric acid or potassium hydroxide to a pH of 6.0 ± 0.1); 500 mL, deaerated

Apparatus 1: 100 rpm

Times: 1, 4, and 8 h for Tablets labeled to contain 0.5 mg or 1 mg; 1, 4, 8, and 16 h for Tablets labeled to contain 2 mg or 3 mg

Mobile phase: Acetonitrile and *Medium* (40:60)

Standard stock solution: 0.5 mg/mL of USP Alprazolam RS in methanol

Standard solution: ($L/500$) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 1- μ m pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 10-cm; 3- μ m or 5- μ m packing L7

Flow rate: 1 mL/min

Injection volume: 100 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of alprazolam ($C_{17}H_{13}ClN_4$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of alprazolam from the *Sample solution* at each time point

r_S = peak response of alprazolam from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_U = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (i)	Time (h)	Amount Dissolved			
		0.5-mg Tablet	1-mg Tablet	2-mg Tablet	3-mg Tablet
1	1	15%–35%	10%–30%	10%–30%	5%–25%
2	4	50%–75%	45%–65%	30%–55%	25%–50%
3	8	NLT 75%	NLT 70%	60%–80%	50%–75%
4	16	—	—	NLT 85%	NLT 80%

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0

g/L of dibasic potassium phosphate in water; adjust with phosphoric acid or potassium hydroxide to a pH of 6.0); 500 mL

Apparatus 1 (20-mesh basket): 100 rpm

Times: 1, 4, 8, and 16 h

Mobile phase: Acetonitrile and *Medium* (32:68)

Standard stock solution: 0.4 mg/mL of USP Alprazolam RS in methanol

Standard solution: ($L/500$) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet. Pass through a suitable filter of 0.45- μ m pore size, and use the filtrate.

Sample solution: At the end of specified time intervals, withdraw a known volume (V_S) of the solution from the dissolution vessel, and replace an equal volume of fresh *Medium* into the dissolution vessel. Pass the withdrawn sample through a suitable filter of 0.45- μ m pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 1.5 mL/min

Injection volume: 100 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of alprazolam ($C_{17}H_{13}N_4$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of alprazolam from the *Sample solution* at each time point

r_S = peak response of alprazolam from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}N_4$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times V] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

V_5 volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (i)	Time (h)	Amount Dissolved			
		0.5-mg Tablet	1-mg Tablet	2-mg Tablet	3-mg Tablet
1	1	NMT 40%	NMT 35%	NMT 35%	NMT 35%
2	4	50%–75%	45%–65%	35%–55%	30%–55%
3	8	NLT 75%	70%–90%	55%–75%	50%–70%
4	16	NLT 85%	NLT 85%	NLT 85%	NLT 75%

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Dissolution* $\langle 711 \rangle$, *Acceptance Table 2*.

- **Test 5:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water; adjust with phosphoric acid to a pH of 6.0); 500 mL

Apparatus 1: 100 rpm

Times: 1, 4, 8, and 16 h

Mobile phase: Acetonitrile, water, and phosphoric acid (350:650:1)

Standard stock solution: 0.5 mg/mL of USP Alprazolam RS in methanol

Standard solution: ($L/500$) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Column temperature: 30 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 50 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of alprazolam ($C_{17}H_{13}ClN_4$) in the sample withdrawn

from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U peak response of alprazolam from the *Sample solution* at each time point

r_S peak response of alprazolam from the *Standard solution*

C_S concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

V_S volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 5*.

Table 5

Time Point (i)	Time (h)	Amount Dissolved
1	1	NMT 25%
2	4	40%–65%
3	8	65%–95%
4	16	NLT 85%

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Dissolution* $\langle 711 \rangle$, *Acceptance Table 2*.

*(RB 1-Apr-2014)

- **Uniformity of Dosage Units** $\langle 905 \rangle$: Meet the requirements

IMPURITIES

Change to read:

- **Organic Impurities**

Buffer: 5.4 g/L of monobasic potassium phosphate (KH_2PO_4) in water. Adjust with phosphoric acid to a pH of 3.4.

Solution A: Acetonitrile, methanol, and *Buffer* (27:10:63)

Solution B: Acetonitrile, methanol, and *Buffer* (7:3:10)

Mobile phase: See *Table 6*.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	95	5
22	95	5
25	15	85
60	15	85
60.1	95	5
70	95	5

System suitability solution: 1 µg/mL each of USP Chlordiazepoxide Related Compound A RS, USP Alprazolam Related Compound A RS, and USP Nordazepam RS; and 0.4 µg/mL of USP Alprazolam RS in methanol

Standard solution: 0.4 µg/mL of USP Alprazolam RS in methanol

Sample solution: From NLT 20 Tablets ground to a fine powder, transfer an amount of powder to a suitable flask to obtain a nominal concentration of 0.2 mg/mL of alprazolam in methanol. [Note—Sonicate for 15 min to dissolve the contents.] Filter a portion, and discard the first 1 mL of filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times are listed in *Table 7*.]

Suitability requirements

Resolution: NLT 1.5 between nordazepam and alprazolam; NLT 1.5 between chlordiazepoxide related compound A and alprazolam related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for the alprazolam peak, *System suitability solution*

Relative standard deviation: NMT 5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of alprazolam in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 7*)

Acceptance criteria: See *Table 7*.

Table 7

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Chlordiazepoxide related compound A ^a	0.36	1.0	— ■ 0.2 ■ 1S (USP39)
Alprazolam related compound A	0.45	0.7	0.5
Nordazepam ^{a,b}	0.8	1.0	— ■ 0.2 ■ 1S (USP39)
Alprazolam	1.0	—	—
2-Amino-5-chloro-benzophenone	1.8	0.9	0.5
Amino-derivative ^c	2.2	1.2	0.5
Any other individual degradation product	—	1.0	0.2
Total impurities	—	—	2.0

a If present meets the requirement for any other individual degradation product.

b ~~7-Chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one (reported as unspecified impurity).~~

■

a If possible from the manufacturing process.

b 7-Chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one.

■ 1S (USP39)

c 7-Chloro-1-methyl-5-phenyl[1,2,4]triazolo[4,3-a]quinolin-4-amine.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at room temperature.

Change to read:

- **Labeling:** When more than one *Dissolution* test is given, the

■ The ■ 1S (USP39)

labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

- **USP Reference Standards** { 11 }

USP Alprazolam RS

USP Alprazolam Related Compound A RS

2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3H-1,4-benzodiazepine.

■ $C_{17}H_{15}ClN_4O$ 326.78 ■ 1S (USP39)

USP Chlordiazepoxide Related Compound A RS

7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.

$C_{15}H_{11}ClN_2O_2$ — 286.72

■ 286.71 ■ 2S (USP39)

USP Nordazepam RS **BRIEFING**

Alprazolam Orally Disintegrating Tablets, USP 38 page 2104. As part of the USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Add an *Identification* test *B* based on the UV spectral agreement for the main peak from the *Assay*.
2. Revise the *Assay* to add the use of a diode array detector to support the proposed *Identification* test *B*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: D. Min.)

Correspondence Number—C153797

Comment deadline: September 30, 2015

Alprazolam Orally Disintegrating Tablets**DEFINITION**

Alprazolam Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam (C₁₇H₁₃ClN₄).

IDENTIFICATION**Change to read:**

-

■ A.**■ 2S (USP39)**

The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. **■ 2S (USP39)**

ASSAY**Change to read:**

- **Procedure**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

Diluent: Acetonitrile and water (60:40)

Mobile phase: Acetonitrile, methanol, and *Buffer* (35:10:55)

Standard solution: 10 µg/mL of USP Alprazolam RS in *Diluent*

Sample solution: Nominally 10 µg/mL of alprazolam from Tablets prepared as follow.

Transfer 10 Tablets into a suitable volumetric flask. Add *Diluent* to volume and pass through a suitable filter. [Note—Sonicate with intermittent shaking to help dissolve, if

necessary.]

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 221 nm.

■ For *Identification* test B, use a diode array detector in the range of 200–400 nm.

■ 2S (USP39)

Column: 4.6-mm × 15-cm; 5-μm packing L7

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 30 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam (C₁₇H₁₃ClN₄) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Alprazolam RS in the *Standard solution* (μg/mL)

C_U nominal concentration of alprazolam in the *Sample solution* (μg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Disintegration { 701 }

Test 1

Time: NMT 60 s

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Disintegration Test 2*.

Time: NMT 30 s

• Dissolution { 711 }

Test 1

Medium: pH 6.0 phosphate buffer (8 g/L of monobasic potassium phosphate and 2 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid or diluted potassium hydroxide to a pH of 6.0 ± 0.1); 900 mL

Apparatus 2: 50 rpm

Time: 10 min

Mobile phase, Chromatographic system, and System suitability: Proceed as directed

in the *Assay*, except use an *Injection volume* of 100 μL .

Standard stock solution: 50 $\mu\text{g}/\text{mL}$ of USP Alprazolam RS in methanol. [Note—Sonicate to help dissolve, if necessary.]

Standard solution: $(L/1000)$ $\mu\text{g}/\text{mL}$ of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in $\mu\text{g}/\text{Tablet}$

Sample solution: Pass a portion of the solution under test through a nylon membrane filter of 0.45- μm pore size, discarding the first few mL.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ($\text{C}_{17}\text{H}_{13}\text{ClN}_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of alprazolam ($\text{C}_{17}\text{H}_{13}\text{ClN}_4$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: pH 6.0 phosphate buffer (8 g/L of monobasic potassium phosphate and 2 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of 6.0 ± 0.1); 500 mL

Apparatus 2: 50 rpm

Time: 10 min

Buffer: 1.36 g/L of monobasic potassium phosphate. Adjust with dilute sodium hydroxide to a pH of 6.0.

Mobile phase: Acetonitrile and *Buffer* (35:65)

Standard stock solution: 50 $\mu\text{g}/\text{mL}$ of USP Alprazolam RS in methanol. [Note—Sonicate to help dissolve, if necessary.]

Standard solution: $(L/500)$ $\mu\text{g}/\text{mL}$ of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in $\mu\text{g}/\text{Tablet}$

Sample solution: Pass a 5-mL aliquot of the solution under test through a suitable filter of 0.45- μm pore size, discarding the first 3 mL.

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 7.5-cm; 5- μm packing L7

Flow rate: 1.5 mL/min

Injection volume: 40 μL

Run time: 3 times the retention time of alprazolam

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam (C₁₇H₁₃ClN₄) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

Tolerances: NLT 70% (Q) of the labeled amount of alprazolam (C₁₇H₁₃ClN₄) is dissolved.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

- **Organic Impurities**

Diluent: Prepare as directed in the *Assay*.

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Solution A: Acetonitrile, methanol, and *Buffer* (25:20:55)

Solution B: Acetonitrile, methanol, and *Buffer* (40:5:55)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
12	100	0
15	0	100
60	0	100
65	100	0
70	100	0

Standard solution: 0.6 µg/mL of USP Alprazolam RS in *Diluent*

Sample solution: 200 µg/mL of alprazolam in *Diluent*. Prepare using 10 Tablets, and pass through a suitable filter.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements**Theoretical plates:** NLT 2000**Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 6.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U peak response of each impurity from the *Sample solution* r_S peak response of alprazolam from the *Standard solution* C_S concentration of USP Alprazolam RS in the *Standard solution* ($\mu\text{g/mL}$) C_U nominal concentration of alprazolam in the *Sample solution* ($\mu\text{g/mL}$) F = relative response factor (see *Table 2*)**Acceptance criteria:** See *Table 2*. Disregard any peaks less than 0.05%.**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Alprazolam related compound A ^{a,b}	0.8	—	—
Alprazolam	1.0	—	—
2-Amino-5-chlorobenzophenone	2.9	1.9	0.5
Any other unknown impurity	—	1.0	0.5
Total impurities	—	—	2.0

^a 2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3H-1,4-benzodiazepine.

^b Disregard the peak due to alprazolam related compound A, because it is a process impurity in alprazolam.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
- **Labeling:** When more than one *Disintegration* test is given, the labeling states the *Disintegration* test used only if *Test 1* is not used. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP Reference Standards** { 11 }
USP Alprazolam RS

BRIEFING

Aminobenzoate Potassium, *USP 38* page 2174; and *PF 41(2)* [Mar.–Apr. 2015]. In preparation for the omission of the flame tests from *Identification Tests—General* { 191 }, proposed in *PF 41(2)*, it is proposed to delete the reference to { 191 } under *Identification* test *C* and include a complete description of the flame test in the monograph. Manufacturers are encouraged to submit a replacement wet-chemistry or an instrumental procedure for the

Expert Committee's consideration.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

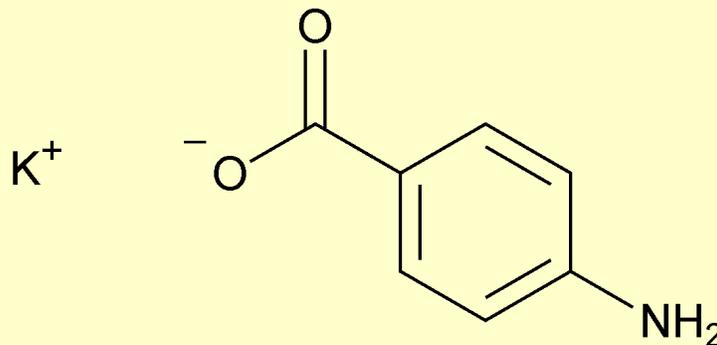
(SM2: H. Cai.)

Correspondence Number—C157466

Comment deadline: September 30, 2015

Aminobenzoate Potassium

Add the following:



$C_7H_6KNO_2$ 175.23

Benzoic acid, 4-amino-, potassium salt;
Potassium 4-aminobenzoate [138-84-1]. ■1S (*USP39*)

DEFINITION

Change to read:

Aminobenzoate Potassium contains NLT 98.5%

■98.0% ■1S (*USP39*)

and NMT 101.0%

■102.0% ■1S (*USP39*)

of aminobenzoate potassium ($C_7H_6KNO_2$), calculated on the dried basis.

IDENTIFICATION

Delete the following:

- ~~A. Ultraviolet Absorption (197U)~~

~~**Solution:** 10 µg/mL in 0.001 N sodium hydroxide~~

~~**Acceptance criteria:** Meets the requirements.~~

■1S (*USP39*)

Add the following:

- **A. Infrared Absorption (197K)** ■1S (*USP39*)

Delete the following:■● ~~B.~~~~**Sample:** 400 mg~~~~**Analysis:** Dissolve the *Sample* in 10 mL of water, add 1 mL of 3 N hydrochloric acid, filter, and wash the precipitate with two 5 mL portions of cold water. Recrystallize the precipitate from alcohol, and dry at 110° for 1 h.~~~~**Acceptance criteria:** The resulting *p*-aminobenzoic acid melts between 186° and 189°.~~~~■1S (USP39)~~**Add the following:**

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■1S (USP39)

Delete the following:

- ~~**C. Identification Tests—General, Potassium** (191):~~ A solution (1 in 100) meets the requirements of the flame test. ■2S (USP39)

Add the following:■● **C.****Sample solution:** A solution (1 in 100)

Acceptance criteria: The *Sample solution* imparts a violet color to a nonluminous flame. Since the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

■2S (USP39)

ASSAY**Change to read:**● **Procedure**~~**Sample:** 500 mg~~~~**Titrimetric system**~~~~**Mode:** Direct titration~~~~**Titrant:** 0.1 M sodium nitrite VS~~~~**Endpoint detection:** Potentiometric~~

~~**Analysis:** Add 25 mL of water and 25 mL of 3 N hydrochloric acid to the *Sample*, and cool in an ice bath. Titrate with *Titrant* using a calomel–platinum electrode system. Each mL of 0.1 M sodium nitrite is equivalent to 17.52 mg of aminobenzoate potassium ($C_7H_6KNO_2$).~~

~~**Acceptance criteria:** 98.5%–101.0%, on the dried basis~~

- **Solution A:** 1.5% Acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid and passing through a suitable filter of 0.45- μ m pore size

Mobile phase: Methanol and *Solution A* (15:85)

Standard solution: 0.1 mg/mL of USP Aminobenzoate Potassium RS in *Mobile phase*.
Sonicate to dissolve.

Sample solution: 0.1 mg/mL of Aminobenzoate Potassium in *Mobile phase*. Sonicate to dissolve.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.0-mm × 15-cm; 3.5-µm packing L11

Flow rate: 0.35 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoate potassium ($C_7H_6KNO_2$) in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Aminobenzoate Potassium RS in the *Standard solution* (mg/mL)

C_U concentration of Aminobenzoate Potassium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ■ 1S (USP39)

IMPURITIES

Delete the following:

- ~~Chloride and Sulfate, Chloride~~ 〈 221 〉

Sample: 1.4 g

Acceptance criteria: Shows no more chloride than corresponds to 0.4 mL of 0.020 N hydrochloric acid (0.02%) ■ 1S (USP39)

Delete the following:

- ~~Chloride and Sulfate, Sulfate~~ 〈 221 〉

Sample: 1.4 g

Acceptance criteria: Shows no more sulfate than corresponds to 0.3 mL of 0.020 N sulfuric acid (0.02%) ■ 1S (USP39)

Delete the following:

- **Heavy Metals, Method II** 〈 231 〉: NMT 0.002% ● (Official 1-Dec-2015)

Delete the following:

- **Volatile Diazotizable Substances**

Standard stock solution: 0.1 mg/mL of *p*-toluidine, prepared by dissolving a quantity of *p*-toluidine in 5% of the flask volume of methanol, and diluting with water to volume

Standard solution: 1 µg/mL of *p*-toluidine from the *Standard stock solution*

Sample solution: Transfer 5.0 g of Aminobenzoate Potassium to a suitable flask, and add a volume of 1.25 N sodium hydroxide that is just sufficient to dissolve the sample and to render the solution just alkaline to phenolphthalein TS. Dilute with water to 50 mL, and steam-distill the solution, collecting 95 mL of the distillate in a 100-mL volumetric flask. Dilute with water to volume.

Blank: Water

Instrumental conditions

Mode: Vis

Analytical wavelength: Wavelength of maximum absorbance at about 405 nm

Analysis

Samples: *Standard solution, Sample solution, and Blank*

Transfer 20.0 mL each of the *Standard solution, Sample solution, and Blank* to three separate 100-mL beakers, and treat each as follows. Add 5.0 mL of 1 N hydrochloric acid, and cool in an ice bath. Add 2.0 mL of 0.1 M sodium nitrite dropwise, with stirring. Allow to stand for 5 min for the diazotization reaction to be complete, add quickly to 10.0 mL of a cold solution of guaiacol (freshly prepared by dissolving 0.20 g of guaiacol in 100 mL of 1 N sodium hydroxide), mix and allow to stand for 30 min. Concomitantly determine the absorbances of the solutions.

Acceptance criteria: The absorbance of the solution from the *Sample solution* does not exceed that of the solution from the *Standard solution*, corresponding to NMT 0.002% of volatile diazotizable substances as *p*-toluidine. ■ 1S (USP39)

Add the following:

- **Limit of Aniline and *p*-Toluidine**

Diluent: Methylene chloride

Standard stock solution: 0.1 mg/mL each of USP Aniline RS and USP *p*-Toluidine RS in *Diluent*

Standard solution: 1 µg/mL each of USP Aniline RS and USP *p*-Toluidine RS in *Diluent* from *Standard stock solution*

Sample solution: 100 mg/mL of Aminobenzoate Potassium in *Diluent* prepared as follows. Add an appropriate quantity of Aminobenzoate Potassium to a suitable volumetric flask and dilute with *Diluent* to volume. Agitate for 10 min on a shaker and centrifuge at 3000 rpm for 5 min. Use the supernatant.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: GC

Detectors**Flame ionization:** 300°**Hydrogen:** 40 mL/min**Air:** 400 mL/min**Column:** 30-m × 0.32-mm fused silica capillary; coated with 0.5-µm film of phase G27**Temperatures****Injection port:** 280°**Detector:** 300°**Column:** See *Table 1*.**Table 1**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	—	130	4
130	20	180	5

Carrier gas: Helium**Flow rate:** 1.0 mL/min**Injection volume:** 2 µL**Injection type:** Split ratio, 1:10**System suitability****Sample:** *Standard solution*[Note—The relative retention times of aniline and *p*-toluidine are about 4.1 and 5.1 min, respectively.]**Suitability requirements****Resolution:** NLT 7.0 between aniline and *p*-toluidine**Tailing factor:** NMT 1.5 for aniline and *p*-toluidine**Relative standard deviation:** NMT 6.0% for aniline and *p*-toluidine**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of *p*-toluidine or aniline in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of *p*-toluidine or aniline from the *Sample solution* r_S = peak response of *p*-toluidine or aniline from the *Standard solution* C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL) C_U = concentration of Aminobenzoate Potassium in the *Sample solution* (mg/mL)**Acceptance criteria****Aniline:** NMT 10 ppm***p*-Toluidine:** NMT 20 ppm

■ 1S (USP39)

Add the following:

Organic Impurities

Solution A: 1.5% Acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid

Solution B: Methanol

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	85	15
4.0	85	15
4.1	45	55
10.0	45	55
10.1	85	15
13	85	15

Diluent: Methanol and water (85:15)

System suitability solution: 1 mg/mL of USP Aminobenzoate Potassium RS, 0.01 mg/mL of USP 4-Nitrobenzoic Acid RS, and 0.01 mg/mL of USP Benzocaine RS in *Diluent* prepared as follows. Transfer 1 mL each of 0.1 mg/mL of USP 4-Nitrobenzoic Acid RS in methanol and 0.1 mg/mL USP of Benzocaine RS in *Diluent* to a 10-mL volumetric flask containing the appropriate amount of USP Aminobenzoate Potassium RS, and dilute with *Diluent* to volume.

Standard solution: 1 µg/mL each of USP Aminobenzoate Potassium RS, USP 4-Nitrobenzoic Acid RS, and USP Benzocaine RS in *Diluent*

Sample solution: 1 mg/mL of Aminobenzoate Potassium in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.0-mm × 15-cm; 3.5-µm packing L11

Flow rate: 0.4 mL/min

Injection volume: 5 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between benzocaine and 4-nitrobenzoic acid, *System suitability solution*

Relative standard deviation: NMT 3% for the aminobenzoate potassium, 4-nitrobenzoic acid, and benzocaine peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 4-nitrobenzoic acid or benzocaine in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 4-nitrobenzoic acid or benzocaine from the *Sample solution*

r_S peak response of 4-nitrobenzoic acid or benzocaine from the *Standard solution*

C_S concentration of USP 4-Nitrobenzoic Acid RS or USP Benzocaine RS in the *Standard solution* (mg/mL)

C_U concentration of Aminobenzoate Potassium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any unspecified impurity from the *Sample solution*

r_S peak response of aminobenzoate from the *Standard solution*

C_S concentration of USP Aminobenzoate Potassium RS in the *Standard solution* (mg/mL)

C_U concentration of Aminobenzoate Potassium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	1.0	—
Benzocaine	2.0	0.2
4-Nitrobenzoic acid	2.1	0.2
Any individual unspecified impurity	—	0.10

■ 1S (USP39)

SPECIFIC TESTS

- pH 〈 791 〉

Sample solution: 50 mg/mL

Acceptance criteria: 8.0–9.0

- Loss on Drying 〈 731 〉

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Aminobenzoate Potassium RS

■

USP Aniline RS

Aniline.

C₆H₇N 93.13

USP Benzocaine RS

USP 4-Nitrobenzoic Acid RS

4-Nitrobenzoic acid.

C₇H₅NO₄ 167.12USP *p*-Toluidine RS

4-Methylaniline.

C₇H₉N 107.15

■ 1S (USP39)

BRIEFING

Aminobenzoate Potassium Capsules, *USP 38* page 2175. As part of the USP monograph modernization effort, the following revisions are proposed:

1. Add *Identification* test *B* based on the retention time agreement of aminobenzoic acid in the proposed HPLC method.
2. Replace the existing UV procedure in the *Assay* with a validated stability-indicating HPLC procedure. The proposed liquid chromatographic procedure is based on analyses performed with the Eclipse XBD-Phenyl brand of L11 column. The typical retention time for aminobenzoic acid is about 6.0 min.
3. Add a test for *Organic Impurities* based on the proposed HPLC procedure in the *Assay*. This method monitors impurities that are consistent with those found in aminobenzoic acid.
4. Add a new Reference Standard and an existing Reference Standard, introduced by the proposed HPLC procedure, to the *USP Reference Standards* section: USP 4-Nitrobenzoic Acid RS and USP Benzocaine RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: C. Anthony.)

Correspondence Number—C88870

Comment deadline: September 30, 2015

Aminobenzoate Potassium Capsules

DEFINITION

Aminobenzoate Potassium Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of aminobenzoate potassium (C₇H₆KNO₂).

IDENTIFICATION

● **A.**

Sample: 1 g of the Capsule contents

Analysis: Dissolve the *Sample* in 25 mL of water, add 5 mL of 3 N hydrochloric acid, and wash the precipitate with two 5-mL portions of cold water. Recrystallize from alcohol the precipitate so obtained, and dry at 110° for 1 h.

Acceptance criteria: The *p*-aminobenzoic acid melts between 186° and 189°.

Add the following:

- ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the

Standard solution, as obtained in the Assay. ■2S (USP39)

ASSAY

Change to read:

● Procedure

~~**Standard solution:** 5 µg/mL of USP Aminobenzoate Potassium RS in water~~

~~**Sample solution:** Nominally 5 µg/mL of aminobenzoate potassium prepared as follows. Remove as completely as possible, and combine, the contents of NLT 20 Capsules. Transfer a portion of the combined contents, equivalent to 100 mg of aminobenzoate potassium, to a 200 mL volumetric flask. Add 150 mL of water, shake by mechanical means for 30 min, dilute with water to volume, and filter. Pipet 2 mL of the filtrate into a 200 mL volumetric flask, and dilute with water to volume.~~

~~Spectrometric conditions-~~

~~**Mode:** UV~~

~~**Analytical wavelength:** maximum absorbance at about 270 nm~~

~~**Blank:** Water~~

~~Analysis-~~

~~**Samples:** *Standard solution* and *Sample solution*~~

~~Concomitantly determine the absorbances of the *Standard solution* and *Sample solution* and calculate the percentage of the labeled amount of aminobenzoate potassium ($C_7H_6KNO_2$) in the portion of Capsule taken:~~

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

~~A_U absorbance of the *Sample solution*~~

~~A_S absorbance of the *Standard solution*~~

~~C_S concentration of USP Aminobenzoate Potassium RS in the *Standard solution* (µg/mL)~~

~~C_U nominal concentration of aminobenzoate potassium in the *Sample solution* (µg/mL)~~

~~**Acceptance criteria:** 90.0%–110.0%~~

■**Solution A:** 1.5% Acetic acid prepared as follows. Mix 690 mL of water with 10 mL of acetic acid and pass through a filter of 0.45-µm pore size.

Mobile phase: Methanol and *Solution A* (15:85)

Standard solution: 0.1 mg/mL of USP Aminobenzoate Potassium RS in *Mobile phase*

Sample solution: Nominally 0.1 mg/mL of aminobenzoate potassium prepared as follows. Remove as completely as possible, and combine the contents of NLT 10 Capsules. Transfer a portion of the combined contents, equivalent to 10 mg of aminobenzoate potassium to a 100-mL volumetric flask, dissolve in 70 mL of *Mobile phase*, sonicate for 3–4 min, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: 280 nm

Column: 3.0-mm × 15-cm; 3.5-µm packing L11

Flow rate: 0.35 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aminobenzoate potassium ($\text{C}_7\text{H}_6\text{KNO}_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Aminobenzoate Potassium RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of aminobenzoate potassium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110% $\pm 2S$ (USP39)

PERFORMANCE TESTS

● **Dissolution** { 711 }

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 270 nm

Standard solution: A known concentration of USP Aminobenzoate Potassium RS in *Medium*

Sample solution: Filter portions of the solution under test, and dilute with *Medium*, if necessary, in comparison with the *Standard solution* concentration.

Analysis: Calculate the percentage of the labeled amount of aminobenzoate potassium ($\text{C}_7\text{H}_6\text{KNO}_2$) dissolved.

Tolerances: NLT 75% (Q) of the labeled amount of aminobenzoate potassium ($\text{C}_7\text{H}_6\text{KNO}_2$) is dissolved.

● **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES**Add the following:****Organic Impurities**

Solution A, Mobile phase, and Chromatographic system: Prepare as directed in the Assay.

Standard solution: 1 µg/mL each of USP Aminobenzoate Potassium RS, USP 4-Nitrobenzoic Acid RS, and USP Benzocaine RS in *Mobile phase*

Sensitivity solution: 0.1 µg/mL of USP Aminobenzoate Potassium RS in *Mobile phase* from *Standard solution*

Sample solution: Nominally 1 mg/mL of aminobenzoate potassium in *Mobile phase* prepared as follows. Remove as completely as possible, and combine, the contents of NLT 10 Capsules. Transfer a portion of the combined contents, equivalent to 10 mg of aminobenzoate potassium, to a 10-mL volumetric flask. Dissolve in 7 mL of *Mobile phase*, sonicate for 3–4 min, and dilute with *Mobile phase* to volume.

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.5 between benzocaine and 4-nitrobenzoic acid, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of any individual unspecified degradation product from the *Sample solution*

r_S peak response of aminobenzoate from the *Standard solution*

C_S concentration of USP Aminobenzoate Potassium RS in the *Standard solution* (mg/mL)

C_U nominal concentration of aminobenzoate potassium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	1.0	—

^a These are process impurities controlled in the API and are included in this table for identification purposes only. They are not reported in the drug product and should not be included in the total impurities.

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzocaine ^a	2.0	—
4-Nitrobenzoic acid ^a	2.1	—
Any individual unspecified degradation product	—	0.10
Total impurities	—	1.0

^a These are process impurities controlled in the API and are included in this table for identification purposes only. They are not reported in the drug product and should not be included in the total impurities.

■ 2S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Aminobenzoate Potassium RS

■ USP Benzocaine RS

USP 4-Nitrobenzoic Acid RS

4-Nitrobenzoic acid.

C₇H₅NO₄ 167.12

■ 2S (USP39)

BRIEFING

Aminobenzoate Sodium, *USP 38* page 2176 and *PF 41(2)* [Mar.–Apr. 2015]. In preparation for the omission of the flame tests from *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)*, it is proposed to delete the reference to 〈 191 〉 under *Identification* test C and include a complete description of the flame test in the monograph. Manufacturers are encouraged to submit a replacement wet-chemistry or an instrumental procedure for the Expert Committee's consideration.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: H. Cai.)

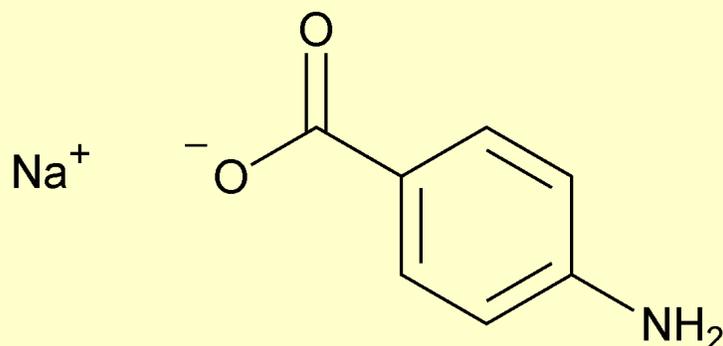
Correspondence Number—C157465

Comment deadline: September 30, 2015

Aminobenzoate Sodium

Add the following:

■



$C_7H_6NNaO_2$ 159.12

Benzoic acid, 4-amino-, sodium salt;
Sodium 4-aminobenzoate [555-06-6]. ■1S (USP39)

DEFINITION

Change to read:

Aminobenzoate Sodium contains NLT 98.5%

■98.0% ■1S (USP39)

and NMT 101.0%

■102.0% ■1S (USP39)

of aminobenzoate sodium ($C_7H_6NNaO_2$), calculated on the dried basis.

IDENTIFICATION

Delete the following:

■● ~~A. Ultraviolet Absorption (197U)~~

~~**Sample solution:** 10 µg/mL in 0.001 N sodium hydroxide~~

~~**Acceptance criteria:** Meets the requirements.~~

■1S (USP39)

Add the following:

■● **A. Infrared Absorption (197K)** ■1S (USP39)

Delete the following:

■● ~~B-~~

~~**Sample:** 400 mg~~

~~**Analysis:** Dissolve the *Sample* in 10 mL of water, add 1 mL of 3 N hydrochloric acid, filter, and wash the precipitate with two 5 mL portions of cold water. Recrystallize the precipitate from alcohol, and dry at 110° for 1 h.~~

~~**Acceptance criteria:** The resulting *p*-aminobenzoic acid melts between 186° and 189°.~~

■1S (USP39)

Add the following:

- ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 1S (USP39)

Delete the following:

- ● **C. Identification Tests—General, Sodium** (191): A solution (1 in 100) meets the requirements of the flame test. ■ 2S (USP39)

Add the following:

- ● **C.**
Sample solution: A solution (1 in 100)
Acceptance criteria: The *Sample solution* imparts an intense yellow color to a nonluminous flame. ■ 2S (USP39)

ASSAY**Change to read:**● **Procedure**~~**Sample:** 500 mg~~~~**Titrimetric system**~~~~**Mode:** Direct titration~~~~**Titrant:** 0.1 M sodium nitrite VS~~~~**Endpoint detection:** Potentiometric~~~~**Analysis:** Add 25 mL of water and 25 mL of 3 N hydrochloric acid to the *Sample* in a suitable vessel, and cool in an ice bath. Titrate with *Titrant* using a calomel–platinum electrode system. Each mL of 0.1 M sodium nitrite is equivalent to 15.91 mg of aminobenzoate sodium ($C_7H_6NNaO_2$).~~~~**Acceptance criteria:** 98.5%–101.0%, on the dried basis~~

- **Solution A:** 1.5% Acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid, and passing through a suitable filter of 0.45- μ m pore size

Mobile phase: Methanol and *Solution A* (15:85)**Standard solution:** 0.1 mg/mL of USP Aminobenzoate Sodium RS in *Mobile phase*. Sonicate to dissolve.**Sample solution:** 0.1 mg/mL of Aminobenzoate Sodium in *Mobile phase*. Sonicate to dissolve.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 280 nm**Column:** 3.0-mm \times 15-cm; 3.5- μ m packing L11**Flow rate:** 0.35 mL/min**Injection volume:** 5 μ L**System suitability****Sample:** *Standard solution***Suitability requirements**

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoate sodium ($C_7H_6NNaO_2$) in the portion of Aminobenzoate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Aminobenzoate Sodium RS in the *Standard solution* (mg/mL)

C_U concentration of Aminobenzoate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ■ 1S (USP39)

IMPURITIES

Delete the following:

- ~~Chloride and Sulfate, Chloride <221>~~

~~Sample:~~ 1.4 g

~~Acceptance criteria:~~ Shows no more chloride than corresponds to 0.4 mL of 0.020 N hydrochloric acid (0.02%). ■ 1S (USP39)

Delete the following:

- ~~Chloride and Sulfate, Sulfate <221>~~

~~Sample:~~ 1.4 g

~~Acceptance criteria:~~ Shows no more sulfate than corresponds to 0.3 mL of 0.020 N sulfuric acid (0.02%). ■ 1S (USP39)

Delete the following:

- ~~Heavy Metals, Method II <231>~~: NMT 0.002% ● (Official 1-Dec-2015)

Delete the following:

- ~~Volatile Diazotizable Substances~~

~~Standard stock solution:~~ 0.1 mg/mL of *p*-toluidine, prepared by dissolving a quantity of *p*-toluidine in 5% of the volumetric flask volume of methanol, and diluting with water to volume

~~Standard solution:~~ 1 µg/mL of *p*-toluidine from the *Standard stock solution*

~~Sample solution:~~ Transfer 5.0 g of Aminobenzoate Sodium to a suitable flask, and add a volume of 1.25 N sodium hydroxide that is just sufficient to dissolve the sample and to render the solution just alkaline to phenolphthalein TS. Dilute with water to 50 mL, and steam distill the solution, collecting 95 mL of the distillate in a 100 mL volumetric flask.

Dilute with water to volume.

Blank: Water

Instrumental conditions

Mode: Vis

Analytical wavelength: Wavelength of maximum absorbance at about 405 nm

Analysis

Samples: *Standard solution, Sample solution, and Blank*

Transfer 20.0 mL each of the *Standard solution, Sample solution, and Blank* to three separate 100 mL beakers, and treat each as follows. Add 5.0 mL of 1 N hydrochloric acid, and cool in an ice bath. Add 2.0 mL of 0.1 M sodium nitrite dropwise, with stirring. Allow to stand for 5 min for the diazotization reaction to be complete, add quickly to 10.0 mL of a cold solution of guaiacol (freshly prepared by dissolving 0.20 g of guaiacol in 100 mL of 1 N sodium hydroxide), and allow to stand for 30 min. Concomitantly determine the absorbances of the solutions.

Acceptance criteria: The absorbance of the solution from the *Sample solution* does not exceed that of the solution from the *Standard solution*, corresponding to NMT 0.002% of volatile diazotizable substances as *p*-toluidine. ■ 1S (USP39)

Add the following:

■ • **Limit of Aniline and *p*-Toluidine**

Diluent: Methylene chloride

Standard stock solution: 0.1 mg/mL each of USP Aniline RS and USP *p*-Toluidine RS in *Diluent*

Standard solution: 1.0 µg/mL each of USP Aniline RS and USP *p*-Toluidine RS in *Diluent* from *Standard stock solution*

Sample solution: 100 mg/mL of Aminobenzoate Sodium in *Diluent* prepared as follows. Add an appropriate quantity of Aminobenzoate Sodium to a suitable volumetric flask and dilute with *Diluent* to volume. Agitate for 10 min on a shaker and centrifuge at 3000 rpm for 5 min. Use the supernatant.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: GC

Detectors

Flame ionization: 300°

Hydrogen: 40 mL/min

Air: 400 mL/min

Column: 30-m × 0.32-mm fused silica capillary; coated with 0.5-µm film of phase G27

Temperatures

Injection port: 280°

Detector: 300°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	—	130	4
130	20	180	5

Carrier gas: Helium

Flow rate: 1 mL/min

Injection volume: 2 µL

Injection type: Split ratio, 1:10

System suitability

Sample: *Standard solution*

[Note—The relative retention times of aniline and *p*-toluidine are about 4.1 and 5.1 min, respectively.]

Suitability requirements

Resolution: NLT 7.0 between aniline and *p*-toluidine

Tailing factor: NMT 1.5 for aniline and *p*-toluidine

Relative standard deviation: NMT 6.0% for aniline and *p*-toluidine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of *p*-toluidine or aniline in the portion of Aminobenzoate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of *p*-toluidine or aniline from the *Sample solution*

r_S = peak response of *p*-toluidine or aniline from the *Standard solution*

C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

C_U = concentration of Aminobenzoate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria

Aniline: NMT 10 ppm

***p*-Toluidine:** NMT 20 ppm

■ 1S (USP39)

Add the following:

■ • Organic Impurities

Solution A: 1.5% Acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid

Solution B: Methanol

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	85	15
4.0	85	15

Time (min)	Solution A (%)	Solution B (%)
4.1	45	55
10.0	45	55
10.1	85	15
13	85	15

Diluent: Methanol and water (85:15)

System suitability solution: 1 mg/mL of USP Aminobenzoate Sodium RS, 0.01 mg/mL of USP 4-Nitrobenzoic Acid RS, and 0.01 mg/mL of USP Benzocaine RS in *Diluent* prepared as follows. Transfer 1 mL each of 0.1 mg/mL of USP 4-Nitrobenzoic Acid RS in methanol and 0.1 mg/mL of USP Benzocaine RS in *Diluent* to a 10-mL volumetric flask containing the appropriate amount of USP Aminobenzoate Sodium RS, and dilute with *Diluent* to volume.

Standard solution: 1 µg/mL each of USP Aminobenzoate Sodium RS, USP 4-Nitrobenzoic Acid RS, and USP Benzocaine RS in *Diluent*

Sample solution: 1 mg/mL of Aminobenzoate Sodium in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.0-mm × 15-cm; 3.5-µm packing L11

Flow rate: 0.4 mL/min

Injection volume: 5 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between benzocaine and 4-nitrobenzoic acid, *System suitability solution*

Relative standard deviation: NMT 3% for the aminobenzoate sodium, 4-nitrobenzoic acid, and benzocaine peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 4-nitrobenzoic acid or benzocaine in the portion of Aminobenzoate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of 4-nitrobenzoic acid or benzocaine from the *Sample solution*

r_S peak response of 4-nitrobenzoic acid or benzocaine from the *Standard solution*

C_S concentration of USP 4-Nitrobenzoic Acid RS or USP Benzocaine RS in the *Standard solution* (mg/mL)

C_U concentration of Aminobenzoate Sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Aminobenzoate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_{\bar{f}}$ peak response of any unspecified impurity from the *Sample solution*

$r_{\bar{s}}$ peak response of aminobenzoate from the *Standard solution*

$C_{\bar{s}}$ concentration of USP Aminobenzoate Sodium RS in the *Standard solution* (mg/mL)

$C_{\bar{f}}$ concentration of Aminobenzoate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	1.0	—
Benzocaine	2.0	0.2
4-Nitrobenzoic acid	2.1	0.2
Any individual unspecified impurity	—	0.10

■ 1S (USP39)

SPECIFIC TESTS

- **pH** 〈 791 〉

Sample solution: 50 mg/mL

Acceptance criteria: 8.0–9.0

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Aminobenzoate Sodium RS

■

USP Aniline RS

Aniline.

C₆H₇N 93.13

USP Benzocaine RS

USP 4-Nitrobenzoic Acid RS

4-Nitrobenzoic acid.

C₇H₅NO₄ 167.12

USP *p*-Toluidine RS

4-Methylaniline.

C₇H₉N 107.16

■ 1S (USP39)

Gamma-Aminobutyric Acid Capsules. Because there is no existing *USP* monograph for this dosage form, a new monograph is being proposed. The liquid chromatographic procedure in the test for *Strength* is based on analyses performed with the Waters XBridge Amide brand of L68 column. Typical retention times for gamma-aminobutyric acid and monosodium glutamate are 7.2 and 8.7 min, respectively.

(DS: N. Davydova.)

Correspondence Number—C135188

Comment deadline: September 30, 2015

Add the following:

■ **Gamma-Aminobutyric Acid Capsules**

DEFINITION

Gamma-Aminobutyric Acid Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of gamma-aminobutyric acid (C₄H₉NO₂).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Strength*.

STRENGTH

● **Procedure**

Buffer solution: 20 mM monobasic sodium phosphate. Adjust with 1 N sodium hydroxide to a pH of 5.5.

Mobile phase: Acetonitrile and *Buffer solution* (78:22)

Diluent: Acetonitrile and water (60:40)

System suitability solution: 1 mg/mL of USP Gamma-Aminobutyric Acid RS and 0.1 mg/mL of monosodium glutamate in *Diluent*

Standard solution: 1 mg/mL of USP Gamma-Aminobutyric Acid RS in *Diluent*

Sample solution: Accurately weigh the contents of NLT 20 Capsules and mix. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of gamma-aminobutyric acid, to a 50-mL volumetric flask. Add about 40 mL of *Diluent* and sonicate for about 10 min. Cool the mixture to room temperature, dilute with *Diluent* to volume, and pass through a nylon membrane filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* 〈621〉, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm \times 15-cm; 3.5- μ m packing L68

Column temperature: 35^o

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for gamma-aminobutyric acid and monosodium

glutamate are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 2.0 between gamma-aminobutyric acid and monosodium glutamate, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of gamma-aminobutyric acid ($C_4H_9NO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Gamma-Aminobutyric Acid RS in the *Standard solution* (mg/mL)

C_U nominal concentration of gamma-aminobutyric acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Disintegration and Dissolution** $\langle 2040 \rangle$: Meet the requirements for *Disintegration*
- **Weight Variation** $\langle 2091 \rangle$: Meet the requirements

CONTAMINANTS

- **Microbial Enumeration Tests** $\langle 2021 \rangle$: The total aerobic microbial count does not exceed 3×10^3 cfu/g, and the total combined molds and yeasts count does not exceed 3×10^2 cfu/g.
- **Absence of Specified Microorganisms** $\langle 2022 \rangle$: Meet the requirements of the test for absence of *Escherichia coli*

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** $\langle 11 \rangle$
USP Gamma-Aminobutyric Acid RS
■ 2S (USP39)

BRIEFING

Chlordiazepoxide Hydrochloride for Injection, USP 38 page 2756. It is proposed to omit this monograph for the following reasons:

1. This dosage form is not currently used for human use in the United States.
2. This dosage form is not currently used for veterinary medicine in the United States.

(SM4: H. Joyce.) Correspondence Number—C155481

Comment deadline: September 30, 2015

Delete the following:

■ Chlordiazepoxide Hydrochloride for Injection

» ~~Chlordiazepoxide Hydrochloride for Injection is Chlordiazepoxide Hydrochloride suitable for parenteral use.~~

Packaging and storage—~~Preserve in Containers for Sterile Solids as described under Injections ~~(1)~~, protected from light.~~

USP Reference standards ~~(11)~~—

~~USP 2-Amino-5-chlorobenzophenone RS—~~

~~C₁₃H₁₀ClNO 231.68~~

~~USP Chlordiazepoxide Hydrochloride RS~~

~~USP Chlordiazepoxide Related Compound A RS—~~

~~7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.~~

~~C₁₅H₁₁ClN₂O₂ 286.72~~

~~USP Endotoxin RS~~

Completeness of solution ~~(64)~~—~~It dissolves in the solvent and in the concentration recommended in the labeling to yield a clear solution.~~

Change to read:

Constituted solution—

• ~~At the time of use, it meets the requirements for~~

~~Constituted Solutions under Injections ~~(1)~~; • (ERR 1 Aug 2014)~~

Bacterial endotoxins ~~(85)~~—~~It contains not more than 3.57 USP Endotoxin Units per mg of chlordiazepoxide hydrochloride.~~

pH

~~(79)~~: between 2.5 and 3.5, in a solution (1 in 100).

Change to read:

Other requirements—

~~It responds to the Identification tests and meets the requirements of the tests for Loss on drying~~

• • (Official 1 Dec 2015)

~~under Chlordiazepoxide Hydrochloride, and the test for Related compounds under~~

~~Chlordiazepoxide. It meets also the requirements for Sterility Tests ~~(71)~~, Uniformity of~~

~~Dosage Units ~~(905)~~, and Labeling under Injections ~~(1)~~.~~

Assay—

~~Proceed with Chlordiazepoxide Hydrochloride for Injection as directed in the Assay under Chlordiazepoxide, except to use USP Chlordiazepoxide Hydrochloride RS to prepare the Standard preparation. ■ 2S (USP39)~~

BRIEFING

Diatrizoate Sodium, *USP 38* page 3070. On the basis of comments received, it is proposed to revise the monograph as follows:

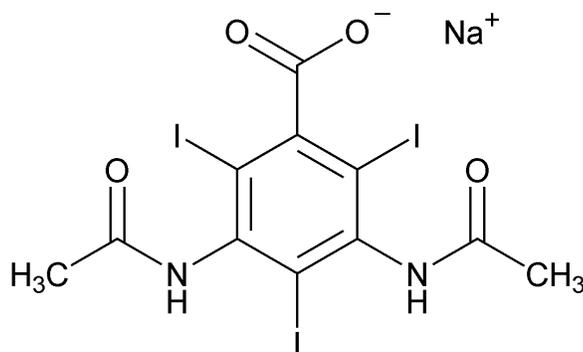
1. Delete *Identification* test *B* because it generates iodine vapors, which are a potential safety hazard because iodine vapors can irritate the nose and eyes.
2. Delete the reference to *Identification Tests—General* { 191 }, *Sodium* in *Identification* test *C* and include a complete description of each flame test in the monograph.
3. Include a calculation formula in the *Assay*.
4. Rename the test for *Organic Impurities, Procedure: Free Aromatic Amine* as *Limit of Free Aromatic Amine*. Reformat the procedure to make it easier to follow and perform.
5. Rename the *Iodine and Iodide* test as *Limit of Iodine and Iodide*. Reformat the procedure to make it easier to follow and perform.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R. Ravichandran.)

Correspondence Number—C157591

Comment deadline: September 30, 2015

Diatrizoate Sodium

$C_{11}H_8I_3N_2NaO_4$ 635.90

Benzoic acid, 3,5-bis(acetylamino)-2,4,6-triiodo-, monosodium salt;
 Monosodium 3,5-diacetamido-2,4,6-triiodobenzoate [737-31-5].

DEFINITION

Diatrizoate Sodium contains NLT 98.0% and NMT 102.0% of diatrizoate sodium ($C_{11}H_8I_3N_2NaO_4$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. Thin-Layer Chromatographic Identification Test** (201)

Solution A: Sodium hydroxide in methanol (0.8 in 1000)

Standard solution: 1 mg/mL of USP Diatrizoic Acid RS in *Solution A*

Sample solution: 1 mg/mL of Diatrizoate Sodium in *Solution A*

Developing solvent system: Methanol, chloroform, and ammonium hydroxide (10:20:2)

Analysis

Samples: *Standard solution* and *Sample solution*

Locate the spots using short-wavelength UV light.

■ **Acceptance criteria:** Meets the requirements ■ 2S (USP39)

Delete the following:

- ● ~~B. Heat 500 mg in a suitable crucible; violet vapors are evolved.~~ ■ 2S (USP39)

Add the following:

- ● B. A 5-mg/mL solution in water imparts an intense yellow color to a nonluminous flame confirming the presence of sodium. ■ 2S (USP39)

Delete the following:

- ● ~~C. Identification Tests—General, Sodium (191):~~ It meets the requirements of the flame test. ■ 2S (USP39)

ASSAY

Change to read:

- **Procedure**

Sample solution: Transfer 300 mg of Diatrizoate Sodium to a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 h. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS.

■ **Titrimetric system**

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.05 N silver nitrate VS

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with the *Titrant* until the yellow precipitate just turns green.

Calculate the percentage of diatrizoate sodium ($C_{11}H_8I_3N_2NaO_4$) in the portion of Diatrizoate Sodium taken:

$$\text{Result} = [(V \times N \times F)/W] \times 100$$

V

= sample *Titrant* volume (mL)

 N

= *Titrant* normality (mEq/mL)

 F

= equivalent weight of diatrizoate sodium, 212 mg/mEq

 W

= weight of Diatrizoate Sodium (mg)

■ 2S (USP39)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Delete the following:

●● Heavy Metals 〈 231 〉

Standard solution: To 2.0 mL of *Standard Lead Solution* (20 µg of Pb) in a 50-mL color-comparison tube, add 5 mL of 1 N sodium hydroxide, and dilute with water to 40 mL.

Sample solution: Dissolve 1.0 g of Diatrizoate Sodium in 20 mL of water and 5 mL of 1 N sodium hydroxide, transfer the solution to a 50-mL color-comparison tube, and dilute with water to 40 mL.

Analysis: To each of the tubes containing the *Standard solution* and the *Sample solution*, add 10 mL of sodium sulfide TS, allow to stand for 5 min, and view downward over a white surface.

Acceptance criteria: The color of the solution from the *Sample solution* is not darker than that of the solution from the *Standard solution* (0.002%). •(Official 1-Dec-2015)

Delete the following:

●● Organic Impurities

● Procedure: Free Aromatic Amine

Sample solution: Transfer 1.0 g to a 50-mL volumetric flask, and add 5 mL of water and 10 mL of 0.1 N sodium hydroxide.

Standard stock solution: Dissolve a suitable quantity of USP Diatrizoic Acid Related Compound A RS in 0.1 N sodium hydroxide. Use 0.2 mL of 0.1 N sodium hydroxide for each 5.0 mg of Standard, and dilute with water to obtain 500 µg/mL.

Standard solution: Transfer 4 mL of water, 10 mL of 0.1 N sodium hydroxide, and 1.0 mL of a *Standard stock solution* to a 50-mL volumetric flask.

Blank: Transfer 5 mL of water and 10 mL of 0.1 N sodium hydroxide to a 50-mL volumetric flask.

Analysis: Treat each flask as follows. Add 25 mL of dimethyl sulfoxide, insert the stopper, and mix by swirling gently. Chill in an ice bath in the dark for 5 min.

[Note—In conducting the following steps, keep the flasks in the ice bath and in the dark as

~~much as possible until all of the reagents have been added. }~~

~~Slowly add 2 mL of hydrochloric acid and allow to stand for 5 min. Add 2 mL of sodium nitrite solution (1 in 50), and allow to stand for 5 min. Add 1 mL of sulfamic acid solution (2 in 25), shake, and allow to stand for 5 min.~~

~~[Note—Considerable pressure is produced.]~~

~~Add 2 mL of a solution (1 in 1000) of *N*-(1-naphthyl)ethylenediamine dihydrochloride in dilute propylene glycol (7 in 10). Remove the flasks from the ice bath and from storage in the dark, and allow to stand in a water bath at 22°–25° for 10 min. Shake gently and occasionally during this period, releasing the pressure by loosening the stopper. Dilute with water to volume.~~

Spectrometric conditions

~~(See *Spectrophotometry and Light Scattering* (851).)~~

~~**Mode:** UV-Vis~~

~~**Analytical wavelength:** About 465 nm~~

~~**Cell:** 1 cm~~

~~**Analysis:** Within 5 min from the time of diluting the solutions in all three flasks to 50 mL, concomitantly determine the absorbances of the solutions.~~

~~**Acceptance criteria:** The absorbance of the *Sample solution* is NMT that of the *Standard solution* (0.05%).~~

■ 2S (USP39)

Add the following:

■ • **Limit of Free Aromatic Amine**

Solution A: 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in a mixture of propylene glycol and water (70:30)

Standard stock solution: 0.5 mg/mL of USP Diatrizoic Acid Related Compound A RS in 0.1 N sodium hydroxide

Standard solution: Transfer 4 mL of water, 10 mL of 0.1 N sodium hydroxide, and 1.0 mL of *Standard stock solution* to a 50-mL volumetric flask.

Sample solution: Transfer 1.0 g of Diatrizoate Sodium to a 50-mL volumetric flask, and add 5 mL of water and 10 mL of 0.1 N sodium hydroxide.

Blank solution: Transfer 5 mL of water and 10 mL of 0.1 N sodium hydroxide to a 50-mL volumetric flask.

Instrumental conditions

Mode: Vis

Analytical wavelength: 465 nm

Cell: 1 cm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank solution*

Treat the *Samples* as follows. Add 25 mL of dimethylsulfoxide. Place the flask in an ice bath for 5 min. Add 2 mL of hydrochloric acid, and allow to stand in the ice bath for 5 min. Add 2.0 mL of sodium nitrite solution (20 mg/mL), and allow to stand in the ice bath for 5 min. Remove the flask from the ice bath, add 1.0 mL of sulfamic acid solution (80 mg/mL), and allow to stand in the ice bath for 5 min. [**Caution**—Considerable pressure is

produced.] Add 2.0 mL of *Solution A*. Remove the flasks from the ice bath and allow to stand in a water bath at room temperature. Shake occasionally, releasing the pressure by loosening the stopper. Dilute with water to volume. Within 5 min, measure the absorbances of the *Standard solution* and *Sample solution* against the blank.

Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (NMT 0.05% of free aromatic amine). ■2S (USP39)

SPECIFIC TESTS

- **Water Determination** { 921 }, *Method I*: NMT 10.0%

Change to read:

- **Iodine and Iodide**

■ **Limit of Iodine and Iodide**

Standard solution: Dissolve 0.5 mg of potassium iodide in 24 mL of water in a 50-mL stoppered centrifuge tube. ■2S (USP39)

Sample solution: Transfer 2.0 g to a 50-mL stoppered centrifuge tube, dilute with water to 24 mL, and shake to dissolve.

Analysis

- **Samples:** *Standard solution* and *Sample solution*

Treat the *Samples* as follows. Add 5 mL of toluene and 5 mL of 2 N sulfuric acid, shake, and centrifuge. The toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), shake, and centrifuge.

Acceptance criteria

1. The *Sample solution* does not show any red color in the toluene layer prior to addition of the sodium nitrite solution.
2. The red color of the toluene layer in the *Sample solution* after the addition of sodium nitrite solution is not darker than the *Standard solution* (NMT 0.02% of iodide).

■2S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at room temperature.
- **USP Reference Standards** { 11 }

USP Diatrizoic Acid RS

USP Diatrizoic Acid Related Compound A RS

5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.

C₉H₇I₃N₂O₃ 571.88

BRIEFING

Diclofenac Potassium, *USP 38* page 3085. In preparation for the omission of the flame tests from general chapter *Identification Tests—General* { 191 }, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to replace the use of the flame test under *Identification* test *C* with the potassium counter ion test in the *European Pharmacopoeia* monograph for *Diclofenac Potassium*.

Additionally, minor editorial changes have been made to update the monograph to current

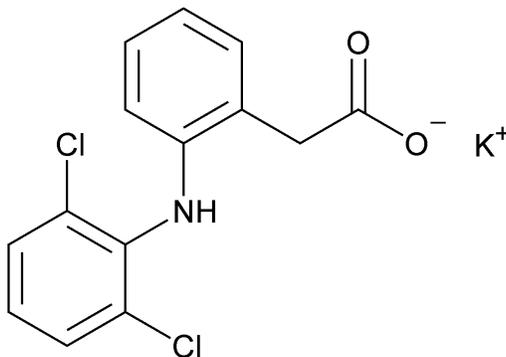
USP style.

(SM2: H. Cai.)

Correspondence Number—C157662

Comment deadline: September 30, 2015

Diclofenac Potassium



$C_{14}H_{10}Cl_2KNO_2$ 334.24

Benzeneacetic acid, 2-[(2,6-dichlorophenyl)amino]-, monopotassium salt;
Potassium [*o*-(2,6-dichloroanilino)phenyl]acetate [15307-81-0].

DEFINITION

Diclofenac Potassium contains NLT 99.0% and NMT 101.0% of diclofenac potassium ($C_{14}H_{10}Cl_2KNO_2$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈197K〉
- **B. Ultraviolet Absorption** 〈197U〉
Solution: 0.01 mg/mL in *Medium*
Medium: Methanol
Acceptance criteria: Meets the requirements

Delete the following:

- ~~C. Identification Tests—General~~ (191), *Potassium*: It meets the requirements of the flame test. ■2S (USP39)

Add the following:

- **C.**
Sample solution: Suspend 0.5 g of Diclofenac Potassium in 10 mL of water. Stir and add water until the substance is dissolved. Add 2 mL of 7 N hydrochloric acid, stir for 1 h, and filter with the aid of a vacuum. Neutralize with 5 N sodium hydroxide.
Analysis: To 1 mL of *Sample solution*, add 1 mL of 2 N acetic acid and 1 mL of a freshly prepared 100-g/L solution of sodium cobaltinitrite.
Acceptance criteria: A yellow or orange yellow precipitate is formed immediately.
 ■2S (USP39)

ASSAY● **Procedure**

Sample solution: Dissolve about 300 mg of Diclofenac Potassium in 50 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* 〈 541 〉.)

Titrant: 0.1 N perchloric acid VS

Analysis: Titrate with *Titrant*, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 33.424 mg of diclofenac potassium ($C_{14}H_{10}Cl_2KNO_2$).

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES**Delete the following:**

●● **Heavy Metals, Method II** 〈 231 〉: NMT 10 ppm ●(Official 1-Dec-2015)

● **Organic Impurities**

Solution A: 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate (1:1). If necessary, adjust with additional portions of the appropriate components to a pH of 2.5 ± 0.2 .

Mobile phase: Methanol and *Solution A* (70:30)

Diluent: Methanol and water (70:30)

System suitability solution: 40 µg/mL of diethyl phthalate, 0.5 mg/mL of USP Diclofenac Potassium RS, and 22.5 µg/mL of USP Diclofenac Related Compound A RS in *Diluent*

Standard stock solution: 0.25 mg/mL of USP Diclofenac Related Compound A RS in methanol

Standard solution: 1.5 µg/mL of USP Diclofenac Related Compound A RS in *Diluent* from *Standard stock solution*

Sample solution: 0.5 mg/mL of Diclofenac Potassium in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 30 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for diethyl phthalate, diclofenac related compound A, and diclofenac are about 0.5, 0.7, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between diethyl phthalate and diclofenac related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis**Samples:** *Standard solution and Sample solution*

Calculate the percentage of diclofenac related compound A in the portion of Diclofenac Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of diclofenac related compound A from the *Sample solution*

r_S peak response of diclofenac related compound A from the *Standard solution*

C_S concentration of USP Diclofenac Related Compound A RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U concentration of Diclofenac Potassium in the *Sample solution* ($\mu\text{g/mL}$)

Calculate the percentage of each other impurity in the portion of Diclofenac Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each individual impurity from the *Sample solution*

r_S peak response of diclofenac related compound A from the *Standard solution*

C_S concentration of USP Diclofenac Related Compound A RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U concentration of Diclofenac Potassium in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria**Diclofenac related compound A:** NMT 0.1%**Each other individual impurity:** NMT 0.1%**Total impurities:** NMT 0.3%**SPECIFIC TESTS**

- **pH** 〈 791 〉

Sample solution: 1% Aqueous solution**Acceptance criteria:** 7.0–8.5

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° under vacuum for 3 h.**Acceptance criteria:** NMT 0.5%**ADDITIONAL REQUIREMENTS**

- **Packaging and Storage:** Preserve in light-resistant containers, and store at controlled room temperature.

- **USP Reference Standards** 〈 11 〉

USP Diclofenac Potassium RS USP Diclofenac Related Compound A RS *N*-(2,6-Dichlorophenyl)indolin-2-one.C₁₄H₉Cl₂NO 278.14**BRIEFING**

Diclofenac Sodium, *USP 38* page 3087. In preparation for the omission of the flame tests from general chapter *Identification Tests—General* 〈191〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to replace the use of the flame test under *Identification* test C with the sodium counter ion test in the *European Pharmacopoeia* monograph for *Diclofenac Sodium*.

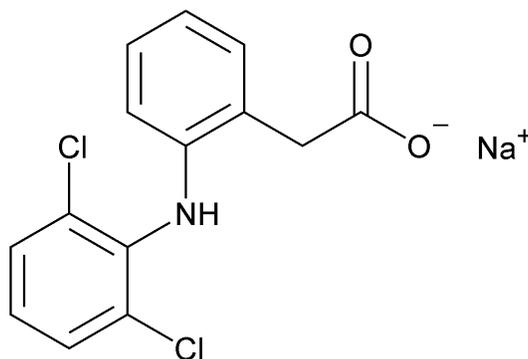
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: H. Cai.)

Correspondence Number—C157661

Comment deadline: September 30, 2015

Diclofenac Sodium



C₁₄H₁₀Cl₂NNaO₂ 318.13

Benzeneacetic acid, 2-[(2,6-dichlorophenyl)amino]-, monosodium salt;
Sodium [*o*-(2,6-dichloroanilino)phenyl]acetate [15307-79-6].

DEFINITION

Diclofenac Sodium contains NLT 99.0% and NMT 101.0% of diclofenac sodium (C₁₄H₁₀Cl₂NNaO₂), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** 〈197K〉
- **B.** The retention time of the diclofenac peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Organic Impurities*.

Delete the following:

- ~~**C. Identification Tests—General** 〈191〉, *Sodium*: The residue obtained by igniting it meets the requirements of the flame test. ■2S (USP39)~~

Add the following:

- **C.**
Sample solution: Dissolve 60 mg of Diclofenac Sodium in 0.5 mL of methanol and add 0.5 mL of water.
Analysis: To 0.5 mL of the *Sample solution* add 1.5 mL of methoxyphenylacetic TS and

cool in ice water for 30 min. A voluminous, white, crystalline precipitate is formed. Place the mixture of precipitate in a water bath at 20° and stir for 5 min. The precipitate does not disappear. Add 1 mL of ammonia TS. The precipitate dissolves completely. Add 1 mL of 158 g/L of ammonium carbonate in water.

Acceptance criteria: No precipitate is formed. ■2S (USP39)

ASSAY

● Procedure

Sample solution: Dissolve about 450 mg of Diclofenac Sodium in 25 mL of glacial acetic acid.

Titrimetric system

Titrant: 0.1 N perchloric acid VS

Analysis: Titrate with *Titrant*, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 31.81 mg of diclofenac sodium (C₁₄H₁₀Cl₂NNaO₂).

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

Delete the following:

●● Heavy Metals, Method II { 231 }

Analysis: To prepare the *Sample solution*, use a 100-mL borosilicate glass beaker or a quartz crucible. If the residue is not completely white after the ignition at 500°–600°, add enough hydrogen peroxide to dissolve it, heat gently until dry, and ignite for 1 h. Repeat the hydrogen peroxide treatment and ignition until the residue is completely white. Proceed as directed for the *Test preparation* in the chapter, beginning with "Cool, add 4 mL of 6 N hydrochloric acid..."

Acceptance criteria: NMT 10 ppm (Official 1-Dec-2015)

● Organic Impurities

Solution A: 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate (1:1). If necessary, adjust with additional portions of the appropriate components to a pH of 2.5 ± 0.2.

Mobile phase: Methanol and *Solution A* (70:30)

Diluent: Methanol and water (70:30)

System suitability solution: 20 µg/mL of diethyl phthalate, 7.5 µg/mL of USP Diclofenac Related Compound A RS, and 0.75 mg/mL of USP Diclofenac Sodium RS in *Diluent*

Standard stock solution: 0.75 mg/mL of USP Diclofenac Related Compound A RS in methanol

Standard solution: 1.5 µg/mL of USP Diclofenac Related Compound A RS in *Diluent* from *Standard stock solution*

Sample solution: 0.75 mg/mL of Diclofenac Sodium in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L7 (end-capped)

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 2.5 times the retention time of diclofenac

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for diethyl phthalate, diclofenac related compound A, and diclofenac are 0.5, 0.6, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.2 between diethyl phthalate and diclofenac related compound A; NLT 6.5 between diclofenac related compound A and diclofenac, *System suitability solution*

Relative standard deviation: NMT 5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of diclofenac related compound A in the portion of Diclofenac Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diclofenac related compound A from the *Sample solution*

r_S = peak response of diclofenac related compound A from the *Standard solution*

C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Diclofenac Sodium in the *Sample solution* (mg/mL)

Calculate the percentage of each other impurity in the portion of Diclofenac Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of diclofenac related compound A from the *Standard solution*

C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Diclofenac Sodium in the *Sample solution* (mg/mL)

Acceptance criteria

Diclofenac related compound A: NMT 0.2%

Each other individual impurity: NMT 0.2%

Total impurities: NMT 0.5%

SPECIFIC TESTS

- **Color of Solution:** A solution (1 of 20) of Diclofenac Sodium in methanol is colorless to faintly yellow, and the absorbance of the solution, determined in a 1-cm cell at 440 nm, is NMT 0.050, methanol being used as the blank.
- **Clarity of Solution:** The solution prepared as directed under *Color of Solution* is not

significantly less clear than an equal volume of methanol contained in a similar vessel and examined similarly.

- **pH** 〈 791 〉

Sample solution: A solution (1 of 100)

Acceptance criteria: 7.0–8.5

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105°–110° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

- **USP Reference Standards** 〈 11 〉

USP Diclofenac Sodium RS

USP Diclofenac Related Compound A RS

N-(2,6-Dichlorophenyl)indolin-2-one.

C₁₄H₉Cl₂NO 278.14

BRIEFING

Doxapram Hydrochloride, *USP 38* page 3207. As part of USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Replace the TLC test for *Organic Impurities* with a stability-indicating HPLC procedure that is capable of identifying and separating doxapram chloroethyl analog and doxapram related compound B. Add the corresponding limits based on the current USP specification. The liquid chromatographic procedure was validated using the Waters Xselect CSH C18 brand of L1 column. The typical retention time for doxapram is about 5.5 min.
2. Replace the current titration *Assay* with a chromatographic procedure using the same parameters as in the proposed test for *Organic Impurities*.
3. Revise the acceptance criteria in the *Definition* and the *Assay* from "NLT 98.0% and NMT 100.5%" to "NLT 98.0% and NMT 102.0%," which are typical for chromatographic procedures.
4. Replace the current *Identification* test *B*, based on UV absorption, with retention time agreement as in the proposed *Assay*.
5. Add *Identification* test *C* for chloride counter ion determination.
6. Add USP Doxapram Related Compound B RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

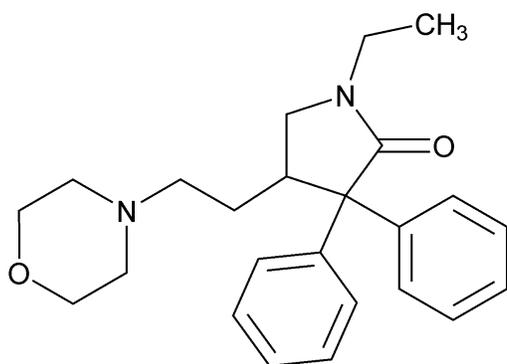
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: D. Min.)

Correspondence Number—C117378

Comment deadline: September 30, 2015

Doxapram Hydrochloride



• HCl • H₂O

C₂₄H₃₀N₂O₂·HCl·H₂O 432.99

C₂₄H₃₀N₂O₂·HCl 414.97

2-Pyrrolidinone, 1-ethyl-4-[2-(4-morpholinyl)ethyl]-3,3-diphenyl-, monohydrochloride, monohydrate, (±)-;
 (±)-1-Ethyl-4-(2-morpholinoethyl)-3,3-diphenyl-2-pyrrolidinone monohydrochloride monohydrate [7081-53-0].
 Anhydrous [113-07-5].

DEFINITION

Change to read:

Doxapram Hydrochloride, dried at 105° for 2 h, contains NLT 98.0% and NMT 100.5%

■ 102.0% ■ 2S (USP39)

of doxapram hydrochloride (C₂₄H₃₀N₂O₂·HCl).

IDENTIFICATION

• A. Infrared Absorption < 197K >

Change to read:

• B. ~~Ultraviolet Absorption < 197U >~~

~~**Analytical wavelength:** 258 nm~~

~~**Sample solution:** 400 µg/mL~~

~~**Medium:** Water~~

~~**Acceptance criteria:** Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.~~

■ The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

Add the following:

■ • C. Identification Tests—General < 191 >, Chloride ■ 2S (USP39)

ASSAY

Change to read:

Change to read.● **Procedure****Sample:** 800 mg of Doxapram Hydrochloride, previously dried**Blank:** 50 mL of glacial acetic acid**Titrimetric system****Mode:** Direct titration**Titrant:** 0.1 N perchloric acid VS**Endpoint detection:** Visual**Analysis:** Dissolve the *Sample* in 50 mL of glacial acetic acid. Add 1 drop of crystal violet TS and 10 mL of mercuric acetate TS, and titrate with *Titrant* to a blue-green endpoint. Perform the *Blank* determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 41.50 mg of doxapram hydrochloride ($C_{24}H_{30}N_2O_2 \cdot HCl$).■ **Solution A:** To each L of water, add 0.1 mL of trifluoroacetic acid.**Solution B:** To each L of acetonitrile, add 0.1 mL of trifluoroacetic acid.**Mobile phase:** See *Table 1*.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20	50	50
25	50	50
25.1	90	10
30	90	10

Diluent: Acetonitrile and water (30:70)**Standard solution:** 0.2 mg/mL of USP Doxapram Hydrochloride RS in *Diluent***Sample solution:** 0.2 mg/mL of Doxapram Hydrochloride in *Diluent***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 220 nm**Column:** 4.6-mm × 5-cm; 2.5-μm packing L1**Column temperature:** 35°**Flow rate:** 1.0 mL/min**Injection volume:** 5 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 0.73%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of doxapram hydrochloride ($C_{24}H_{30}N_2O_2 \cdot HCl$) in the portion of Doxapram Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of doxapram from the *Sample solution*

 r_S

= peak response of doxapram from the *Standard solution*

 C_S

= concentration of USP Doxapram Hydrochloride RS in the *Standard solution* (mg/mL)

 C_U

= concentration of Doxapram Hydrochloride in the *Sample solution* (mg/mL)

■ 2S (USP39)

Acceptance criteria: 98.0%–100.5%

■ 102.0%, previously ■ 2S (USP39)

dried at 105° for 2 h

IMPURITIES

- **Residue on Ignition** (281): NMT 0.3%

Delete the following:

- **Heavy Metals, Method II** (231): 20 ppm ● (Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

Standard solution A: Dissolve 57 mg of USP Doxapram Hydrochloride RS in 0.5 mL of 0.1 N-sodium hydroxide, add 1.0 mL of chloroform, and shake.

Standard solution B: Dissolve 11.4 mg of USP Doxapram Hydrochloride RS in 0.5 mL of 0.1 N-sodium hydroxide, add 100 mL of chloroform, and shake.

Sample solution: Dissolve 57 mg of Doxapram Hydrochloride in 0.5 mL of 0.1 N-sodium hydroxide, add 1.0 mL of chloroform, and shake.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Isopropyl alcohol and 1 N ammonium hydroxide (4:1)

Dragendorff stock reagent A: Dissolve 17 g of bismuth subnitrate and 200 g of tartaric acid in 800 mL of water.

Dragendorff stock reagent B: Dissolve 160 g of potassium iodide in 400 mL of water.

Dragendorff reagent: Combine *Dragendorff stock reagent A* and *Dragendorff stock reagent B*. To a 25-mL aliquot of the resulting mixture add 50 g of tartaric acid and 250

mL of water.

Analysis

Samples: *Standard solution A, Standard solution B, and Sample solution*

Proceed as directed in the chapter. Allow the spots to dry, and develop the chromatogram in a chromatographic chamber lined with paper and equilibrated with the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with *Dragendorff reagent* to visualize the spots.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that from *Standard solution A*, and no spot other than the principal spot of the *Sample solution* is larger or more intense than the principal spot from *Standard solution B* (0.2%).

■ Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system:

Proceed as directed in the *Assay*.

System suitability solution: 2 mg/mL of USP Doxapram Hydrochloride RS and 0.04 mg/mL of USP Doxapram Related Compound B RS in *Diluent*

Standard solution: 0.004 mg/mL each of USP Doxapram Hydrochloride RS and USP Doxapram Related Compound B RS in *Diluent*

Sample solution: 2 mg/mL of Doxapram Hydrochloride in *Diluent*

System suitability

Samples: *System suitability solution and Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between doxapram related compound B and doxapram, *System suitability solution*

Relative standard deviation: NMT 5.0% for doxapram related compound B and doxapram, *Standard solution*

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of doxapram related compound B in the portion of Doxapram Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of doxapram related compound B from the *Sample solution*

r_S

= peak response of doxapram related compound B from the *Standard solution*

C_S

= concentration of USP Doxapram Related Compound B RS in the *Standard solution* (mg/mL)

C_U

= concentration of Doxapram Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of doxapram chloroethyl analog or any individual unspecified impurity in the portion of Doxapram Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of doxapram chloroethyl analog or any other individual impurity from the *Sample solution*

r_S

= peak response of doxapram from the *Standard solution*

C_S

= concentration of USP Doxapram Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= concentration of Doxapram Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Doxapram related compound B	0.9	0.2
Doxapram	1.0	—
Doxapram chloroethyl analog ^a	3.9	0.2
Any individual impurity	—	0.2
Total impurities	—	1.0
^a	4-(2-Chloroethyl)-1-ethyl-3,3-diphenylpyrrolidin-2-one.	

■ 2S (USP39)

SPECIFIC TESTS

- **pH** 〈 791 〉

Sample solution: 10 mg/mL of Doxapram Hydrochloride in water

Acceptance criteria: 3.5–5.0

- **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 2 h.

Acceptance criteria: 3.0%–4.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

Change to read:

- **USP Reference Standards** (11)

USP Doxapram Hydrochloride RS

- USP Doxapram Related Compound B RS

1-Ethyl-4- {2- [(2-hydroxyethyl)amino]ethyl}-3,3-diphenylpyrrolidin-2-one hydrochloride.

C₂₂H₂₉N₂O₂ 388.93

- 2S (USP39)

BRIEFING

Doxapram Hydrochloride Injection, *USP 38* page 3208. As part of USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Add a stability-indicating HPLC procedure for the *Organic Impurities* test. The HPLC procedure was validated using the Waters Xselect CSH C18 brand of L1 column. The typical retention time for doxapram is about 5.5 min. The *Acceptance criteria* are consistent with the *Doxapram Hydrochloride* revision proposal, appearing elsewhere in this issue of *PF*. Manufacturers are encouraged to submit their approved specifications to USP if they are different from those proposed in this revision.
2. Replace the current HPLC procedure in the *Assay*, which uses an internal standard, with a chromatographic procedure that uses the same parameters as in the proposed test for *Organic Impurities*.
3. Revise the current *Identification* test *A* based on the proposed *Assay*.
4. Replace the current *Identification* test *B*, based on the UV spectrum from the sample extraction using chloroform, with the UV spectral agreement for the main peak from the proposed *Assay*.
5. Include a storage requirement in the *Packaging and Storage* section based on the approved storage conditions for drug products containing doxapram hydrochloride.
6. Add USP Doxapram Related Compound B RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: D. Min.)

Correspondence Number—C146490

Comment deadline: September 30, 2015

Doxapram Hydrochloride Injection**DEFINITION**

Doxapram Hydrochloride Injection is a sterile solution of Doxapram Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of doxapram hydrochloride monohydrate (C₂₄H₃₀N₂O₂·HCl·H₂O).

IDENTIFICATION

Change to read:

- **A.** The retention time of the major peak of doxapram from
 ■ 2S (USP39)

the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

Change to read:

- **B.**

Standard solution: Transfer 50 mg of USP Doxapram Hydrochloride RS to a separator containing 5 mL of water. Add 1 mL of a saturated solution of sodium chloride to the separator, insert the stopper, and mix. Add 5 mL of 2.5 N sodium hydroxide, and extract with three 15 mL portions of chloroform. Pass each extract through a pledget of glass wool, combine the filtrates in a 50 mL volumetric flask, and dilute with chloroform to volume. Evaporate about 5 mL of this solution to dryness. Dissolve the residue in 0.01 N sulfuric acid, and dilute with 0.01 N sulfuric acid to 100 mL.

Sample solution: Transfer a volume of Injection, equivalent to 50 mg of doxapram hydrochloride monohydrate, to a separator containing 5 mL of water. Add 1 mL of a saturated solution of sodium chloride to the separator, insert the stopper, and mix. Add 5 mL of 2.5 N sodium hydroxide, and extract with three 15 mL portions of chloroform. Pass each extract through a pledget of glass wool, combine the filtrates in a 50 mL volumetric flask, and dilute with chloroform to volume. Evaporate about 5 mL of this solution to dryness. Dissolve the residue in 0.01 N sulfuric acid, and dilute with 0.01 N sulfuric acid to 100 mL.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*.

- The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ■ 2S (USP39)

ASSAY**Change to read:**

- **Procedure**

Buffer: 2.8 g/L of monobasic potassium phosphate in water. Adjust with 50% phosphoric acid or 1 N potassium hydroxide to a pH of 3.0 ± 0.1 .

Mobile phase: Acetonitrile and *Buffer* (35:65)

Internal standard solution: 1.5 mg/mL of diphenhydramine hydrochloride in water

Standard stock solution: 2 mg/mL of USP Doxapram Hydrochloride RS in water

Standard solution: 0.2 mg/mL of doxapram hydrochloride from *Standard stock solution* prepared as follows. Transfer 5.0 mL each of *Standard stock solution* and *Internal standard solution* to a 50 mL volumetric flask, and dilute with water to volume.

Sample stock solution: Nominally 2 mg/mL of doxapram hydrochloride monohydrate from Injection in water.

Sample solution: Nominally 0.2 mg/mL of doxapram hydrochloride monohydrate from *Sample stock solution* prepared as follows. Transfer 5.0 mL each of *Sample stock solution* and *Internal standard solution* to a 50 mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ~~(621)~~, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6 mm × 15 cm; 5 μm packing L10

Column temperature: 40 °

Flow rate: 1.5 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for doxapram and diphenhydramine are about 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 3.0 between doxapram and diphenhydramine

Tailing factor: NMT 2.0 for doxapram and diphenhydramine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxapram hydrochloride monohydrate ($C_{24}H_{30}N_2O_2 \cdot HCl \cdot H_2O$) in the portion of injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of doxapram to the internal standard from the *Sample solution*

R_S = peak response ratio of doxapram to the internal standard from the *Standard solution*
 S • (ERR 1 Aug 2014)

C_S = concentration of USP Doxapram Hydrochloride RS in the *Standard solution* (mg/mL) C_U = nominal concentration of doxapram hydrochloride monohydrate in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of doxapram hydrochloride monohydrate, 432.98 M_{r2} = molecular weight of anhydrous doxapram hydrochloride, 414.98

Solution A: To each L of water, add 0.1 mL of trifluoroacetic acid.

Solution B: To each L of acetonitrile, add 0.1 mL of trifluoroacetic acid.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20	50	50
25	50	50
25.1	90	10
30	90	10

Diluent: Acetonitrile and water (30:70)

Standard solution: 0.2 mg/mL of USP Doxapram Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.2 mg/mL of doxapram hydrochloride monohydrate, equivalent to

0.19 mg/mL of doxapram hydrochloride from Injection in *Diluent*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 220 nm. For *Identification* test B, use a diode array detector in the range of 190–300 nm.

Column: 4.6-mm × 5-cm; 2.5-μm packing L1

Column temperature: 35°

Flow rate: 1.0 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxapram hydrochloride monohydrate ($C_{24}H_{30}N_2O_2 \cdot HCl \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of doxapram from the *Sample solution*

r_S = peak response of doxapram from the *Standard solution*

C_S = concentration of USP Doxapram Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of doxapram hydrochloride monohydrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of doxapram hydrochloride monohydrate, 432.99

M_{r2} = molecular weight of anhydrous doxapram hydrochloride, 414.97

■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

■ • Organic Impurities

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 2 mg/mL of USP Doxapram Hydrochloride RS and 0.04 mg/mL of USP Doxapram Related Compound B RS in *Diluent*

Standard solution: 0.004 mg/mL of USP Doxapram Hydrochloride RS in *Diluent*

Sample solution: Nominally 2 mg/mL of doxapram hydrochloride monohydrate, equivalent to 1.9 mg/mL of doxapram hydrochloride from Injection in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between doxapram related compound B and doxapram, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of doxapram related compound B, doxapram chloroethyl analog, or any individual unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each corresponding impurity from the *Sample solution*

r_S peak response of doxapram from the *Standard solution*

C_S concentration of USP Doxapram Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of doxapram hydrochloride monohydrate in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard any peak below 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Doxapram related compound B	0.9	0.2
Doxapram	1.0	—
Doxapram chloroethyl analog ^a	3.9	0.2
Any individual unspecified degradation product	—	0.2
Total impurities	—	1.0
^a 4-(2-Chloroethyl)-1-ethyl-3,3-diphenylpyrrolidin-2-one.		

■ 2S (USP39)

SPECIFIC TESTS

- **pH** 〈 791 〉: 3.5–5.0
- **Bacterial Endotoxins Test** 〈 85 〉: It contains NMT 3.3 USP Endotoxin Units/mg of doxapram hydrochloride.
- **Other Requirements:** It meets the requirements in *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

■ Store at controlled room temperature. ■ 2S (USP39)

Change to read:

- **USP Reference Standards** { 11 }

USP Doxapram Hydrochloride RS

- USP Doxapram Related Compound B RS

1-Ethyl-4- {2- [(2-hydroxyethyl)amino]ethyl}-3,3-diphenylpyrrolidin-2-one hydrochloride.

C₂₂H₂₉N₂O₂ 388.93

- 2S (USP39)

USP Endotoxin RS

BRIEFING

Doxycycline Hyclate Capsules, USP 38 page 3226. As part of USP monograph modernization efforts, it is proposed to make the following revisions:

1. Replace the TLC *Identification* procedure, *Identification—Tetracyclines* { 193 }, with a more specific FTIR procedure.
2. Add a second *Identification* test based on retention time using the HPLC procedure in the *Assay*.
3. A validated stability-indicating HPLC procedure for the *Organic Impurities* test is added. The proposed procedure uses the Acquity UPLC BEH C8 brand of L7 column manufactured by Waters, where doxycycline elutes at about 3 min. A guard-column of the same brand of stationary phase was used in the validation.
4. Replace the *Assay* procedure with one that uses a new chromatographic system, which will also be used in the *Organic Impurities* test.
5. Acceptance limits for 4-epidoxycycline and any other unspecified impurity are proposed to strengthen the monograph. Manufacturers with different limits are encouraged to submit their approved specifications to USP.
6. USP Reference Standards are added to the *USP Reference Standards* section to support the revised procedures. USP Doxycycline Monohydrate RS is introduced as the Reference Standard for *Identification* test A. USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS are introduced in the *Assay* and the *Organic Impurities* tests for system suitability evaluation and peak identification.
7. The test for *Water Determination* is deleted because the acceptance criterion for water is formulation specific.
8. The *Packaging and Storage* section is updated to include storage conditions consistent with the product label.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM1: A. Potts.)

Correspondence Number—C113525; C113526

Comment deadline: September 30, 2015

Doxycycline Hyclate Capsules**DEFINITION**

Doxycycline Hyclate Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

Change to read:

- **A.**

- **Infrared Absorption** $\langle 197A \rangle$ ■_{2S} (USP39)

~~**Test solution:** Nominally 1 mg/mL of doxycycline in methanol prepared from Capsule contents. Pass through a filter, and use the filtrate.~~

~~**Analysis:** Proceed as directed in *Identification—Tetracyclines* $\langle 193 \rangle$, *Method II*.~~

~~**Acceptance criteria:** Meet the requirements~~

- **Standard:** Transfer about 25 mg of USP Doxycycline Monohydrate RS to a suitable container. Add 25 mL of acetonitrile and mix for approximately 5 min with a magnetic stir bar. Pass through a suitable filter, evaporate the solvent, and dry under vacuum.

- **Sample:** Place about 2 g of Capsules contents in a suitable flask. Add 25 mL of acetonitrile and mix for approximately 5 min with a magnetic stir bar. Pass through a suitable filter and evaporate the acetonitrile under vacuum.

- **Analysis:** Examine the spectra of the *Standard* and the *Sample* in the range between 4000 and 650 cm^{-1} .

- **Acceptance criteria:** The *Sample* exhibits bands at about 1685, 1610, 1585, 1531, 1000, 958, 935, and 620 cm^{-1} , similar to the *Standard*. ■_{2S} (USP39)

Add the following:

- • **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■_{2S} (USP39)

ASSAY

Change to read:

- **Procedure**

~~Throughout the following sections, protect the *Standard solution* and the *Sample solution* from light.~~

~~**Mobile phase:** Transfer 2.72 g of monobasic potassium phosphate, 0.74 g of sodium hydroxide, 0.50 g of tetrabutylammonium hydrogen sulfate, and 0.40 g of edetate disodium to a 1000 mL volumetric flask. Add 850 mL of water, and stir to dissolve. Add 60 g of tertiary butyl alcohol with the aid of water, dilute with water to volume, and adjust with 1 N sodium hydroxide to a pH of 8.0 ± 0.1 . Pass this solution through a filter of 0.5 μm or finer pore size, and degas before using. Decreasing the proportion of tertiary butyl alcohol results in a longer retention time of doxycycline and improved separation of doxycycline from the related compounds.~~

~~**Diluent:** 0.01 N hydrochloric acid~~

~~**System suitability stock solution:** 6 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Diluent*~~

System suitability solution: Transfer 5 mL of *System suitability stock solution* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in 0.01 N hydrochloric acid, and dilute with *Diluent* to volume. Pass a portion of this solution through a filter of 0.5- μ m or finer pore size, and use the filtrate. This solution contains a mixture of 4-epidoxycycline, 6-epidoxycycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.

Standard solution: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.

Sample solution: Nominally 1 mg/mL of doxycycline in *Diluent*, prepared as follows: Remove as completely as possible the contents of NLT 20 Capsules. Mix the combined contents, and transfer a suitable portion of the powder to a suitable volumetric flask; add 75% of the final volume of *Diluent*, sonicate for 5 min, shake for 15 min, and dilute with *Diluent* to volume. Pass through a membrane filter of 0.5- μ m or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6 mm \times 25 cm; packing L21

Column temperature: 60 \pm 1 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: 1.7 times the retention time of doxycycline

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for 4-epidoxycycline (the main degradation product), 6-epidoxycycline, and doxycycline are about 0.4, 0.7, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the 4-epidoxycycline peak and the doxycycline peak; *System suitability solution*

Tailing factor: NMT 2.0 for doxycycline, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

P = potency of doxycycline in USP Doxycycline Hyclate RS (μ g/mg)

F = unit conversion factor, 0.001 mg/ μ g

Acceptance criteria: ~~90.0%–120.0%~~

- Protect solutions containing doxycycline from light.

Solution A: Transfer 3.1 g of *monobasic potassium phosphate*, 0.5 g of *edetate disodium*, and 0.5 mL of *triethylamine* to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with *1 N sodium hydroxide* to a pH of 8.5 ± 0.2 .

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
2.0	90	10
4.0	60	40
6.0	90	10
9.0	90	10

Diluent: 0.01 N hydrochloric acid

System suitability stock solution 1: 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS in *Diluent*

System suitability stock solution 2: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*

System suitability solution: Transfer 5 mL of *System suitability stock solution 2* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in *Diluent*, add 0.5 mL of *System suitability stock solution 1*, and dilute with *Diluent* to volume. Pass the solution through a suitable filter and use the filtrate.

This solution contains a mixture of 4-epidoxycycline, doxycycline related compound A, methacycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.

Standard solution: 0.3 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.

Sample solution: Nominally 0.25 mg/mL of doxycycline in *Diluent*, prepared as follows. Empty as completely as possible the contents of NLT 20 Capsules. Mix the combined contents and transfer a suitable portion of the powder to a suitable volumetric flask. Add 75% of the final volume of *Diluent*, sonicate for about 5 min, shake for about 15 min, and dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of 0.2- μm pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 350 nm

Column: 2.1-mm \times 5-cm; 1.7- μm packing L7. [Note—A 1.7- μm guard column with packing L7 was used during method validation.]

Column temperature: 60 $^{\circ}$

Flow rate: 0.6 mL/min

Injection volume: 5 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 1.5 between doxycycline related compound A and doxycycline, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of doxycycline in the *Sample solution* (mg/mL)

P

= potency of doxycycline in USP Doxycycline Hyclate RS (μ g/mg)

F

= conversion factor, 0.001 mg/ μ g

Acceptance criteria: 90.0%–120.0% \pm 2S (USP39)

PERFORMANCE TESTS

Change to read:

- **Dissolution** { 711 }

Medium: Water; 900 mL

Apparatus 2: 75 rpm, the distance between the blade and the inside bottom of the vessel

being maintained at 4.5 ± 0.5 cm during the test

Time: 30 min

Standard solution: USP Doxycycline Hyclate RS in *Medium*

Sample solution: Sample per *Dissolution* 〈 711 〉. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: 276 nm

■ **Analysis**

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U

= absorbance of the *Sample solution*

A_S

= absorbance of the *Standard solution*

C_S

= concentration of doxycycline in the *Standard solution* (mg/mL)

L

= label claim (mg/Capsule)

V

= volume of *Medium*, 900 mL

■ **2S (USP39)**

Tolerances: NLT 80% (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

- **Uniformity of Dosage Units** 〈 905 〉: Meet the requirements

IMPURITIES

Add the following:

■ • **Organic Impurities**

Mobile phase, Diluent, System suitability solution, Sample solution, and

Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 1.5 $\mu\text{g/mL}$ of USP Doxycycline Hyclate RS in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 1.5 between doxycycline related compound A and doxycycline, *System suitability solution*

Relative standard deviation: NMT 5.0% for the doxycycline peak, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_S peak response of doxycycline from the *Standard solution*

C_S concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

C_U nominal concentration of doxycycline in the *Sample solution* (mg/mL)

P = potency of doxycycline in USP Doxycycline Hyclate RS ($\mu\text{g}/\text{mg}$)

F = conversion factor, 0.001 mg/ μg

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.2%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methacycline ^{a,b}	0.64	—
4-Epidoxycycline ^c	0.79	0.5
Doxycycline related compound A (6-epidoxycycline) ^{a,d}	0.88	—
Doxycycline	1.0	—
Any individual unspecified impurity	—	0.5
Total impurities	—	2.0

^a Process impurities are controlled in the drug substance and are not to be reported here. They are not included in total impurities.

^b (4*S*,4*aR*,5*S*,5*aR*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenicarboxamide.

^c (4*R*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenicarboxamide.

^d (4*S*,4*aR*,5*S*,5*aR*,6*S*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenicarboxamide.

■ 2*S* (USP39)

SPECIFIC TESTS

Delete the following:

■ **Water Determination**, *Method I* (921): NMT 8.5% ■ 2*S* (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- Store at controlled room temperature. ■ 2S (USP39)

Change to read:● **USP Reference Standards** 〈 11 〉USP Doxycycline Hyclate RS

■ USP Doxycycline Monohydrate RS

USP Doxycycline Related Compound A RS

(4*S*,4*aR*,5*S*,5*aR*,6*S*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenicarboxamide.

C₂₂H₂₄N₂O₈ 444.43

USP Methacycline Hydrochloride RS

■ 2S (USP39)

BRIEFING

Doxycycline Hyclate Tablets, USP 38 page 3232. As part of USP monograph modernization efforts, it is proposed to make the following revisions:

1. Replace the TLC *Identification* procedure, *Identification—Tetracyclines* 〈 193 〉, with a more specific FTIR procedure.
2. Add a second *Identification* test based on retention time using the HPLC procedure in the *Assay*.
3. A validated stability-indicating HPLC procedure for the *Organic Impurities* test is added. The proposed procedure uses the Acquity UPLC BEH C8 brand of L7 column manufactured by Waters, where doxycycline elutes at about 3 min. A guard-column of the same brand of stationary phase was used in the validation.
4. Replace the *Assay* procedure with one that uses a new chromatographic system which will also be used in the *Organic Impurities* test.
5. Acceptance limits for 4-epidoxycycline and any other unspecified impurity are proposed to strengthen the monograph. The proposed limits are consistent with limits in the drug substance monograph. Manufacturers with different limits are encouraged to submit their approved specifications to USP.
6. *Dissolution Test 1* is revised to include a calculation and to remove notation on the use of low actinic glassware for protection from light. A statement is added to require that solutions be protected from light.
7. *Dissolution Test 2* is revised to remove the specific filter pore size in the *Sample solution* preparation. A suitable filter is to be used.
8. USP Reference Standards are added to the *USP Reference Standards* section to support the revised procedures. USP Doxycycline Monohydrate RS is introduced as the Reference Standard for *Identification* test A. USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS are introduced in the *Assay* and the *Organic Impurities* tests for system suitability evaluation and peak identification.
9. The test for *Water Determination* is deleted because the acceptance criterion for water is formulation specific.

10. The *Packaging and Storage* section is updated to include storage conditions consistent with the product label.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM1: A. Potts.)

Correspondence Number—C113525; C113526

Comment deadline: September 30, 2015

Doxycycline Hyclate Tablets

DEFINITION

Doxycycline Hyclate Tablets contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

Change to read:

- **A.**

■ **Infrared Absorption** (197A) ■_{2S} (USP39)

~~**Sample solution:** Shake a suitable quantity of finely ground Tablets with methanol to obtain a solution containing the equivalent of 1 mg/mL of doxycycline, and filter. Use the filtrate as the *Sample solution*.~~

~~**Analysis:** Proceed as directed under *Identification—Tetracyclines* (193), *Method II*.~~

~~**Acceptance criteria:** Meet the requirements~~

■ **Standard:**

Transfer about 25 mg of USP Doxycycline Monohydrate RS to a suitable flask. Add 25 mL of acetonitrile and mix for approximately 5 min with a magnetic stir bar. Pass through a suitable filter, evaporate the solvent, and dry under vacuum.

Sample: Place about 2 g of finely powdered Tablets in a suitable flask. Add 25 mL of acetonitrile and mix for approximately 5 min with a magnetic stir bar. Pass through a suitable filter and evaporate the acetonitrile under vacuum.

Analysis: Examine the spectra of the *Standard* and the *Sample* in the range between 4000 and 650 cm^{-1} .

Acceptance criteria: The *Sample* exhibits bands at about 1685, 1610, 1585, 1531, 1000, 958, 935, and 620 cm^{-1} , similar to the *Standard*. ■_{2S} (USP39)

Add the following:

- • **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■_{2S} (USP39)

ASSAY

Change to read:

- **Procedure**

Throughout the following sections, protect the *Standard solution* and the *Sample solution* from light.

Mobile phase: Transfer 2.72 g of monobasic potassium phosphate, 0.74 g of sodium hydroxide, 0.50 g of tetrabutylammonium hydrogen sulfate, and 0.40 g of edetate disodium to a 1000-mL volumetric flask. Add 850 mL of water, and stir to dissolve. Add 60 g of tertiary butyl alcohol with the aid of water, dilute with water to volume, and adjust with 1 N sodium hydroxide to a pH of 8.0 ± 0.1 . Decreasing the proportion of tertiary butyl alcohol results in a longer retention time of doxycycline and improved separation of doxycycline from the related compounds.

Diluent: 0.01 N hydrochloric acid

System suitability solution: Prepare 6 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Diluent*. Transfer 5 mL of this solution to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in 0.01 N hydrochloric acid, dilute with *Diluent* to volume, and filter. This solution contains a mixture of 4-epidoxycycline, 6-epidoxycycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.

Standard solution: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. [Note—Sonicate as necessary to dissolve.]

Sample solution: Nominally 1 mg/mL of doxycycline in *Diluent*, prepared as follows. Transfer nominally equivalent to 100 mg of doxycycline, from finely powdered Tablets (NLT 20), to a 100-mL volumetric flask, add 75 mL of *Diluent*, sonicate for 5 min, shake for 15 min, dilute with *Diluent* to volume, and mix. Pass through a suitable filter.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6 mm × 25 cm; packing L21

Column temperature: $60 \pm 1^\circ$

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: 1.7 times the retention time of doxycycline

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for 4-epidoxycycline (the main degradation product), 6-epidoxycycline, and doxycycline are about 0.4, 0.7, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the 4-epidoxycycline peak and the doxycycline peak; *System suitability solution*

Tailing factor: NMT 2.0 for doxycycline, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

~~r_U~~ = peak response from the *Sample solution*

~~r_S~~ = peak response from the *Standard solution*

~~C_S~~ = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

~~C_U~~ = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

~~P~~ = potency of doxycycline in USP Doxycycline Hyclate RS ($\mu\text{g}/\text{mg}$)

~~F~~ = unit conversion factor, 0.001 mg/ μg

Acceptance criteria: ~~90.0%–120.0%~~

- Protect solutions containing doxycycline from light.

Solution A: Transfer 3.1 g of *monobasic potassium phosphate*, 0.5 g of *edetate disodium*, and 0.5 mL of *triethylamine* to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with *1 N sodium hydroxide* to a pH of 8.5 \pm 0.2.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
2.0	90	10
4.0	60	40
6.0	90	10
9.0	90	10

Diluent: 0.01 N hydrochloric acid

System suitability stock solution 1: 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS in *Diluent*

System suitability stock solution 2: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*

System suitability solution: Transfer 5 mL of *System suitability stock solution 2* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in *Diluent*, add 0.5 mL of *System suitability stock solution 1*, and dilute with *Diluent* to volume. Pass the solution through a suitable filter and use the filtrate.

This solution contains a mixture of 4-epidoxycycline, doxycycline related compound A, methacycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.

Standard solution: 0.3 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.

Sample solution: Nominally 0.25 mg/mL of doxycycline in *Diluent*, prepared as follows. Transfer a suitable portion of NLT 20 finely powdered Tablets to a suitable volumetric flask. Add 50% of the final volume of *Diluent*, sonicate for about 5 min, shake for about 15 min, and dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of 0.2- μm pore size.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 350 nm

Column: 2.1-mm × 5-cm; 1.7-μm packing L7. [Note—A 1.7-μm guard column with packing L7 was used during method validation.]

Column temperature: 60°

Flow rate: 0.6 mL/min

Injection volume: 5 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 1.5 between doxycycline related compound A and doxycycline, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline (C₂₂H₂₄N₂O₈) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of doxycycline in the *Sample solution* (mg/mL)

P

= potency of doxycycline in USP Doxycycline Hyclate RS (μg/mg)

F

= conversion factor, 0.001 mg/μg

Acceptance criteria: 90.0%–120.0% ■_{2S} (USP39)

PERFORMANCE TESTS**Change to read:**

- **Dissolution** 〈 711 〉 [~~Note—Use low-actinic glassware to prepare the solutions.~~]

- Protect solutions containing doxycycline from light.

- 2S (USP39)

Test 1

Medium: Water; 900 mL

Apparatus 2: 75 rpm, the distance between the blade and the inside bottom of the vessel being maintained at 4.5 ± 0.5 cm during the test

Time: 90 min

Standard solution: USP Doxycycline Hyclate RS in *Medium*

Sample solution: Dilute with *Medium*, if necessary, to a concentration that is similar to the *Standard solution*.

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* 〈 851 〉.)

Mode: UV-Vis

Analytical wavelength: 276 nm

Analysis

Samples: *Standard solution* and *Sample solution*

- Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U

= absorbance of the *Sample solution*

A_S

= absorbance of the *Standard solution*

C_S

= concentration of doxycycline in the *Standard solution* (mg/mL)

L

= label claim (mg/Tablet)

V

= volume of *Medium*, 900 mL

- 2S (USP39)

Tolerances: NLT 85% (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Water; 900 mL

Apparatus 2: 50 rpm, the distance between the blade and the inside bottom of the vessel

being maintained at 4.5 ± 0.5 cm during the test

Time: 30 min

Standard solution: 22 µg/mL of doxycycline from USP Doxycycline Hyclate RS, in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. ~~of 0.45-µm pore size~~

■ **2S (USP39)**

Blank: *Medium*

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* { 851 } .)

Mode: UV-Vis

Analytical wavelength: 276 nm

Cell: 0.5 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U absorbance of the *Sample solution*

A_S absorbance of the *Standard solution*

C_S concentration of doxycycline in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (Q) of the labeled amount of doxycycline is dissolved.

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

Add the following:

■ • Organic Impurities

Mobile phase, Diluent, System suitability solution, Sample solution, and

Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 1.5 µg/mL of USP Doxycycline Hyclate RS in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 1.5 between doxycycline related compound A and doxycycline, *System suitability solution*

Relative standard deviation: NMT 5.0% for the doxycycline peak, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of doxycycline from the *Standard solution*

C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

P = potency of doxycycline in USP Doxycycline Hyclate RS ($\mu\text{g}/\text{mg}$)

F = conversion factor, 0.001 mg/ μg

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.2%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methacycline ^{a,b}	0.64	—
4-Epidoxycycline ^c	0.79	0.5
Doxycycline related compound A (6-epidoxycycline) ^{a,d}	0.88	—
Doxycycline	1.0	—
Any individual unspecified impurity	—	0.5
Total impurities	—	2.0

^a Process impurities are controlled in the drug substance and are not to be reported here. They are not included in total impurities.

^b (4*S*,4*aR*,5*S*,5*aR*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenicarboxamide.

^c (4*R*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenicarboxamide.

^d (4*S*,4*aR*,5*S*,5*aR*,6*S*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenicarboxamide.

■ 2*S* (USP39)

SPECIFIC TESTS

Delete the following:

● **Water Determination**, *Method I (921)*: NMT 5.0% ■ 2*S* (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

● **Packaging and Storage:** Preserve in tight, light-resistant containers.

■ Store at controlled room temperature. ■ 2*S* (USP39)

- **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

- **USP Reference Standards** { 11 }

USP Doxycycline Hyclate RS

- USP Doxycycline Monohydrate RS

USP Doxycycline Related Compound A RS

(4*S*,4*aR*,5*S*,5*aR*,6*S*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenicarboxamide.
C₂₂H₂₄N₂O₈ 444.43

USP Methacycline Hydrochloride RS

- 2*S* (USP39)

BRIEFING

Multiple Electrolytes Injection Type 1, *USP 38* page 3273. It is proposed to make the following revisions to the monograph:

1. In preparation for the omission of the flame tests from *Identification Tests—General* { 191 }, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to delete the reference to *Sodium* and *Potassium* in *Identification* test *A* and include a complete description of each flame test in the monograph. Manufacturers are encouraged to submit a replacement wet chemistry or an instrumental procedure for consideration by the Expert Committee.
2. An equation is added to calculate the amount of chloride in the *Assay for Chloride*.
3. Concentration units for the *Standard solution* and *Sample solution* are revised to mEq/mL in the *Assay for Phosphate* to eliminate the need for the molecular weight correction term in the equation for the calculation of the amount of phosphate.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: M. Koleck.)

Correspondence Number—C157936

Comment deadline: September 30, 2015

Multiple Electrolytes Injection Type 1

DEFINITION

Multiple Electrolytes Injection Type 1 is a sterile solution of suitable salts in Water for Injection to provide sodium, potassium, magnesium, and chloride ions. In addition, the salts provide ions of acetate, acetate and gluconate, or acetate, gluconate, and phosphate. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), chloride (Cl), acetate (C₂H₃O₂), gluconate (C₆H₁₁O₇), and phosphate (PO₄). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

IDENTIFICATION**Change to read:**

- **A. Identification Tests—General** < 191 >, ~~**Sodium**~~ < 191 >, ~~**Potassium**~~ < 191 >

- **2S** (USP39)

Magnesium and **Chloride**: Meets the requirements of the flame tests for ~~*Sodium*~~ and ~~*Potassium*~~, and for the tests for ~~*Magnesium*~~ and ~~*Chloride*~~

- **2S** (USP39)

Add the following:

- **B. Sodium:** The sample imparts an intense yellow color to a nonluminous flame. ■ **2S** (USP39)

Add the following:

- **C. Potassium:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

- **2S** (USP39)

Change to read:

- **B**

- **D.** ■ **2S** (USP39)

The retention time of the acetate peak of the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the *Assay for Acetate*.

Change to read:

- **€**

- **E.** ■ **2S** (USP39)

Where gluconate is purported to be present, the retention time of the gluconate peak of the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the *Assay for Gluconate*.

Change to read:

- **Đ**

- **F.** ■ **2S** (USP39)

Where phosphate is purported to be present, proceed as follows.

Sample solution: Add 5 mL of Injection and 1 mL of ammonium molybdate TS to a test tube and mix.

Acceptance criteria: A yellow precipitate, which is soluble in 6 N ammonium hydroxide, is formed.

ASSAY

- **Acetate**

- Mobile phase:** 0.05 N sulfuric acid

- Standard solution:** 1.2 mg/mL of sodium acetate trihydrate (0.0088 mEq/mL of acetate) in water

- Sample solution:** Nominally 0.0088 mEq/mL of acetate from a volume of Injection in water

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Columns

Guard: 4.6-mm × 3-cm; packing L17

Analytical: 7.8-mm × 30-cm; packing L17

Column temperature: 60°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetate (C₂H₃O₂) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of acetate from the *Sample solution*

r_S peak response of acetate from the *Standard solution*

C_S concentration of acetate in the *Standard solution* (mEq/mL)

C_U nominal concentration of acetate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

- **Chloride**

Sample solution: Transfer a volume of Injection equivalent to 55 mg of chloride (1.55 mEq) to a suitable conical flask, and add water, if necessary, to bring the volume to 10 mL. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

Each mL of *Titrant* is equivalent to 3.545 mg (0.1 mEq) of chloride (Cl).

■ Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the *Sample solution* (mL) N = actual normality of the *Titrant* (mEq/mL) F

= equivalency factor, 35.45 mg/mEq

 W = nominal amount of chloride in the *Sample solution* (mg)

■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

- **Gluconate** (if present)

Mobile phase: 0.05 N sulfuric acid**Standard solution:** 1 mg/mL of USP Potassium Gluconate RS (0.0043 mEq/mL of gluconate) in water**Sample solution:** Nominally 0.004 mEq/mL of gluconate from a volume of Injection in water**Chromatographic system**(See *Chromatography* { 621 }, *System Suitability*.)**Mode:** LC**Detector:** UV 210 nm**Columns****Guard:** 4.6-mm × 3-cm; packing L17**Analytical:** 7.8-mm × 30-cm; packing L17**Flow rate:** 0.8 mL/min**Injection volume:** 20 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of gluconate ($C_6H_{11}O_7$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U peak response of gluconate from the *Sample solution* r_S peak response of gluconate from the *Standard solution* C_S concentration of USP Potassium Gluconate RS in the *Standard solution* (mEq/mL) C_U nominal concentration of gluconate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

• **Magnesium**

[Note—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Solution B: Mix 678 mL of hydrochloric acid with water to make 3000 mL.

Standard stock solution A: 1.00 mg/mL of magnesium (Mg) prepared as follows. Transfer 1.00 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water. Slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with *Solution B* to volume.

Standard stock solution B: 100 µg/mL of magnesium (Mg) prepared as follows. Transfer 10.0 mL of *Standard stock solution A* to a 100-mL volumetric flask, and dilute with *Solution B* to volume.

Standard solutions: 10.0, 15.0, and 20.0 µg/mL of magnesium (Mg) prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution B*. Dilute the contents of each flask with *Solution B* to volume.

Sample solution: Nominally 20.0 µg/mL of magnesium from Injection prepared as follows. Transfer a volume of Injection, equivalent to 20 mg (~~1.65 mEq~~)

■ ■ 2S (USP39)

of magnesium (Mg) to a 1000-mL volumetric flask containing 50.0 mL of *Solution A*. Dilute with *Solution B* to volume.

Blank: 5.0 mL of *Solution A* diluted with *Solution B* to 100.0 mL

Instrumental conditions

(See *Spectrophotometry and Light Scattering* { 851 } .)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Magnesium emission line at 285.2 nm

Lamp: Magnesium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of magnesium (Mg), and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (*C*) in µg/mL, of magnesium (Mg) in the *Sample solution*.

Calculate the percentage of the labeled amount of magnesium (Mg) in the portion of Injection taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of magnesium (Mg) in the *Sample solution* (µg/mL)

C_U = nominal concentration of magnesium (Mg) in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

● **Phosphate** (if present)

Solution A: 50 g/L of ammonium molybdate prepared as follows. Transfer a suitable amount of ammonium molybdate to an appropriate volumetric flask. Add 60% of the final flask volume of water, and swirl to dissolve. Add 15% of the final flask volume of sulfuric acid, and swirl. Allow to cool, and dilute with water to volume.

Solution B: Dissolve 0.5 g of hydroquinone in 100 mL of water, and add 1 drop of sulfuric acid. Prepare this solution fresh daily.

Solution C: Dissolve 1 g of sodium sulfite in water to make 5 mL. Prepare this solution fresh daily.

Standard solution: 0.11 mg/mL of monobasic potassium phosphate

■ (0.0008 mEq/mL of phosphate) ■ 2S (USP39)

in water

Sample solution: ~~Transfer a volume of Injection equivalent to 4 mg (0.126 mEq) of phosphate to a 50 mL volumetric flask, and dilute with water to volume.~~

■ Nominally 0.0008 mEq/mL of phosphate from a volume of Injection in water ■ 2S (USP39)

Blank: Water

Instrumental conditions

Mode: Vis

Analytical wavelength: 640 nm

Analysis

Samples: *Standard solution, Sample solution, and Blank*

Use water to zero the instrument. Transfer 2.0 mL each of the *Standard solution, Sample solution, and the Blank* to separate test tubes. To each test tube add 1.0 mL of *Solution A*, mix, and allow to stand for 3 min. Add 1.0 mL of *Solution B*, and mix. Add 1.0 mL of *Solution C*, mix, and allow to stand for 30 min.

Calculate the percentage of the labeled amount of phosphate (PO_4) in the portion of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

■
$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100 \text{ ■ 2S (USP39)}$$

A_U = absorbance of the *Sample solution*, corrected for any absorbance of the solution from the *Blank*

A_S = absorbance of the *Standard solution*, corrected for any absorbance of the solution from the *Blank*

C_S = concentration of phosphate in the *Standard solution* (mg

■ mEq/ ■ 2S (USP39)

■ mL)

- C_U = nominal concentration of phosphate (PO_4) in the *Sample solution* (~~mg~~
 ■ mEq/ ■ 2S (USP39)
 mL)
- ~~$M_{r\pm}$ = molecular weight of phosphate, 94.97~~
- ~~M_{r2} = molecular weight of monobasic potassium phosphate, 136.09~~
 ■
 ■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

● **Potassium and Sodium**

Internal standard solution: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Add a suitable nonionic surfactant, and then dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride (1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Standard stock solution: 0.0391J mg/mL of potassium (K) from *Potassium stock solution* and 0.02299J' mg/mL of sodium (Na) from *Sodium stock solution* prepared as follows. Transfer 0.1J mL of *Potassium stock solution* and 0.1J' mL of *Sodium stock solution* to a 100-mL volumetric flask, where J and J' are the labeled amounts, in mEq/L, of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Dilute 5.0 mL of the *Standard stock solution* with *Internal standard solution* to 500.0 mL.

Sample solution: Dilute 5.0 mL of Injection with *Internal standard solution* to 500.0 mL.

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: Maximum at 766 nm

Sodium: Maximum at 589 nm

Lithium: Maximum at 671 nm

Blank: *Internal standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{U}}$ emission reading ratio of potassium to lithium from the *Sample solution*

$R_{\bar{S}}$ emission reading ratio of potassium to lithium from the *Standard solution*

$C_{\bar{S}}$ concentration of potassium (K) in the *Standard solution* (mg/mL)

$C_{\bar{U}}$ nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[Note—Each mg of potassium (K) is equivalent to 0.02558 mEq of potassium (K).]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U emission reading ratio of sodium to lithium from the *Sample solution*

R_S emission reading ratio of sodium to lithium from the *Standard solution*

C_S concentration of sodium (Na) in the *Standard solution* (mg/mL)

C_U nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[Note—Each mg of sodium (Na) is equivalent to 0.04350 mEq of sodium (Na).]

Acceptance criteria

Potassium: 90.0%–110.0%

Sodium: 90.0%–110.0%

SPECIFIC TESTS

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 0.5 USP Endotoxin Units/mL
- **pH** 〈 791 〉: 4.0–8.0
- **Other Requirements:** It meets the requirements in *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers of Type I or Type II glass are preferable.
- **Labeling:** The label states the content of each electrolyte in terms of milliequivalents (mEq) in a given volume. The label states the total osmolar concentration in mOsmol/L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.
- **USP Reference Standards** 〈 11 〉
 - USP Endotoxin RS
 - USP Potassium Gluconate RS

BRIEFING

Multiple Electrolytes Injection Type 2, *USP 38* page 3274. It is proposed to make the following revisions to the monograph:

1. In preparation for the omission of the flame tests from *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to delete the reference to *Sodium* and *Potassium* in *Identification test A* and include a complete description of each flame test in the monograph. Manufacturers are encouraged to submit a replacement wet chemistry or an instrumental procedure for consideration by the Expert Committee.
2. An equation is added to calculate the amount of chloride in the *Assay for Chloride*.

Additionally, minor editorial changes have been made to update the monograph to current

USP style.

(SM4: M. Koleck.)

Correspondence Number—C157937

Comment deadline: September 30, 2015

Multiple Electrolytes Injection Type 2

DEFINITION

Multiple Electrolytes Injection Type 2 is a sterile solution of suitable salts in Water for Injection to provide sodium, potassium, calcium, magnesium, and chloride ions. In addition, the salts provide ions of either acetate and citrate, or acetate and lactate. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), chloride (Cl), acetate (C₂H₃O₂), citrate (C₆H₅O₇), and lactate (C₃H₅O₃). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

IDENTIFICATION

Change to read:

- **A. Identification Tests—General** { 191 }, ~~Sodium~~, ~~Potassium~~, ~~Calcium~~

■ 2S (USP39)

Magnesium and **Chloride**: Meets the requirements of the flame tests for *Sodium* and *Potassium*, of the oxalate test for *Calcium*, and of the tests for *Magnesium* and *Chloride*

■ 2S (USP39)

Add the following:

- **B. Sodium**: The sample imparts an intense yellow color to a nonluminous flame. ■ 2S (USP39)

Add the following:

- **C. Potassium**: The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

■ 2S (USP39)

Add the following:

- **D. Identification Tests—General** { 191 }, **Calcium**: Meets the requirements of the oxalate test ■ 2S (USP39)

Change to read:

- **B**

■ **E.** ■ 2S (USP39)

The retention time of the acetate peak of the *Sample solution* corresponds to that of the

Standard solution, obtained as directed in the *Assay for Acetate*.

Change to read:

- €

■F. ■2S (USP39)

Where citrate is purported to be present, the retention time of the citrate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Citrate*.

Change to read:

- Đ

■G. ■2S (USP39)

Where lactate is purported to be present, the retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Lactate*.

ASSAY

- Acetate

Mobile phase: 0.05 N sulfuric acid

Standard solution: 1.2 mg/mL of sodium acetate trihydrate (0.0088 mEq/mL of acetate) in water

Sample solution: Nominally 0.0088 mEq/mL of acetate from a volume of Injection in water

Chromatographic system

(See *Chromatography* 〈621〉, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Columns

Guard: 4.6-mm × 3-cm; packing L17

Analytical: 7.8-mm × 30-cm; packing L17

Column temperature: 60°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetate (C₂H₃O₂) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetate from the *Sample solution*

r_S = peak response of acetate from the *Standard solution*

C_S = concentration of acetate in the *Standard solution* (mEq/mL)

C_U = nominal concentration of acetate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

• **Calcium**

[Note—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Solution B: Mix 678 mL of hydrochloric acid with water to make 3000 mL.

Standard stock solution: 1000 µg/mL of calcium prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of *Solution B*, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 10.0, 15.0, and 20.0 µg/mL of calcium prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 1.0, 1.5, and 2.0 mL, respectively, of *Standard stock solution*. Dilute the contents of each flask with *Solution B* to volume.

Sample solution: Nominally 20.0 µg/mL of calcium from Injection prepared as follows. Transfer a volume of Injection, equivalent to 20 mg (~~1 mEq~~)

■ ■ 2S (USP39)

of calcium, to a 1000-mL volumetric flask containing 50.0 mL of *Solution A*. Dilute with *Solution B* to volume.

Blank: 5.0 mL of *Solution A* diluted with *Solution B* to 100.0 mL

Instrumental conditions

(See *Spectrophotometry and Light Scattering* (851).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of calcium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (*C*), in µg/mL, of calcium in the *Sample solution*.

Calculate the percentage of the labeled amount of calcium (Ca) in the portion of Injection taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of calcium in the *Sample solution* (µg/mL), interpolated from the graph obtained

C_U = nominal concentration of calcium in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

- **Chloride**

Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride (1.55 mEq), to a suitable conical flask, and add water, if necessary, to bring the volume to 10 mL. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

Each mL of *Titrant* is equivalent to 3.545 mg (0.1 mEq) of chloride (Cl).

- Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V

= *Titrant* volume consumed by the *Sample solution* (mL)

N

= actual normality of the *Titrant* (mEq/mL)

F

= equivalency factor, 35.45 mg/mEq

W

= nominal amount of chloride in the *Sample solution* (mg)

■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

- **Citrate** (if present)

Mobile phase and Chromatographic system: Proceed as directed in the *Assay for Citric Acid/Citrate and Phosphate* 〈 345〉.

Standard solution: 0.3 mEq/L of citrate (C₆H₅O₇) from USP Citric Acid RS in freshly prepared 1 mM sodium hydroxide

Sample solution: Nominally 0.3 mEq/L of citrate in 1 mM sodium hydroxide from a volume of Injection diluted with freshly prepared sodium hydroxide

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in 〈 345〉.

Calculate the percentage of the labeled amount of citrate (C₆H₅O₇) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of citrate from the *Sample solution*

r_S peak response of citrate from the *Standard solution*

C_S concentration of citrate in the *Standard solution* (mEq/L)

C_U nominal concentration of citrate in the *Sample solution* (mEq/L)

Acceptance criteria: 90.0%–110.0%

Change to read:

• **Lactate** (if present)

Mobile phase: Formic acid, dicyclohexylamine, and water (1:1:998)

System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water

Standard solution: 2 mg/mL of USP Sodium Lactate RS

■(0.018 mEq/mL of lactate)■_{2S} (USP39)

in water

Sample solution

For Injections containing >20 mEq/L of lactate: Nominally 0.02 mEq/mL of lactate from Injection in water

For Injections containing ≤20 mEq/L of lactate: Use the undiluted Injection.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between acetate and lactate, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lactate ($C_3H_5O_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of lactate from the *Sample solution*

r_S peak response of lactate from the *Standard solution*

C_S concentration of lactate in the *Standard solution* (mEq/mL)

C_U nominal concentration of lactate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:● **Magnesium**

[Note—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Solution B: Mix 678 mL of hydrochloric acid with water to make 3000 mL.

Standard stock solution A: 1.00 mg/mL of magnesium (Mg) prepared as follows. Transfer 1.00 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water. Slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with *Solution B* to volume.

Standard stock solution B: 100 µg/mL of magnesium (Mg) prepared as follows. Transfer 10.0 mL of *Standard stock solution A* to a 100-mL volumetric flask, and dilute with *Solution B* to volume.

Standard solutions: 10.0, 15.0, and 20.0 µg/mL of magnesium (Mg) prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution B*. Dilute the contents of each flask with *Solution B* to volume.

Sample solution: Nominally 20.0 µg/mL of magnesium from Injection prepared as follows. Transfer a volume of Injection, equivalent to 20 mg (~~1.65 mEq~~)

■ ■ 2S (USP39)

of magnesium (Mg) to a 1000-mL volumetric flask containing 50.0 mL of *Solution A*. Dilute with *Solution B* to volume.

Blank: 5.0 mL of *Solution A* diluted with *Solution B* to 100.0 mL

Instrumental conditions

(See *Spectrophotometry and Light Scattering* (851).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Magnesium emission line at 285.2 nm

Lamp: Magnesium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of magnesium (Mg), and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (*C*) in µg/mL, of magnesium (Mg) in the *Sample solution*.

Calculate the percentage of the labeled amount of magnesium (Mg) in the portion of Injection taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of magnesium (Mg) in the *Sample solution* (µg/mL)

C_U = nominal concentration of magnesium (Mg) in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

- **Potassium and Sodium**

Internal standard solution: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Add a suitable nonionic surfactant, and then dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride (1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Standard stock solution: 0.0391J mg/mL of potassium (K) from *Potassium stock solution* and 0.02299J' mg/mL of sodium (Na) from *Sodium stock solution* prepared as follows. Transfer 0.1J mL of *Potassium stock solution* and 0.1J' mL of *Sodium stock solution* to a 100-mL volumetric flask, where J and J' are the labeled amounts, in mEq/L, of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Dilute 5.0 mL of *Standard stock solution* with *Internal standard solution* to 500.0 mL.

Sample solution: Dilute 5.0 mL of Injection with *Internal standard solution* to 500.0 mL.

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: Maximum at 766 nm

Sodium: Maximum at 589 nm

Lithium: Maximum at 671 nm

Blank: *Internal standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\overline{K}}$ emission reading ratio of potassium to lithium from the *Sample solution*

$R_{\overline{S}}$ emission reading ratio of potassium to lithium from the *Standard solution*

$C_{\overline{S}}$ concentration of potassium (K) in the *Standard solution* (mg/mL)

$C_{\overline{U}}$ nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[Note—Each mg of potassium (K) is equivalent to 0.02558 mEq of potassium (K).]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\overline{Na}}$ emission reading ratio of sodium to lithium from the *Sample solution*

$R_{\overline{S}}$ emission reading ratio of sodium to lithium from the *Standard solution*

C_s concentration of sodium (Na) in the *Standard solution* (mg/mL)

C_f nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[Note—Each mg of sodium (Na) is equivalent to 0.04350 mEq of sodium (Na).]

Acceptance criteria

Potassium: 90.0%–110.0%

Sodium: 90.0%–110.0%

SPECIFIC TESTS

- **Bacterial Endotoxins Test** $\langle 85 \rangle$: NMT 0.5 USP Endotoxin Units/mL
- **pH** $\langle 791 \rangle$: 4.0–8.0
- **Other Requirements:** It meets the requirements in *Injections* $\langle 1 \rangle$.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers of Type I or Type II glass are preferable.
- **Labeling:** The label states the content of each electrolyte in terms of milliequivalents (mEq) in a given volume. The label states the total osmolar concentration in mOsmol/L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.
- **USP Reference Standards** $\langle 11 \rangle$
 - USP Citric Acid RS
 - USP Endotoxin RS
 - USP Sodium Lactate RS

BRIEFING

Multiple Electrolytes and Dextrose Injection Type 1, *USP 38* page 3276. It is proposed to make the following revisions to the monograph:

1. In preparation for the omission of the flame tests from *Identification Tests—General* $\langle 191 \rangle$, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to delete the references to *Sodium* and *Potassium* in *Identification test B* and include a complete description of each flame test in the monograph. Manufacturers are encouraged to submit a replacement wet chemistry or an instrumental procedure for consideration by the Expert Committee.
2. An equation is added to calculate the amount of chloride in the *Assay for Chloride*.
3. Concentration units for the *Standard solution* and *Sample solution* are revised to mEq/mL in the *Assay for Phosphate* to eliminate the need for the molecular weight correction term in the equation for the calculation of the amount of phosphate.
4. An equation is added to calculate the amount of sulfate in the *Assay for Sulfate*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: M. Koleck.)

Correspondence Number—C156774

Comment deadline: September 30, 2015

Multiple Electrolytes and Dextrose Injection Type 1

DEFINITION

Multiple Electrolytes and Dextrose Injection Type 1 is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, potassium, magnesium, and chloride ions. In addition, the salts provide ions of acetate, or acetate and gluconate, or acetate and phosphate, or phosphate and lactate, or phosphate and sulfate. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), acetate ($C_2H_3O_2$), gluconate ($C_6H_{11}O_7$), phosphate (PO_4), lactate ($C_3H_5O_3$), and sulfate (SO_4); NLT 90.0% and NMT 120.0% of the labeled amount of chloride (Cl); and NLT 90.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

IDENTIFICATION

- **A.**

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

Change to read:

- **B. Identification Tests—General** ~~(191)~~, ~~**Sodium**~~ ~~(191)~~, ~~**Potassium**~~ ~~(191)~~,

- **2S** (USP39)

Magnesium and **Chloride**: Meets the requirements of the flame tests for ~~Sodium~~ and ~~Potassium~~, and of the tests for ~~Magnesium~~ and ~~Chloride~~

- **2S** (USP39)

Add the following:

- **C. Sodium:** The sample imparts an intense yellow color to a nonluminous flame. ■ **2S** (USP39)

Add the following:

- **D. Potassium:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

- **2S** (USP39)

Change to read:

- **€**

- **E.** ■ **2S** (USP39)

Where acetate is purported to be present, the retention time of the acetate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay for Acetate.

Change to read:

- **D**

- **F.** 2S (USP39)

Where gluconate is purported to be present, the retention time of the gluconate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Gluconate*.

Change to read:

- **E**

- **G.** 2S (USP39)

Where phosphate is purported to be present, proceed as follows.

Sample solution: Add 5 mL of Injection and 1 mL of ammonium molybdate TS to a test tube and mix.

Acceptance criteria: A yellow precipitate, which is soluble in 6 N ammonium hydroxide, is formed.

Change to read:

- **F**

- **H.** 2S (USP39)

Where lactate is purported to be present, the retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Lactate*.

Change to read:

- **G**

- **I.** 2S (USP39)

Identification Tests—General $\langle 191 \rangle$, *Sulfate*: Where sulfate is purported to be present, it meets the requirements of the barium chloride test.

ASSAY

- **Acetate** (if present)

Mobile phase: 0.05 N sulfuric acid

Standard solution: 1.2 mg/mL of sodium acetate trihydrate (0.0088 mEq/mL of acetate) in water

Sample solution: Nominally 0.0088 mEq/mL of acetate from a volume of Injection in water

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Columns

Guard: 4.6-mm × 3-cm; packing L17

Analytical: 7.8-mm × 30-cm; packing L17

Column temperature: 60°

Flow rate: 0.8 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetate ($C_2H_3O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of acetate from the *Sample solution*

r_S peak response of acetate from the *Standard solution*

C_S concentration of acetate in the *Standard solution* (mEq/mL)

C_U nominal concentration of acetate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%**Change to read:**● **Chloride**

Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride (1.55 mEq), to a suitable conical flask, and add water, if necessary, to bring the volume to 10 mL. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS.

Titrimetric system**Mode:** Direct titration**Titrant:** 0.1 N silver nitrate VS**Endpoint detection:** Visual**Analysis****Sample:** *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

Each mL of *Titrant* is equivalent to 3.545 mg (0.1 mEq) of chloride (Cl).

■ Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V

= *Titrant* volume consumed by the *Sample solution* (mL)

N

= actual normality of the *Titrant* (mEq/mL)

F

= equivalency factor, 35.45 mg/mEq

W

= nominal amount of chloride in the *Sample solution* (mg)

■ 2S (USP39)

Acceptance criteria: 90.0%–120.0%

- **Dextrose**

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis

Sample: *Sample solution*

Determine the angular rotation in a polarimeter tube (see *Optical Rotation* 〈 781 〉).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution*, in degrees

l = length of the polarimeter tube, in decimeters

α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution*, g/100 mL

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–105.0%

Change to read:

- **Gluconate** (if present)

Mobile phase: Dissolve 7.0 g of calcium acetate in 1900 mL of water, and adjust with glacial acetic acid to a pH of 4.5 ± 0.1 . Dilute with water to 2000 mL, mix, pass through a filter of 0.5- μm pore size or finer, and degas. Maintain the *Mobile phase* at $70 \pm 2^\circ$ at all times.

System suitability solution: 5 mg/mL each of USP Sodium Lactate RS and USP Potassium Gluconate RS in water

Standard solution: 4.6 mg/mL of USP Potassium Gluconate RS

■ (0.02 mEq/mL of gluconate) ■ 2S (USP39)

in water

Sample solution: Nominally 0.023 mEq/mL of gluconate from a volume of Injection in water

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 7.8-mm \times 30-cm; packing L19 in the calcium form. Condition the column with *Mobile phase* for 60 min.

Column temperature: $70 \pm 2^\circ$

Flow rate: 1 mL/min

Injection volume: 50 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between lactate and gluconate, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of gluconate ($C_6H_{11}O_7$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of gluconate from the *Sample solution*

r_S peak response of gluconate from the *Standard solution*

C_S concentration of gluconate in the *Standard solution* (mEq/mL)

C_U nominal concentration of gluconate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

- **Lactate** (if present)

Mobile phase: Formic acid, dicyclohexylamine, and water (1:1:998)

System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water

Standard solution: 2 mg/mL of USP Sodium Lactate RS

■ (0.018 mEq/mL of lactate) ■ 2S (USP39)

in water

Sample solution

For Injections containing >20 mEq/L of lactate: Nominally 0.02 mEq/mL of lactate from Injection in water

For Injections containing ≤20 mEq/L of lactate: Use the undiluted Injection.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between acetate and lactate, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lactate ($C_3H_5O_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_{\bar{C}}$ peak response of lactate from the *Sample solution*

$r_{\bar{S}}$ peak response of lactate from the *Standard solution*

$C_{\bar{S}}$ concentration of lactate in the *Standard solution* (mEq/mL)

$C_{\bar{C}}$ nominal concentration of lactate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

• **Magnesium**

[Note—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Solution B: Mix 678 mL of hydrochloric acid with water to make 3000 mL.

Standard stock solution A: 1.00 mg/mL of magnesium (Mg) prepared as follows. Transfer 1.00 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water. Slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with *Solution B* to volume.

Standard stock solution B: 100 µg/mL of magnesium (Mg) prepared as follows. Transfer 10.0 mL of *Standard stock solution A* to a 100-mL volumetric flask, and dilute with *Solution B* to volume.

Standard solutions: 10.0, 15.0, and 20.0 µg/mL of magnesium (Mg) prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution B*. Dilute the contents of each flask with *Solution B* to volume.

Sample solution: Transfer a volume of Injection, equivalent to 20 mg (1.65 mEq)

■ ■ 2S (USP39)

of magnesium (Mg) to a 1000-mL volumetric flask containing 50.0 mL of *Solution A*. Dilute with *Solution B* to volume.

Blank: 5.0 mL of *Solution A* diluted with *Solution B* to 100.0 mL

Instrumental conditions

(See *Spectrophotometry and Light Scattering* { 851 } .)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Magnesium emission line at 285.2 nm

Lamp: Magnesium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of magnesium (Mg), and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (C) in µg/mL, of magnesium (Mg) in the *Sample solution*.

Calculate the percentage of the labeled amount of magnesium (Mg) in the portion of Injection

taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of magnesium (Mg) in the *Sample solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of magnesium (Mg) in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–110.0%

Change to read:

• **Phosphate** (if present)

Solution A: 50 g/L of ammonium molybdate prepared as follows. Transfer a suitable amount of ammonium molybdate to an appropriate volumetric flask. Add 60% of the final flask volume of water, and swirl to dissolve. Add 15% of the flask volume of sulfuric acid, and swirl. Allow to cool, and dilute with water to volume.

Solution B: Dissolve 0.5 g of hydroquinone in 100 mL of water, and add 1 drop of sulfuric acid. Prepare this solution fresh daily.

Solution C: Dissolve 1 g of sodium sulfite in water to make 5 mL. Prepare this solution fresh daily.

Standard solution: 0.11 mg/mL of monobasic potassium phosphate

■ (0.0008 mEq/mL of phosphate) ■ 2S (USP39)

in water

Sample solution: Transfer a volume of Injection equivalent to 4 mg (0.126 mEq) of phosphate to a 50 mL volumetric flask, and dilute with water to volume.

■ Nominally 0.0008 mEq/mL of phosphate from a volume of Injection in water ■ 2S (USP39)

Blank: Water

Instrumental conditions

Mode: Vis

Analytical wavelength: 640 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use water to zero the instrument. Transfer 2.0 mL each of the *Standard solution*, *Sample solution*, and the *Blank* to separate test tubes. To each test tube add 1.0 mL of *Solution A*, mix, and allow to stand for 3 min. Add 1.0 mL of *Solution B*, and mix. Add 1.0 mL of *Solution C*, mix, and allow to stand for 30 min.

Calculate the percentage of the labeled amount of phosphate (PO_4) in the portion of the Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

■
$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100 \text{ ■ 2S (USP39)}$$

A_U = absorbance of the *Sample solution*, corrected for any absorbance of the solution from the *Blank*

A_S = absorbance of the *Standard solution*, corrected for any absorbance of the solution from the *Blank*

C_S = concentration of phosphate in the *Standard solution* (~~mg~~
~~■ mEq/ ■ 2S (USP39)~~
 mL)

C_U = nominal concentration of phosphate (PO_4) in the *Sample solution* (~~mg~~
~~■ mEq/ ■ 2S (USP39)~~
 mL)

~~M_{r1} = molecular weight of phosphate, 94.97~~
~~■~~

~~M_{r2} = molecular weight of monobasic potassium phosphate, 136.09 ■ 2S (USP39)~~

Acceptance criteria: 90.0%–110.0%

● **Potassium and Sodium**

Internal standard solution: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Add a suitable nonionic surfactant, and then dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride (1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Standard stock solution: 0.0391J mg/mL of potassium (K) from *Potassium stock solution* and 0.02299J' mg/mL of sodium (Na) from *Sodium stock solution* prepared as follows. Transfer 0.1J mL of *Potassium stock solution* and 0.1J' mL of *Sodium stock solution* to a 100-mL volumetric flask, where J and J' are the labeled amounts, in mEq/L, of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Dilute 5.0 mL of *Standard stock solution* with *Internal standard solution* to 500.0 mL.

Sample solution: Dilute 5.0 mL of Injection with *Internal standard solution* to 500.0 mL.

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: Maximum at 766 nm

Sodium: Maximum at 589 nm

Lithium: Maximum at 671 nm

Blank: *Internal standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\overline{K}}$ emission reading ratio of potassium to lithium from the *Sample solution*

$R_{\bar{S}}$ emission reading ratio of potassium to lithium from the *Standard solution*

$C_{\bar{S}}$ concentration of potassium (K) in the *Standard solution* (mg/mL)

$C_{\bar{I}}$ nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[Note—Each mg of potassium (K) is equivalent to 0.02558 mEq of potassium (K).]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{I}}$ emission reading ratio of sodium to lithium from the *Sample solution*

$R_{\bar{S}}$ emission reading ratio of sodium to lithium from the *Standard solution*

$C_{\bar{S}}$ concentration of sodium (Na) in the *Standard solution* (mg/mL)

$C_{\bar{I}}$ nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[Note—Each mg of sodium (Na) is equivalent to 0.04350 mEq of sodium (Na).]

Acceptance criteria

Potassium: 90.0%–110.0%

Sodium: 90.0%–110.0%

Change to read:

● Sulfate (if present)

Sample solution: Equivalent to 120 mg (1.25 mEq) of sulfate from a volume of injection.

Dilute, if necessary, to 200 mL, and add 1 mL of hydrochloric acid.

Analysis: Heat the *Sample solution* to boiling, and gradually add in small portions and while constantly stirring, an excess of hot barium chloride TS (about 8 mL). Heat the mixture on a steam bath for 1 h, collect the precipitate of barium sulfate on a tared filtering crucible, wash until free from chloride, dry, ignite, and weigh. ~~The weight of the barium sulfate, multiplied by 0.4116, represents its equivalent of sulfate (SO₄). Each mg of barium sulfate represents 0.004285 mEq of sulfate (SO₄).~~

Calculate the percentage of the labeled amount of sulfate (SO₄) in the portion of Injection taken:

$$\text{Result} = [(W \times (M_{r1}/M_{r2}) \times (1/M_{r1})) / (L \times V)] \times 100$$

W

= weight of barium sulfate after ignition (mg)

M_{r1}

= molecular weight of sulfate, 96.06

M_{r2}

= molecular weight of barium sulfate, 233.43

L

= label claim of sulfate in the Injection (mEq/mL)

V

= volume of Injection in the *Sample solution* (mL)

■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%**SPECIFIC TESTS**

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 0.5 USP Endotoxin Units/mL
- **pH** 〈 791 〉: 4.0–6.5
- **Other Requirements:** Meets the requirements in *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **Labeling:** The label states the content of each electrolyte in terms of milliequivalents (mEq) in a given volume. The label states the total osmolar concentration in mOsmol/L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.
- **USP Reference Standards** 〈 11 〉
 - USP Endotoxin RS
 - USP Potassium Gluconate RS
 - USP Sodium Lactate RS

BRIEFING

Multiple Electrolytes and Dextrose Injection Type 2, *USP 38* page 3278. It is proposed to make the following revisions to the monograph.

1. In preparation for the omission of the flame tests from *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to delete the references to *Sodium* and *Potassium* in *Identification test B* and include a complete description of each flame test in the monograph. Manufacturers are encouraged to submit a replacement wet chemistry or an instrumental procedure for consideration by the Expert Committee.
2. An equation is added to calculate the amount of chloride in the *Assay for Chloride*.
3. An equation is added to calculate the amount of sulfate in the *Assay for Sulfate*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: M. Koleck.)

Correspondence Number—C156775

Comment deadline: September 30, 2015

Multiple Electrolytes and Dextrose Injection Type 2**DEFINITION**

Multiple Electrolytes and Dextrose Injection Type 2 is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, potassium, magnesium, calcium, and chloride ions. In addition, the salts provide ions of acetate, or acetate and citrate, or acetate and lactate, or gluconate and sulfate. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), acetate (C₂H₃O₂), citrate (C₆H₅O₇), lactate (C₃H₅O₃), gluconate (C₆H₁₁O₇), and sulfate (SO₄); NLT 90.0% and NMT 120.0% of the labeled amount of chloride (Cl); and NLT 90.0% and NMT 105.0% of the labeled amount of dextrose (C₆H₁₂O₆·H₂O). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

IDENTIFICATION

• A.

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

Change to read:

• B. Identification Tests—General $\langle 191 \rangle$, ~~Sodium $\langle 191 \rangle$ Potassium $\langle 191 \rangle$,~~

■ ■ 2S (USP39)

~~Magnesium and Chloride~~; and ~~Calcium $\langle 191 \rangle$~~

■ ■ 2S (USP39)

Meets the requirements of the flame tests for ~~Sodium and Potassium~~, of the tests for ~~Magnesium and Chloride~~, and of the oxalate test for ~~Calcium~~

■ ■ 2S (USP39)

Add the following:

• C. Sodium: The sample imparts an intense yellow color to a nonluminous flame. ■ 2S (USP39)

Add the following:

- D. Potassium: The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

■ 2S (USP39)

Add the following:

- E. Identification Tests—General $\langle 191 \rangle$, Calcium: Meets the requirements of the oxalate test ■ 2S (USP39)

Change to read:

• F.

■ F. ■ 2S (USP39)

Where acetate is purported to be present, the retention time of the acetate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Acetate*.

Change to read:

- **D**

G. 2S (USP39)

Where citrate is purported to be present, the retention time of the citrate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Citrate*.

Change to read:

- **E**

H. 2S (USP39)

Where lactate is purported to be present, the retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Lactate*.

Change to read:

- **F**

I. 2S (USP39)

Where gluconate is purported to be present, the retention time of the gluconate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Gluconate*.

Change to read:

- **G**

J. 2S (USP39)

Identification Tests—General $\langle 191 \rangle$, *Sulfate*: Where sulfate is purported to be present, it meets the requirements of the barium chloride test.

ASSAY

- **Acetate** (if present)

Mobile phase: 0.05 N sulfuric acid

Standard solution: 1.2 mg/mL of sodium acetate trihydrate (0.0088 mEq/mL of acetate) in water

Sample solution: Nominally 0.0088 mEq/mL of acetate from a volume of Injection in water

Chromatographic system

(See *Chromatography* $\langle 621 \rangle$, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Columns

Guard: 4.6-mm \times 3-cm; packing L17

Analytical: 7.8-mm \times 30-cm; packing L17

Column temperature: 60 $^{\circ}$

Flow rate: 0.8 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetate ($C_2H_3O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetate from the *Sample solution*

r_S = peak response of acetate from the *Standard solution*

C_S = concentration of acetate in the *Standard solution* (mEq/mL)

C_U = nominal concentration of acetate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

- **Calcium**

[Note—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Solution B: Mix 678 mL of hydrochloric acid with water to make 3000 mL.

Standard stock solution: 1000 $\mu\text{g/mL}$ of calcium prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of *Solution B*, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 10.0, 15.0, and 20.0 $\mu\text{g/mL}$ of calcium prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 1.0, 1.5, and 2.0 mL, respectively, of *Standard stock solution*. Dilute the contents of each flask with *Solution B* to volume.

Sample solution: Transfer a volume of Injection, equivalent to 20 mg (1 mEq)

■ ■ 2S (USP39)

of calcium, to a 1000-mL volumetric flask containing 50.0 mL of *Solution A*. Dilute with *Solution B* to volume.

Blank: 5.0 mL of *Solution A* diluted with *Solution B* to 100.0 mL

Instrumental conditions

(See *Spectrophotometry and Light Scattering* { 851 } .)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in $\mu\text{g/mL}$, of calcium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (C), in $\mu\text{g/mL}$, of calcium in the *Sample solution*.

Calculate the percentage of the labeled amount of calcium (Ca) in the portion of Injection taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of Calcium in the *Sample solution* ($\mu\text{g/mL}$), interpolated from the graph obtained

C_U = nominal concentration of Calcium in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–110.0%

Change to read:

• **Chloride**

Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride (1.55 mEq), to a suitable conical flask, and add water, if necessary, to bring the volume to 10 mL. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

Each mL of *Titrant* is equivalent to 3.545 mg (0.1 mEq) of chloride (Cl).

■ Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V

= Titrant volume consumed by the *Sample solution* (mL)

N

= actual normality of the *Titrant* (mEq/mL)

F

= equivalency factor, 35.45 mg/mEq

W

= nominal amount of chloride in the *Sample solution* (mg)

■ 2S (USP39)

Acceptance criteria: 90.0%–120.0%

• **Dextrose**

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis

Sample: *Sample solution*

Determine the angular rotation in a polarimeter tube (see *Optical Rotation* { 781 }.)
Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution*, in degrees

l = length of the polarimeter tube, in decimeters

α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution*, g/100 mL

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–105.0%

- **Citrate** (if present)

Mobile phase and Chromatographic system: Proceed as directed in the *Assay for Citric Acid/Citrate and Phosphate* { 345 }.

Standard solution: 0.3 mEq/L of citrate ($C_6H_5O_7$) from USP Citric Acid RS in freshly prepared 1 mM sodium hydroxide

Sample solution: Nominally 0.3 mEq/L of citrate in 1 mM sodium hydroxide from a volume of Injection diluted with freshly prepared sodium hydroxide

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in { 345 }.

Calculate the percentage of the labeled amount of citrate ($C_6H_5O_7$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of citrate from the *Sample solution*

r_S = peak response of citrate from the *Standard solution*

C_S = concentration of citrate in the *Standard solution* (mEq/L)

C_U = nominal concentration of citrate in the *Sample solution* (mEq/L)

Acceptance criteria: 90.0%–110.0%

Change to read:

- **Gluconate** (if present)

Mobile phase: Dissolve 7.0 g of calcium acetate in 1900 mL of water, and adjust with glacial acetic acid to a pH of 4.5 ± 0.1 . Dilute with water to 2000 mL, mix, pass through a filter of 0.5- μm pore size or finer, and degas. Maintain the *Mobile phase* at $70 \pm 2^\circ$ at all times.

System suitability solution: 5 mg/mL each of USP Sodium Lactate RS and USP Potassium Gluconate RS in water

Standard solution: 4.6 mg/mL of USP Potassium Gluconate RS

■(0.02 mEq/mL of gluconate) ■_{2S} (USP39)

in water

Sample solution: Nominally 0.023 mEq/mL of gluconate from a volume of Injection in water

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 7.8-mm × 30-cm; packing L19 in the calcium form. Condition the column with *Mobile phase* for 60 min.

Column temperature: 70 ± 2°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between lactate and gluconate, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of gluconate (C₆H₁₁O₇) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of gluconate from the *Sample solution*

r_S = peak response of gluconate from the *Standard solution*

C_S = concentration of USP Potassium Gluconate RS in the *Standard solution* (mEq/mL)

C_U = nominal concentration of gluconate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

- **Lactate** (if present)

Mobile phase: Formic acid, dicyclohexylamine, and water (1:1:998)

System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water

Standard solution: 2 mg/mL of USP Sodium Lactate RS

■(0.018 mEq/mL of lactate) ■_{2S} (USP39)

in water

Sample solution

For Injections containing >20 mEq/L of lactate: Nominally 0.02 mEq/mL of lactate from Injection in water

For Injections containing ≤20 mEq/L of lactate: Use the undiluted Injection.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between acetate and lactate, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lactate (C₃H₅O₃) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of lactate from the *Sample solution*

r_S peak response of lactate from the *Standard solution*

C_S concentration of lactate in the *Standard solution* (mEq/mL)

C_U nominal concentration of lactate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

• Magnesium

[Note—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Solution B: Mix 678 mL of hydrochloric acid with water to make 3000 mL.

Standard stock solution A: 1.00 mg/mL of magnesium (Mg) prepared as follows. Transfer 1.00 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water. Slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with *Solution B* to volume.

Standard stock solution B: 100 µg/mL of magnesium (Mg) prepared as follows. Transfer 10.0 mL of *Standard stock solution A* to a 100-mL volumetric flask, and dilute with *Solution B* to volume.

Standard solutions: 10.0, 15.0, and 20.0 µg/mL of magnesium (Mg) prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution B*. Dilute the contents of each flask with *Solution B* to volume.

Sample solution: Transfer a volume of Injection, equivalent to 20 mg (1.65 mEq)

■ ■ 2S (USP39)

of magnesium (Mg) to a 1000-mL volumetric flask containing 50.0 mL of *Solution A*. Dilute with *Solution B* to volume.

Blank: 5.0 mL of *Solution A* diluted with *Solution B* to 100.0 mL

Instrumental conditions

(See *Spectrophotometry and Light Scattering* (851).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Magnesium emission line at 285.2 nm

Lamp: Magnesium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in $\mu\text{g/mL}$, of magnesium (Mg), and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (C) in $\mu\text{g/mL}$, of magnesium (Mg) in the *Sample solution*.

Calculate the percentage of the labeled amount of magnesium (Mg) in each portion of Injection taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of magnesium (Mg) in the *Sample solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of magnesium (Mg) in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–110.0%

• **Potassium and Sodium**

Internal standard solution: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Add a suitable nonionic surfactant, and then dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride (1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Standard stock solution: 0.0391J mg/mL of potassium (K) from *Potassium stock solution* and 0.02299J' mg/mL of sodium (Na) from *Sodium stock solution* prepared as follows. Transfer 0.1J mL of *Potassium stock solution* and 0.1J' mL of *Sodium stock solution* to a 100-mL volumetric flask, where J and J' are the labeled amounts, in mEq/L, of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Dilute 5.0 mL of *Standard stock solution* with *Internal standard solution* to 500.0 mL.

Sample solution: Dilute 5.0 mL of Injection with *Internal standard solution* to 500.0 mL.

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: Maximum at 766 nm

Sodium: Maximum at 589 nm

Lithium: Maximum at 671 nm

Blank: *Internal standard solution*

Analysis

Samples: *Standard solution, Sample solution, and Blank*

Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{U}}$ emission reading ratio of potassium to lithium from the *Sample solution*

$R_{\bar{S}}$ emission reading ratio of potassium to lithium from the *Standard solution*

$C_{\bar{S}}$ concentration of potassium (K) in the *Standard solution* (mg/mL)

$C_{\bar{U}}$ nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[Note—Each mg of potassium (K) is equivalent to 0.02558 mEq of potassium (K).]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{U}}$ emission reading ratio of sodium to lithium from the *Sample solution*

$R_{\bar{S}}$ emission reading ratio of sodium to lithium from the *Standard solution*

$C_{\bar{S}}$ concentration of sodium (Na) in the *Standard solution* (mg/mL)

$C_{\bar{U}}$ nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[Note—Each mg of sodium (Na) is equivalent to 0.04350 mEq of sodium (Na).]

Acceptance criteria

Potassium: 90.0%–110.0%

Sodium: 90.0%–110.0%

Change to read:

- **Sulfate** (if present)

Sample solution: Equivalent to 120 mg (1.25 mEq) of sulfate, from a volume of Injection.

Dilute, if necessary, to 200 mL, and add 1 mL of hydrochloric acid.

Analysis

Sample: *Sample solution*

Heat the *Sample solution* to boiling, and gradually add, in small portions and while constantly stirring, an excess of hot barium chloride TS (about 8 mL). Heat the mixture on a steam bath for 1 h, collect the precipitate of barium sulfate on a tared filtering crucible, wash until free from chloride, dry, ignite, and weigh. ~~The weight of the barium sulfate, multiplied by 0.4116, represents its equivalent of sulfate (SO₄). Each mg of barium sulfate represents 0.004285 mEq of sulfate (SO₄).~~

Calculate the percentage of the labeled amount of sulfate (SO₄) in the portion of Injection taken:

$$\text{Result} = \{[W \times (M_{r1}/M_{r2}) \times (1/M_{r1})]/(L \times V)\} \times 100$$

W

= weight of barium sulfate after ignition (mg)

M_{r1}

= molecular weight of sulfate, 96.06

M_{r2}

= molecular weight of barium sulfate, 233.43

L

= label claim of sulfate in the Injection (mEq/mL)

V

= volume of Injection in the *Sample solution* (mL)

■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **Bacterial Endotoxins Test** 〈 85 〉: It contains NMT 0.5 USP Endotoxin Units/mL.
- **pH** 〈 791 〉: 4.0–6.5
- **Other Requirements:** It meets the requirements in *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **Labeling:** The label states the content of each electrolyte in terms of milliequivalents (mEq) in a given volume. The label states the total osmolar concentration in mOsmol/L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.
- **USP Reference Standards** 〈 11 〉
 - USP Citric Acid RS
 - USP Endotoxin RS
 - USP Potassium Gluconate RS
 - USP Sodium Lactate RS

BRIEFING

Multiple Electrolytes and Dextrose Injection Type 3, *USP 38* page 3279. It is proposed to make the following revisions to the monograph.

1. In preparation for the omission of the flame tests from *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to delete the references to *Sodium* and *Potassium* in *Identification test B* and include a complete

description of each flame test in the monograph. Manufacturers are encouraged to submit a replacement wet chemistry or an instrumental procedure for consideration by the Expert Committee.

2. An equation is added to calculate the amount of ammonium in the *Assay for Ammonium*.
3. An equation is added to calculate the amount of chloride in the *Assay for Chloride*.
4. Concentration units for the *Standard solution* and *Sample solution* are revised to mEq/mL in the *Assay for Phosphate* to eliminate the need for the molecular weight correction term in the equation for the calculation of the amount of phosphate.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: M. Koleck.)

Correspondence Number—C156776

Comment deadline: September 30, 2015

Multiple Electrolytes and Dextrose Injection Type 3

DEFINITION

Multiple Electrolytes and Dextrose Injection Type 3 is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, potassium, and chloride ions. In addition, the salts provide ions of ammonium, or acetate and phosphate, or phosphate and lactate. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of sodium (Na), potassium (K), ammonium (NH₄), acetate (C₂H₃O₂), phosphate (PO₄), and lactate (C₃H₅O₃); NLT 90.0% and NMT 120.0% of the labeled amount of chloride (Cl); and NLT 90.0% and NMT 105.0% of the labeled amount of dextrose (C₆H₁₂O₆·H₂O). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

IDENTIFICATION

• **A.**

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

Change to read:

• **B. Identification Tests—General** < 191 >, ~~**Sodium**~~ < 191 >, ~~**Potassium**~~ < 191 >,

■ 2S (USP39)

Chloride and **Ammonium**: Meets the requirements of the flame tests for ~~*Sodium*~~ and ~~*Potassium*~~, and of the tests for ~~*Chloride*~~ and ~~*Ammonium*~~

■ 2S (USP39)

Add the following:

• **C. Sodium:** The sample imparts an intense yellow color to a nonluminous flame. ■ 2S (USP39)

Add the following:

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetate ($C_2H_3O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of acetate from the *Sample solution*

r_S peak response of acetate from the *Standard solution*

C_S concentration of acetate in the *Standard solution* (mEq/mL)

C_U nominal concentration of acetate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

- **Ammonium** (if present)

Sample solution: Transfer a volume of Injection, equivalent to 63 mg (3.5 mEq) of ammonium, to a 500-mL Kjeldahl flask. Dilute with water to 200 mL, and add 50 mL of sodium hydroxide solution (2 in 5).

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sulfuric acid VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Immediately connect the flask containing the *Sample solution* by means of a distillation trap to a well-cooled condenser, the delivery tube of which dips into 40 mL of boric acid solution (1 in 25) contained in a suitable receiver. Heat to boiling, and distill about 200 mL. Cool the liquid in the receiver, if necessary, then add methyl red TS, and titrate with *Titrant*. Perform a blank determination, and make any necessary correction. ~~Each mL of *Titrant* is equivalent to 1.804 mg (0.1 mEq) of ammonium (NH_4).~~

Calculate the percentage of the labeled amount of ammonium (NH_4) in the portion of Injection taken:

$$\text{Result} = (V_S - V_B) \times N \times (F/W) \times 100$$

V_S

= *Titrant* volume consumed by the *Sample solution* (mL)

V_B

= *Titrant* volume consumed by the blank (mL)

N

= actual normality of the *Titrant* (mEq/mL)

F

= equivalency factor, 18.04 mg/mEq

W

= nominal amount of ammonium in the *Sample solution* (mg)

■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

Change to read:

• **Chloride**

Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride (1.55 mEq), to a suitable conical flask and add water, if necessary, to bring the volume to about 10 mL. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

Each mL of *Titrant* is equivalent to 3.545 mg (0.1 mEq) of chloride (Cl).

■ Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V

= *Titrant* volume consumed by the *Sample solution* (mL)

N

= actual normality of the *Titrant* (mEq/mL)

F

= equivalency factor, 35.45 mg/mEq

W

= nominal amount of chloride in the *Sample solution* (mg)

■ 2S (USP39)

Acceptance criteria: 90.0%–120.0%

• **Dextrose**

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis**Sample:** *Sample solution*

Determine the angular rotation in a polarimeter tube (see *Optical Rotation* 〈 781 〉). Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution*, in degrees

l = length of the polarimeter tube, in decimeters

α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution*, g/100 mL

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–105.0%

Change to read:

- **Lactate** (if present)

Mobile phase: Formic acid, dicyclohexylamine, and water (1:1:998)

System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water

Standard solution: 2 mg/mL of USP Sodium Lactate RS

■(0.018 mEq/mL of lactate)■_{2S} (USP39)

in water

Sample solution

For Injections containing >20 mEq/L of lactate: Nominally 0.02 mEq/mL of lactate from Injection in water

For Injections containing ≤20 mEq/L of lactate: Use the undiluted Injection.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between acetate and lactate, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lactate ($C_3H_5O_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of lactate from the *Sample solution*

r_S peak response of lactate from the *Standard solution*

C_S concentration of lactate in the *Standard solution* (mEq/mL)

C_U nominal concentration of lactate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

• **Phosphate** (if present)

Solution A: 50 g/L of ammonium molybdate prepared as follows. Transfer a suitable amount of ammonium molybdate to an appropriate volumetric flask. Add 60% of the final flask volume of water, and swirl to dissolve. Add 15% of the flask volume of sulfuric acid, and swirl. Allow to cool, and dilute with water to volume.

Solution B: Dissolve 0.5 g of hydroquinone in 100 mL of water, and add 1 drop of sulfuric acid. Prepare this solution fresh daily.

Solution C: Dissolve 1 g of sodium sulfite in water to make 5 mL. Prepare this solution fresh daily.

Standard solution: 0.11 mg/mL of monobasic potassium phosphate

■ (0.0008 mEq/mL of phosphate) ■ 2S (USP39)

in water

Sample solution: ~~Transfer a volume of Injection equivalent to 4 mg (0.126 mEq) of phosphate to a 50 mL volumetric flask, and dilute with water to volume.~~

■ Nominally 0.0008 mEq/mL of phosphate from a volume of Injection ■ 2S (USP39)

Blank: Water

Instrumental conditions

Mode: Vis

Analytical wavelength: 640 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use water to zero the instrument. Transfer 2 mL each of the *Standard solution*, the *Sample solution*, and the *Blank* to separate test tubes. To each test tube add 1 mL of *Solution A*, and allow to stand for 3 min. Add 1 mL of *Solution B*. Add 1 mL of *Solution C*, and allow to stand for 30 min.

Calculate the percentage of the labeled amount of phosphate (PO_4) in the portion of the Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

■ $\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$ ■ 2S (USP39)

A_U = absorbance of the *Sample solution*, corrected for any absorbance of the solution from the *Blank*

A_S = absorbance of the *Standard solution*, corrected for any absorbance of the solution from the *Blank*

C_S = concentration of phosphate in the *Standard solution* (~~mg~~
 ■ mEq/ ■ 2S (USP39)
 mL)

C_U = nominal concentration of phosphate (PO_4) in the *Sample solution* (~~mg~~
 ■ mEq/ ■ 2S (USP39)
 mL)

M_{r1} = molecular weight of phosphate, 94.97
 ■

M_{r2} = molecular weight of monobasic potassium phosphate, 136.09 ■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

● **Potassium and Sodium**

Internal standard solution: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, then dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride (1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Standard stock solution: 0.0391J mg/mL of potassium (K) from *Potassium stock solution* and 0.02299J' mg/mL of sodium (Na) from *Sodium stock solution* prepared as follows. Transfer 0.1J mL of *Potassium stock solution* and 0.1J' mL of *Sodium stock solution* to a 100-mL volumetric flask, where J and J' are the labeled amounts, in mEq/L, of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Dilute 5.0 mL of *Standard stock solution* with *Internal standard solution* to 500.0 mL.

Sample solution: Dilute 5.0 mL of Injection with *Internal standard solution* to 500.0 mL.

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: Maximum at 766 nm

Sodium: Maximum at 589 nm

Lithium: Maximum at 671 nm

Blank: *Internal standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{K}}$ emission reading ratio of potassium to lithium from the *Sample solution*

$R_{\bar{S}}$ emission reading ratio of potassium to lithium from the *Standard solution*

$C_{\bar{S}}$ concentration of potassium (K) in the *Standard solution* (mg/mL)

$C_{\bar{K}}$ nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[Note—Each mg of potassium (K) is equivalent to 0.02558 mEq of potassium (K).]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{Na}}$ emission reading ratio of sodium to lithium from the *Sample solution*

$R_{\bar{S}}$ emission reading ratio of sodium to lithium from the *Standard solution*

$C_{\bar{S}}$ concentration of sodium (Na) in the *Standard solution* (mg/mL)

$C_{\bar{Na}}$ nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[Note—Each mg of sodium (Na) is equivalent to 0.04350 mEq of sodium (Na).]

Acceptance criteria

Potassium: 90.0%–110.0%

Sodium: 90.0%–110.0%

SPECIFIC TESTS

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 0.5 USP Endotoxin Units/mL
- **pH** 〈 791 〉: 4.0–6.5
- **Other Requirements:** It meets the requirements in *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **Labeling:** The label states the content of each electrolyte in terms of milliequivalents (mEq) in a given volume. The label states the total osmolar concentration in mOsmol/L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.
- **USP Reference Standards** 〈 11 〉
 - USP Endotoxin RS
 - USP Sodium Lactate RS

BRIEFING

Multiple Electrolytes and Dextrose Injection Type 4, *USP 38* page 3281. It is proposed to omit this monograph for the following reasons. No drug products formulated as defined under Multiple Electrolytes and Dextrose Injection Type 4 are currently marketed in the United States. The drug product is currently not used in veterinary medicine in the United States.

(SM4: M. Koleck.) Correspondence Number—C157334

Comment deadline: September 30, 2015

Delete the following:**Multiple Electrolytes and Dextrose Injection Type 4**

» Multiple Electrolytes and Dextrose Injection Type 4 is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, magnesium, calcium, chloride, gluconate, and sulfate ions. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sodium (Na), magnesium (Mg), calcium (Ca), gluconate ($C_6H_{11}O_7$), and sulfate (SO_4), not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloride (Cl), and not less than 90.0 percent and not more than 105.0 percent of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

Labeling—The label states the content of each electrolyte in terms of milliequivalents in a given volume. The label states the total osmolar concentration in mOsmol per L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

USP Reference standards ~~(11)~~—

USP Endotoxin RS

USP Potassium Gluconate RS

Identification—

A: It responds to the *Identification* test under *Dextrose*.

B: It responds to the flame test for *Sodium* ~~(191)~~, to the tests for *Magnesium* ~~(191)~~ and *Chloride* ~~(191)~~, to the oxalate test for *Calcium* ~~(191)~~, and to the barium chloride test for *Sulfate* ~~(191)~~.

C: The retention time of the gluconate peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay for gluconate*.

Bacterial endotoxins ~~(85)~~—It contains not more than 0.5 USP Endotoxin Unit per mL.

pH ~~(791)~~: between 4.2 and 5.2.

Other requirements—It meets the requirements under *Injections* ~~(1)~~.

Assay for sodium—

Internal standard solution—Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, dilute with water to volume, and mix.

Standard preparation— Transfer 7.31 g of sodium chloride, previously dried at 105 ° for 2 hours and accurately weighed, to a 250 mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100 mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500 mL volumetric flask, dilute with *Internal standard solution* to volume, and mix. Each mL of this solution contains 0.0292 mg of sodium chloride, equivalent to 0.0115 mg (0.0005 mEq) of sodium (Na).

Assay preparation— Dilute an accurately measured volume of Injection quantitatively with *Internal standard solution* to obtain a solution containing about 0.0115 mg (0.0005 mEq) of sodium per mL.

Procedure— Using a flame photometer, adjusted to read zero with *Internal standard solution*, concomitantly determine the flame emission readings for the *Standard preparation* and the *Assay preparation* at the wavelengths of maximum emission for sodium and lithium (589 nm and 671 nm, respectively). Calculate the quantity, in mg, of sodium (Na) in each mL of the Injection taken by the formula:

$$(22.99 / 58.44)(C)(L / D)(R_{U,589} / R_{U,671})(R_{S,671} / R_{S,589})$$

in which 22.99 is the atomic weight of sodium, 58.44 is the molecular weight of sodium chloride, *C* is the concentration, in mg per mL, of sodium chloride in the *Standard preparation*, *L* is the labeled quantity, in mg, of sodium in each mL of the Injection, *D* is the concentration, in mg per mL, of sodium in the *Assay preparation*, based on the volume of Injection taken and the extent of dilution, and $R_{U,589}$ and $R_{U,671}$ are the emission readings at the wavelengths identified by the subscript numbers obtained for the *Assay preparation*, and $R_{S,589}$ and $R_{S,671}$ are the emission readings at the wavelengths identified by the subscript numbers obtained from the *Standard preparation*. Each mg of sodium is equivalent to 0.0435 of mEq of sodium.

Assay for magnesium— [Note—Concentrations of the *Standard preparations* and the *Assay preparation* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Lanthanum chloride solution, *Dilute hydrochloric acid*, *Blank solution*, *Magnesium stock solution*, and *Standard preparations*—Prepare as directed in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*.

Assay preparation— Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1.65 mEq) of magnesium, to a 1000 mL volumetric flask containing 50.0 mL of *Lanthanum chloride solution*. Dilute the contents of the flask with *Dilute hydrochloric acid* to volume, and mix.

Procedure— Proceed as directed for *Procedure* in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*. Calculate the quantity, in µg, of magnesium (Mg) in each mL of Injection taken by the formula:

$$1000(C / V)$$

in which the terms are as defined therein.

Assay for calcium— [Note—Concentrations of the *Standard preparations* and the *Assay preparation* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Lanthanum chloride solution, *Dilute hydrochloric acid*, and *Blank solution*— Prepare as directed

~~in the Assay for magnesium under Multiple Electrolytes Injection Type 1.~~

~~Calcium stock solution and Standard preparations— Prepare as directed in the Assay for calcium under Multiple Electrolytes Injection Type 2.~~

~~Assay preparation— Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1 mEq) of calcium, to a 1000 mL volumetric flask containing 50.0 mL of Lanthanum chloride solution. Dilute the contents of the flask with Dilute hydrochloric acid to volume, and mix.~~

~~Procedure— Proceed as directed for Procedure in the Assay for calcium under Multiple Electrolytes Injection Type 2. Calculate the quantity, in µg, of calcium in each mL of Injection taken by the formula:~~

$$1000(C/V)$$

~~in which the terms are as defined therein.~~

~~**Assay for chloride**— Using the Injection, proceed as directed in the Assay for chloride under Multiple Electrolytes Injection Type 1.~~

Assay for gluconate—

~~Mobile phase, Standard preparation, Resolution solution, and Chromatographic system— Prepare as directed in the Assay for gluconate under Multiple Electrolytes and Dextrose Injection Type 1.~~

~~Assay preparation— Dilute an accurately measured volume of Injection quantitatively with water to obtain a solution containing about 0.023 mEq of gluconate per mL.~~

~~Procedure— Proceed as directed for Procedure in the Assay for gluconate under Multiple Electrolytes and Dextrose Injection Type 1. Calculate the quantity, in mEq per liter, of gluconate ($C_6H_{11}O_7$) in the Injection taken by the formula:~~

$$(C / 234.25)(L / D)(r_U / r_S)$$

~~in which the terms are as defined therein.~~

~~**Assay for sulfate**— Using the Injection, proceed as directed in the Assay for sulfate under Multiple Electrolytes and Dextrose Injection Type 1.~~

~~**Assay for dextrose**— Using the Injection, proceed as directed in the Assay for dextrose under Multiple Electrolytes and Dextrose Injection Type 1. ■ 2S (USP39)~~

BRIEFING

Multiple Electrolytes and Invert Sugar Injection Type 1, USP 38 page 3282. It is proposed to omit this monograph for the following reasons. No drug products formulated as defined under Multiple Electrolytes and Invert Sugar Injection Type 1 are currently marketed in the United States. The drug product is currently not used in veterinary medicine in the United States.

(SM4: M. Koleck.) Correspondence Number—C157330

Comment deadline: September 30, 2015

Delete the following:**■ Multiple Electrolytes and Invert Sugar Injection Type 1**

» Multiple Electrolytes and Invert Sugar Injection Type 1 is a sterile solution of a mixture of equal amounts of Dextrose and Fructose, or an equivalent solution produced by the hydrolysis of Sucrose, and suitable salts in Water for Injection to provide sodium, potassium, magnesium, chloride, phosphate, and lactate ions. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), phosphate (PO_4), lactate ($\text{C}_3\text{H}_5\text{O}_3$), and invert sugar ($\text{C}_6\text{H}_{12}\text{O}_6$), and not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloride (Cl). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

Labeling—The label states the content of each electrolyte in terms of milliequivalents in a given volume. The label states the total osmolar concentration in mOsmol per L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

USP Reference standards ~~(11)~~—

USP Sodium Lactate RS

Identification—

A: It responds to the *Identification* test under *Invert Sugar Injection*.

B: It responds to the flame tests for *Sodium* ~~(191)~~ and *Potassium* ~~(191)~~, and to the tests for *Magnesium* ~~(191)~~ and *Chloride* ~~(191)~~.

C: Add 5 mL of the Injection and 1 mL of ammonium molybdate TS to a test tube, and mix: a yellow precipitate, which is soluble in 6-N ammonium hydroxide, is formed (*presence of phosphate*).

D: The retention time of the lactate peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay for lactate*.

Pyrogen—It meets the requirements of the *Pyrogen Test* ~~(151)~~. [Note—Dilute, with Water for Injection, Injection containing more than 10% of invert sugar to give a concentration of 10% invert sugar.]

pH ~~(791)~~: between 3.0 and 6.0.

Completeness of inversion—

Mobile phase, Standard preparation, and Chromatographic system— Prepare as directed in the

~~test for Completeness of inversion under Invert Sugar Injection.~~

~~Test preparation—~~ Transfer an accurately measured volume of Injection, equivalent to about 2.5 g of invert sugar, to a 100 mL volumetric flask, dilute with water to volume, and mix.

~~Procedure—~~ Proceed as directed for *Procedure* in the test for *Completeness of inversion under Invert Sugar Injection*. Calculate the quantity, in mg, of sucrose in the volume of the Injection taken by the formula:

$$100C(r_U/r_S)$$

in which the terms are as defined therein. Not more than 1.5% of the quantity of invert sugar in the volume of the Injection taken, based on the labeled amount, is found.

Other requirements—It meets the requirements under *Injections* ~~(1)~~.

Assay for potassium and sodium—

~~Internal standard solution, Potassium stock solution, Sodium stock solution, Stock standard preparation, and Standard preparation—~~ Prepare as directed in the *Assay for potassium and sodium under Potassium Chloride in Sodium Chloride Injection*.

~~Assay preparation—~~ Transfer 5.0 mL of Injection to a 500 mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

~~Procedure—~~ Proceed as directed for *Procedure* in the *Assay for potassium and sodium under Potassium Chloride in Sodium Chloride Injection*. Calculate the quantity, in mg, of potassium (K) in each mL of the Injection taken by the formula:

$$C(R_{U,766} + R_{U,671})(R_{S,671} + R_{S,766})$$

in which the terms are as defined therein. Each mg of potassium is equivalent to 0.02558 mEq of potassium. Calculate the quantity, in mg, of sodium (Na) in each mL of the Injection taken by the formula:

$$C(R_{U,589} + R_{U,671})(R_{S,671} + R_{S,589})$$

in which the terms are as defined therein. Each mg of sodium is equivalent to 0.04350 mEq of sodium.

Assay for magnesium— [Note—Concentrations of the *Standard preparations* and the *Assay preparation* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

~~Lanthanum chloride solution, Dilute hydrochloric acid, Blank solution, Magnesium stock solution, and Standard preparations—~~ Prepare as directed in the *Assay for magnesium under Multiple Electrolytes Injection Type 1*.

~~Assay preparation—~~ Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1.65 mEq) of magnesium, to a 1000 mL volumetric flask containing 50.0 mL of *Lanthanum chloride solution*. Dilute the contents of the flask with *Dilute hydrochloric acid* to volume, and mix.

~~Procedure—~~ Proceed as directed for *Procedure* in the *Assay for magnesium under Multiple Electrolytes Injection Type 1*. Calculate the quantity, in µg, of magnesium (Mg) in each mL of the Injection taken by the formula:

$$1000(C/V)$$

in which the terms are as defined therein.

Assay for chloride—Using the Injection, proceed as directed in the *Assay for chloride* under *Multiple Electrolytes Injection Type 1*.

Assay for phosphate—

Ammonium molybdate solution, Hydroquinone solution, Sodium sulfite solution, Standard preparation, and Blank— Prepare as directed in the *Assay for phosphate* under *Multiple Electrolytes Injection Type 1*.

Assay preparation— Transfer an accurately measured volume of Injection, equivalent to about 4 mg (0.126 mEq) of phosphate, to a 50-mL volumetric flask, dilute with water to volume, and mix.

Procedure— Proceed as directed for *Procedure* in the *Assay for phosphate* under *Multiple Electrolytes Injection Type 1*. Calculate the quantity, in mg, of phosphate (PO_4) in each mL of the Injection taken by the formula:

$$50(94.97 / 136.09)(C/V)(A_U / A_S)$$

in which the terms are as defined therein.

Assay for lactate—

Mobile phase, Resolution solution, Standard preparation, and Chromatographic system— Prepare as directed in the *Assay for lactate* under *Multiple Electrolytes Injection Type 2*.

Assay preparation— Where the labeled quantity of lactate is greater than 20 mEq per liter, dilute an accurately measured volume of Injection quantitatively with water to obtain a solution containing about 0.02 mEq of lactate per mL. Where the labeled quantity of lactate is 20 mEq or less per liter, use the undiluted Injection as the *Assay preparation*.

Procedure— Proceed as directed for *Procedure* in the *Assay for lactate* under *Multiple Electrolytes Injection Type 2*. Calculate the quantity, in mEq per liter, of lactate ($\text{C}_3\text{H}_5\text{O}_3$) in the Injection taken by the formula:

$$(C / 112.06)(L / D)(r_U / r_S)$$

in which the terms are as defined therein.

Assay for invert sugar—Tare a clean, medium porosity filtering crucible containing several carborundum boiling chips or glass beads. Transfer 50.0 mL of freshly mixed alkaline cupric tartrate TS to a 400-mL beaker. To the beaker add the boiling chips or glass beads from the tared crucible, 48 mL of water, and 2.0 mL of Injection that has been diluted quantitatively with water, if necessary, to a 5.0% concentration of invert sugar. Heat the beaker and contents over a burner that has been adjusted to cause boiling of the solution to start in 3.5 to 4 minutes. Boil the solution for 2.0 minutes, accurately timed, and filter immediately through the tared crucible, taking care to transfer all of the boiling chips or glass beads to the crucible. Wash the precipitate with hot water and 10 mL of alcohol. Dry the crucible and contents at 110° to constant weight, and make any necessary correction. The corrected weight of the precipitate so obtained is not less than 193.6 mg and not more than 234.5 mg, corresponding to between 90.0 and 110.0 mg of invert sugar ($\text{C}_6\text{H}_{12}\text{O}_6$). ■ 2S (USP39)

BRIEFING

Multiple Electrolytes and Invert Sugar Injection Type 2, *USP 38* page 3283. It is proposed to omit this monograph for the following reasons. No drug products formulated as defined under Multiple Electrolytes and Invert Sugar Injection Type 2 are currently marketed in the United States. The drug product is currently not used in veterinary medicine in the United States.

(SM4: M. Koleck.) Correspondence Number—C157331

Comment deadline: September 30, 2015

Delete the following:

Multiple Electrolytes and Invert Sugar Injection Type 2

» ~~Multiple Electrolytes and Invert Sugar Injection Type 2 is a sterile solution of a mixture of equal amounts of Dextrose and Fructose, or an equivalent solution produced by the hydrolysis of Sucrose, and suitable salts in Water for Injection to provide sodium, potassium, magnesium, calcium, chloride, and lactate ions. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), lactate ($C_3H_5O_3$), and invert sugar ($C_6H_{12}O_6$), and not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloride (Cl). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.~~

Packaging and storage—~~Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.~~

Labeling—~~The label states the content of each electrolyte in terms of milliequivalents in a given volume. The label states the total osmolar concentration in mOsmol per L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.~~

USP Reference standards ~~(11)~~—

USP Sodium Lactate RS

Identification—

A: ~~It responds to the *Identification* test under *Invert Sugar Injection*.~~

B: ~~It responds to the flame tests for *Sodium* (191) and *Potassium* (191), to the tests for *Magnesium* (191) and *Chloride* (191), and to the oxalate test for *Calcium* (191).~~

C: ~~The retention time of the lactate peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay for lactate*.~~

Pyrogen—~~It meets the requirements of the *Pyrogen Test* (151). [Note—Dilute, with Water for Injection, Injection containing more than 10% of invert sugar to give a concentration of~~

~~±0% invert sugar.]~~

~~pH (791):~~ between 4.5 and 6.0.

~~Completeness of inversion~~

~~Mobile phase, Standard preparation, and Chromatographic system~~— Prepare as directed in the test for *Completeness of inversion* under *Invert Sugar Injection*.

~~Test preparation~~— Transfer an accurately measured volume of Injection, equivalent to about 2.5 g of invert sugar, to a 100-mL volumetric flask, dilute with water to volume, and mix.

~~Procedure~~— Proceed as directed for *Procedure* in the test for *Completeness of inversion* under *Invert Sugar Injection*. Calculate the quantity, in mg, of sucrose in the volume of Injection taken by the formula:

$$100C(r_U/r_S)$$

in which the terms are as defined therein. Not more than 1.5% of the quantity of invert sugar in the volume of Injection taken, based on the labeled amount, is found.

~~Other requirements~~— It meets the requirements under *Injections* (1).

~~Assay for potassium and sodium~~

~~Internal standard solution, Potassium stock solution, Sodium stock solution, Stock standard preparation, and Standard preparation~~— Prepare as directed in the *Assay for potassium and sodium* under *Potassium Chloride in Sodium Chloride Injection*.

~~Assay preparation~~— Transfer 5.0 mL of Injection to a 500-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

~~Procedure~~— Proceed as directed for *Procedure* in the *Assay for potassium and sodium* under *Potassium Chloride in Sodium Chloride Injection*. Calculate the quantity, in mg, of potassium (K) in each mL of the Injection taken by the formula:

$$C(R_{U,766}/R_{U,671})(R_{S,671}/R_{S,766})$$

in which the terms are as defined therein. Each mg of potassium is equivalent to 0.02558 mEq of potassium. Calculate the quantity, in mg, of sodium (Na) in each mL of the Injection taken by the formula:

$$C(R_{U,589}/R_{U,671})(R_{S,671}/R_{S,589})$$

in which the terms are as defined therein. Each mg of sodium is equivalent to 0.04350 mEq of sodium.

~~Assay for magnesium~~— [Note—Concentrations of the *Standard preparations* and the *Assay preparation* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

~~Lanthanum chloride solution, Dilute hydrochloric acid, Blank solution, Magnesium stock solution, and Standard preparations~~— Prepare as directed in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*.

~~Assay preparation~~— Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1.65 mEq) of magnesium, to a 1000-mL volumetric flask containing 50.0 mL of

~~Lanthanum chloride solution.~~ Dilute the contents of the flask with ~~Dilute hydrochloric acid~~ to volume, and mix.

~~Procedure~~— Proceed as directed for ~~Procedure~~ in the ~~Assay for magnesium~~ under ~~Multiple Electrolytes Injection Type 1~~. Calculate the quantity, in µg, of magnesium (Mg) in each mL of the Injection taken by the formula:

$$1000(C/V)$$

in which the terms are as defined therein.

Assay for calcium— [Note—Concentrations of the ~~Standard preparations~~ and the ~~Assay preparation~~ may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

~~Lanthanum chloride solution, Dilute hydrochloric acid, and Blank solution~~— Prepare as directed in the ~~Assay for magnesium~~ under ~~Multiple Electrolytes Injection Type 1~~.

~~Calcium stock solution and Standard preparations~~— Prepare as directed in the ~~Assay for calcium~~ under ~~Multiple Electrolytes Injection Type 2~~.

~~Assay preparation~~— Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1 mEq) of calcium, to a 1000 mL volumetric flask containing 50.0 mL of ~~Lanthanum chloride solution~~. Dilute the contents of the flask with ~~Dilute hydrochloric acid~~ to volume, and mix.

~~Procedure~~— Proceed as directed for ~~Procedure~~ in the ~~Assay for calcium~~ under ~~Multiple Electrolytes Injection Type 2~~. Calculate the quantity, in µg, of calcium in each mL of the Injection taken by the formula:

$$1000(C/V)$$

in which the terms are as defined therein.

Assay for chloride— Using the Injection, proceed as directed in the ~~Assay for chloride~~ under ~~Multiple Electrolytes Injection Type 1~~.

Assay for lactate—

~~Mobile phase, Resolution solution, Standard preparation, and Chromatographic system~~— Prepare as directed in the ~~Assay for lactate~~ under ~~Multiple Electrolytes Injection Type 2~~.

~~Assay preparation~~— Where the labeled quantity of lactate is greater than 20 mEq per liter, dilute an accurately measured volume of Injection quantitatively with water to obtain a solution containing about 0.02 mEq of lactate per mL. Where the labeled quantity of lactate is 20 mEq or less per liter, use the undiluted Injection as the ~~Assay preparation~~.

~~Procedure~~— Proceed as directed for ~~Procedure~~ in the ~~Assay for lactate~~ under ~~Multiple Electrolytes Injection Type 2~~. Calculate the quantity, in mEq per liter, of lactate ($C_3H_5O_3$) in the Injection taken by the formula:

$$(C/112.06)(L/D)(r_U/r_S)$$

in which the terms are as defined therein.

Assay for invert sugar— Using the Injection, proceed as directed in the ~~Assay for invert sugar~~ under ~~Multiple Electrolytes and Invert Sugar Injection Type 1~~. ■ 2S (USP39)

BRIEFING

Multiple Electrolytes and Invert Sugar Injection Type 3, *USP 38* page 3284. It is proposed to omit this monograph for the following reasons. No drug products formulated as defined under Multiple Electrolytes and Invert Sugar Injection Type 3 are currently marketed in the United States. The drug product is currently not used in veterinary medicine in the United States.

(SM4: M. Koleck.) Correspondence Number—C157332

Comment deadline: September 30, 2015

Delete the following:

■ **Multiple Electrolytes and Invert Sugar Injection Type 3**

» ~~Multiple Electrolytes and Invert Sugar Injection Type 3 is a sterile solution of a mixture of equal amounts of Dextrose and Fructose, or an equivalent solution produced by the hydrolysis of Sucrose, and suitable salts in Water for Injection to provide sodium, potassium, chloride, and ammonium ions. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sodium (Na), potassium (K), ammonium (NH₄), and invert sugar (C₆H₁₂O₆), and not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloride (Cl). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.~~

Packaging and storage—~~Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.~~

Labeling—~~The label states the content of each electrolyte in terms of milliequivalents in a given volume. The label states the total osmolar concentration in mOsmol per L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.~~

Identification—

A: ~~It responds to the *Identification* test under *Invert Sugar Injection*.~~

B: ~~It responds to the flame tests for *Sodium* (191) and *Potassium* (191), and to the tests for *Chloride* (191) and *Ammonium* (191).~~

Pyrogen—~~It meets the requirements of the *Pyrogen Test* (151). [Note—Dilute, with Water for Injection, Injection containing more than 10% of invert sugar to give a concentration of 10% invert sugar.]~~

pH (791): ~~between 3.0 and 5.5.~~

Completeness of inversion—

~~Mobile phase, Standard preparation, and Chromatographic system— Prepare as directed in the test for Completeness of inversion under Invert Sugar Injection.~~

~~Test preparation— Transfer an accurately measured volume of Injection, equivalent to about 2.5 g of invert sugar, to a 100-mL volumetric flask, dilute with water to volume, and mix.~~

~~Procedure— Proceed as directed for Procedure in the test for Completeness of inversion under Invert Sugar Injection. Calculate the quantity, in mg, of sucrose in the volume of Injection taken by the formula:~~

$$100C(r_U/r_S)$$

~~in which the terms are as defined therein. Not more than 1.5% of the quantity of invert sugar in the volume of Injection taken, based on the labeled amount, is found.~~

~~**Other requirements**—It meets the requirements under Injections ~~(1)~~.~~

Assay for potassium and sodium—

~~Internal standard solution, Potassium stock solution, Sodium stock solution, Stock standard preparation, and Standard preparation— Prepare as directed in the Assay for potassium and sodium under Potassium Chloride in Sodium Chloride Injection.~~

~~Assay preparation— Transfer 5.0 mL of Injection to a 500-mL volumetric flask, dilute with Internal standard solution to volume, and mix.~~

~~Procedure— Proceed as directed for Procedure in the Assay for potassium and sodium under Potassium Chloride in Sodium Chloride Injection. Calculate the quantity, in mg, of potassium (K) in each mL of the Injection taken by the formula:~~

$$C(R_{U,766}/R_{U,671})(R_{S,671}/R_{S,766})$$

~~in which the terms are as defined therein. Each mg of potassium is equivalent to 0.02558 mEq of potassium. Calculate the quantity, in mg, of sodium (Na) in each mL of the Injection taken by the formula:~~

$$C(R_{U,589}/R_{U,671})(R_{S,671}/R_{S,589})$$

~~in which the terms are as defined therein. Each mg of sodium is equivalent to 0.04350 mEq of sodium.~~

~~**Assay for chloride**—Using the Injection, proceed as directed in the Assay for chloride under Multiple Electrolytes Injection Type 1.~~

~~**Assay for ammonium**—Using the Injection, proceed as directed in the Assay for ammonium under Multiple Electrolytes and Dextrose Injection Type 3.~~

~~**Assay for invert sugar**—Using the Injection, proceed as directed in the Assay for invert sugar under Multiple Electrolytes and Invert Sugar Injection Type 1. ■ 2S (USP39)~~

BRIEFING

Febantel. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on the *European Pharmacopoeia* monograph, is proposed. The HPLC procedure in the Assay and the test for *Organic Impurities* is based on analyses performed with the Nova-Pak C-18 or LiChrospher 100 RP-18 brands of L1 column. The typical retention

time for febantel is about 32 min.

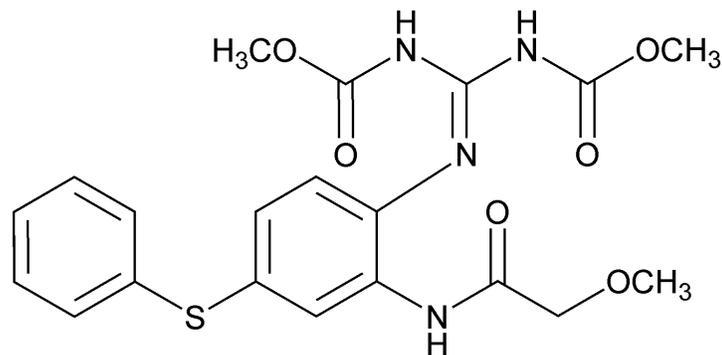
(SM3: M. Puderbaugh.)

Correspondence Number—C132963

Comment deadline: September 30, 2015

Add the following:

■ **Febantel**



C₂₀H₂₂N₄O₆S 446.48

Carbamic acid, [[2-[(methoxyacetyl)amino]-4-(phenylthio)phenyl]carbonimidoyl]bis-, dimethyl ester;

Dimethyl {[2-(2-methoxyacetamido)-4-(phenylthio)phenyl]imidocarbonyl}dicarbamate [58306-30-2].

DEFINITION

Febantel contains NLT 97.5% and NMT 102.0% of febantel (C₂₀H₂₂N₄O₆S), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K): If a difference appears in the IR spectra of the analyte and the standard, dissolve equal portions of the test specimen and the Reference Standard in equal volumes of acetone and evaporate the solutions to dryness. Repeat the test on the residues.
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Buffer: 6.8 g/L of monobasic potassium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (35:65)

Diluent: Acetonitrile and tetrahydrofuran (50:50)

Standard solution: 0.5 mg/mL of USP Febantel RS in *Diluent*

Sample solution: 0.5 mg/mL of Febantel in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 15-cm; 5-μm packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of febantel (C₂₀H₂₂N₄O₆S) in the portion of Febantel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Febantel RS in the *Standard solution* (mg/mL)

C_U = concentration of Febantel in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%
- **Organic Impurities**

Buffer, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 0.01 mg/mL each of USP Febantel Related Compound A RS, USP Febantel Related Compound B RS, and USP Fenbendazole RS in *Diluent*

Standard solution: 0.01 mg/mL of USP Febantel RS in *Diluent*

Sample solution: 10.0 mg/mL of Febantel in *Diluent*

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 3.0 between febantel related compound A and febantel related compound B; NLT 4.0 between febantel related compound B and fenbendazole

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Febantel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of febantel from the *Standard solution*

C_S = concentration of USP Febantel RS in the *Standard solution* (mg/mL)

$C_{\bar{F}}$ concentration of Febantel in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. The reporting level for impurities is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Febantel related compound A	0.19	0.1
Febantel related compound B	0.29	0.1
Fenbendazole	0.39	0.1
Febantel	1.00	—
Any other individual impurity	—	0.20
Total impurities	—	0.5

SPECIFIC TESTS

- **Loss on Drying** 〈 731 〉

Sample: 1.0 g

Analysis: Dry the *Sample* at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at room temperature.
- **Labeling:** Label it to indicate that it is for veterinary use only.
- **USP Reference Standards** 〈 11 〉

USP Febantel RS

USP Febantel Related Compound A RS

Methyl [[2-[(methoxyacetyl)amino]-4-(phenylthio)phenyl]carbamimidoyl]carbamate.

C₁₈H₂₀N₄O₄S 388.44

USP Febantel Related Compound B RS

2-(Methoxymethyl)-5-(phenylthio)-1*H*-benzimidazole.

C₁₅H₁₄N₂OS 270.35

USP Fenbendazole RS

Methyl 5-(phenylthio)-2-benzimidazolecarbamate.

C₁₅H₁₃N₃O₂S 299.35

■ 2S (USP39)

BRIEFING

Iopanoic Acid, *USP 38* page 3916. It is proposed to omit this monograph for the following reasons.

This drug substance is not used in any dosage form for human use in the United States.
This drug substance is currently not used in veterinary medicine in the United States.

(SM4: R. Ravichandran.) Correspondence Number—C135175

Comment deadline: September 30, 2015

Delete the following:

■ **Iopanoic Acid**

$C_{11}H_{12}I_3NO_2$ 570.93

Benzenepropanoic acid, 3-amino-~~α~~-ethyl-2,4,6-triiodo-, (±)-.
(±)-3-Amino-~~α~~-ethyl-2,4,6-triiodohydrocinnamic acid [[96-83-3]].

» Iopanoic Acid contains an amount of iodine equivalent to not less than 97.0 percent and not more than 101.0 percent of $C_{11}H_{12}I_3NO_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

Identification—Mix about 100 mg with 500 mg of sodium carbonate in a crucible, and heat until thoroughly charred. Cool, add 5 mL of hot water, heat on a steam bath for 5 minutes, and filter: the solution responds to the tests for *Iodide* ~~(191)~~.

Melting range ~~(741)~~: between 152° and 158°, with decomposition.

Loss on drying ~~(731)~~—Dry it at 105° for 1 hour: it loses not more than 1.0% of its weight.

Residue on ignition ~~(281)~~: not more than 0.1%.

Free iodine—Shake about 200 mg with 2 mL of water and 2 mL of chloroform for 1 minute: the chloroform layer shows no violet color.

Halide ions—Place about 500 mg in a glass-stoppered, 50-mL cylinder, add 10 mL of 2-N nitric acid and 15 mL of water, shake for 5 minutes, and filter through paper: 10 mL of the filtrate shows no greater turbidity than corresponds to 0.05 mL of 0.020-N hydrochloric acid (see *Chloride and Sulfate* ~~(221)~~).

Delete the following:

• **Heavy metals, Method II** ~~(231)~~: 0.002%. • (Official 1-Dec-2015)

Assay—

Transfer about 250 mg of Iopanoic Acid, accurately weighed, to a glass-stoppered, 250-mL conical flask. Add 30 mL of 1.25-N sodium hydroxide and 500 mg of powdered zinc, and reflux the mixture for 30 minutes. Cool to room temperature, wash the condenser with 20 mL of water, and filter the mixture. Wash the flask and the filter with small portions of water, adding the washings to the filtrate. Add to the filtrate 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05-N silver nitrate VS until the color of the yellow precipitate just changes to green. Each mL of 0.05-N silver nitrate is equivalent to 9.516 mg of $C_{11}H_{12}I_3NO_2$. ■ 2S (USP39)

Iopanoic Acid Tablets, *USP 38* page 3916. It is proposed to omit this monograph for the following reasons.

This dosage form is not used for human use in the United States.

This dosage form is currently not used in veterinary medicine in the United States.

(SM4: R. Ravichandran.) Correspondence Number—C135176

Comment deadline: September 30, 2015

Delete the following:

■ Iopanoic Acid Tablets

» ~~Iopanoic Acid Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{11}H_{12}I_3NO_2$.~~

Packaging and storage—~~Preserve in tight, light-resistant containers.~~

Identification—~~Triturate a quantity of finely powdered Tablets, equivalent to about 1 g of iopanoic acid, with two 10-mL portions of solvent hexane, and decant and discard the liquid. Allow the residue to dry spontaneously, triturate with 15 mL of acetone, and filter. Repeat the trituration with another 15-mL portion of acetone, evaporate the combined filtrates on a steam bath to a volume of not more than 1 mL, add, with constant stirring, 20 mL of water, filter, wash the precipitate with two 5-mL portions of water, and dry at 105° for 2 hours: the iopanoic acid so obtained melts between 150° and 158°, with decomposition, and responds to the *Identification* test under *Iopanoic Acid*.~~

Disintegration ~~(701)~~: 30 minutes.

Uniformity of dosage units ~~(905)~~: meet the requirements.

Halide ions—~~A portion of the powdered Tablets prepared for the *Assay*, equivalent to about 500 mg of iopanoic acid, meets the requirements of the test for *Halide ions* under *Iopanoic Acid*.~~

Assay—~~Weigh and finely powder not fewer than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 1 g of iopanoic acid, and triturate with 10 mL of solvent hexane. Allow the mixture to settle, decant the hexane through a small filter, repeat the trituration with 10 mL of solvent hexane, filter through the same filter, and discard the filtrates. Warm the residue with 10 mL of neutralized alcohol at 70°, filter through the same filter, and wash the undissolved residue with four 10-mL portions of neutralized alcohol at 70°, passing the washings through the same filter. Cool the combined filtrate and washings to room temperature, add 3 to 5 drops of thymol blue TS, and titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 57.09 mg of $C_{11}H_{12}I_3NO_2$.~~ ■ 2S (*USP39*)

BRIEFING

Iodate Sodium, *USP 38* page 3932. It is proposed to omit this monograph for the following reasons. This drug substance is not used in any dosage form for human use in the United States. This drug substance is currently not used in veterinary medicine in the United States.

(SM4: R. Ravichandran.) Correspondence Number—C157587

*Comment deadline: September 30, 2015***Delete the following:****■ Ipodate Sodium**

$C_{12}H_{12}I_3N_2NaO_2$	619.94	<input type="text"/>
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Benzenepropanoic acid, 3-[[[(dimethylamino)methylene]amino]-2,4,6-triiodo-], sodium salt.
Sodium 3-[[[(dimethylamino)methylene]amino]-2,4,6-triiodohydrocinnamate [[1221-56-3]].

» Ipodate Sodium contains not less than 97.5 percent and not more than 102.5 percent of $C_{12}H_{12}I_3N_2NaO_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ~~(11)~~—

USP Ipodate Sodium RS

Identification—**A:** *Infrared Absorption* ~~(197K)~~—**B:** *Ultraviolet Absorption* ~~(197U)~~—*Solution:* 10 µg per mL.*Medium:* methanol.

Absorptivities at 235 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: Heat about 500 mg in a porcelain crucible over a free flame: violet vapors of iodine are evolved.**D:** It responds to the flame test for *Sodium* ~~(191)~~—**Loss on drying** ~~(731)~~—Dry it in vacuum at 60° for 3 hours: it loses not more than 0.5% of its weight.

Iodide or iodine—Dissolve about 200 mg in 10 mL of 6 N acetic acid, add 2 mL of 1 N sulfuric acid and 15 mL of chloroform, and shake vigorously. Allow the layers to separate: the chloroform layer shows not more than a faint violet color. Add 1 mL of 0.1 N potassium iodate, shake vigorously, and allow the layers to separate: the chloroform layer shows at most a slight trace of violet color.

Delete the following:

• **Heavy metals, Method II** ~~(231)~~: 0.003%. • (Official 1-Dec-2015)

Assay—

Transfer about 300 mg of Ipodate Sodium, accurately weighed, to a 250 mL flask, add 30 mL of

~~1.25 N sodium hydroxide and 0.5 g of powdered zinc, and reflux the mixture for 60 minutes. Cool, wash the condenser with 20 mL of water, and filter the mixture. Wash the flask and the filter with small portions of water, adding the washings to the filtrate. Add to the filtrate 5 mL of glacial acetic acid and 3 mL of a mixture of 2 drops of nitric acid in 5 mL of water, then add 3 drops of eosin Y TS, and titrate with 0.05 N silver nitrate VS until the entire mixture changes to a permanent pink color. Each mL of 0.05 N silver nitrate is equivalent to 10.33 mg of $C_{12}H_{12}I_3N_2NaO_2 \cdot 2S$ (USP39)~~

BRIEFING

Ipodate Sodium Capsules, USP 38 page 3932. It is proposed to omit this monograph for the following reasons. This dosage form is not for human use in the United States. This dosage form is currently not used in veterinary medicine in the United States.

(SM4: R. Ravichandran.) Correspondence Number—C157588

Comment deadline: September 30, 2015

Delete the following:

■ Ipodate Sodium Capsules

~~» Ipodate Sodium Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{12}H_{12}I_3N_2NaO_2$.~~

~~**Packaging and storage**—Preserve in tight containers.~~

~~**Identification**~~

~~**A:** Transfer a portion of the contents of Capsules, equivalent to about 2 g of ipodate sodium, to a 250 mL separator, add 100 mL of water and 50 mL of solvent hexane, and shake. Transfer the aqueous layer to a beaker, add 5 mL of 3 N hydrochloric acid, and mix. Filter (retain the filtrate), and wash the precipitate with several portions of water. Dry the precipitate in vacuum at 60° for 4 hours. A 1 in 100,000 solution of the residue so obtained, in a 1 in 100 mixture of 2 N hydrochloric acid in methanol, exhibits an UV absorbance maximum at 242 ± 2 nm.~~

~~**B:** The residue obtained in *Identification* test A responds to *Identification* test C under *Ipodate Sodium*.~~

~~**C:** The filtrate obtained in *Identification* test A responds to the flame test for *Sodium* (191).~~

~~**Uniformity of dosage units** (905): meet the requirements.~~

~~**Assay**—Place a number of Capsules, equivalent to about 5 g of ipodate sodium, in a 400 mL beaker, add 200 mL of 1 N sodium hydroxide and 50 mL of solvent hexane, and stir by mechanical means until the capsules have completely disintegrated. Transfer the mixture to a 500 mL separator, wash the beaker with a total of 25 mL of 1 N sodium hydroxide in divided portions, and add the washings to the separator. Allow the layers to separate, and transfer the aqueous layer to a 500 mL volumetric flask. Wash the solvent hexane layer with two 50 mL portions of 1 N sodium hydroxide, add the washings to the volumetric flask, dilute with 1 N sodium hydroxide to volume, and mix. Pipet 25 mL of the solution, which may be milky in appearance, into a 250 mL conical flask, add 500 mg of powdered zinc, and proceed as~~

directed in the ~~Assay under Ipodate Sodium~~, beginning with “and reflux the mixture for 60 minutes.” ■ 2S (USP39)

BRIEFING

Lisinopril, USP 38 page 4111. As a part of USP monograph modernization efforts, the following changes are proposed:

- A new stability-indicating test for *Organic Impurities* is added. The liquid chromatographic procedure is validated using the Lichrosorb RP 8 brand of L7 column. Alternatively, the Zorbax C8 brand of L7 column could be used. The typical retention time for lisinopril is about 8.5 min under the specified conditions.
- The system suitability requirement for the *Relative standard deviation* in the *Assay* is revised to be consistent with *Chromatography* (621), *System Suitability*.
- The system suitability requirement for the *Column efficiency* in the *Assay* is deleted as the other suitability requirements can adequately establish the suitability of the system.

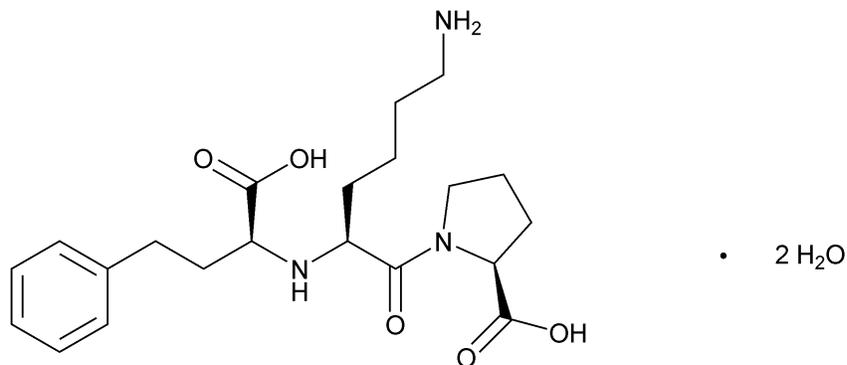
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM2: S. Ramakrishna.)

Correspondence Number—C143450

Comment deadline: September 30, 2015

Lisinopril



C₂₁H₃₁N₃O₅·2H₂O 441.52

L-Proline, 1-[N²-(1-carboxy-3-phenylpropyl)-L-lysyl]-, dihydrate, (S)-;

1-[N²-[(S)-1-Carboxy-3-phenylpropyl]-L-lysyl]-L-proline dihydrate [83915-83-7].

DEFINITION

Lisinopril contains NLT 98.0% and NMT 102.0% of lisinopril (C₂₁H₃₁N₃O₅), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** (197M)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the

Standard solution, as obtained in the Assay.

ASSAY

Change to read:

● Procedure

Solution A: 2.76 g/L of monobasic sodium phosphate in water prepared as follows. Dissolve 2.76 g of monobasic sodium phosphate in about 900 mL of water in a 1000-mL volumetric flask. Adjust with 1 N sodium hydroxide to a pH of 5.0 and dilute with water to volume.

Mobile phase: Acetonitrile and *Solution A* (4:96)

Standard solution: 0.3 mg/mL of USP Lisinopril RS in water

Sample solution: 0.3 mg/mL of Lisinopril in water

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: ~~NLT 180 theoretical plates~~

■ 2S (USP39)

Tailing factor: NMT 1.7

Relative standard deviation: NMT ~~±.0%~~

■ 0.73% ■ 2S (USP39)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lisinopril (C₂₁H₃₁N₃O₅) in the portion of Lisinopril taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lisinopril from the *Sample solution*

r_S = peak response of lisinopril from the *Standard solution*

C_S = concentration of USP Lisinopril RS in the *Standard solution* (mg/mL)

C_U = concentration of Lisinopril in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

● **Residue on Ignition** 〈 281 〉: NMT 0.1%

~~Delete the following:~~

~~Delete the following.~~

●● **Heavy Metals, Method II** { 231 } : 0.001% ● (Official 1-Dec-2015)

Add the following:

●● **Organic Impurities**

Buffer: 3.53 g/L of monobasic sodium phosphate dihydrate in water adjusted with phosphoric acid to a pH of 4.1

Solution A: Acetonitrile and *Buffer* (7:193)

Solution B: Acetonitrile and *Buffer* (20:80)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	60	40
55	60	40
60	100	0

Standard solution: 0.006 mg/mL of USP Lisinopril RS in *Solution A*

Sensitivity solution 1.0 µg/mL of USP Lisinopril RS in *Solution A* from *Standard solution*

Sample solution: 2 mg/mL of Lisinopril in *Solution A*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 45°

Flow rate: 1.8 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Relative standard deviation: NMT 10.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Lisinopril taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_S peak response of lisinopril from the *Standard solution*

C_S concentration of USP Lisinopril RS in the *Standard solution* (mg/mL)

C_U concentration of Lisinopril in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See Table 2. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
<i>N</i> -Alkyl- <i>l</i> -lysine ^a	0.57	0.35	0.15
<i>DL</i> -Homophenylalanine ^b	0.72	1.08	0.15
Lisinopril	1.00	1.00	—
Lisinopril epimer ^c	1.33	0.76	0.30
Lisinopril cyclohexyl analog ^d	2.93	0.39	0.15
<i>R,S,S</i> -Diketopiperazine ^e	3.88	0.79	0.15
<i>S,S,S</i> -Diketopiperazine (lisinopril related compound A) ^f	4.04	0.76	0.15
<i>N</i> -Alkyl lisinopril ^g	4.60	0.86	0.15
Any individual unspecified impurity	—	1.00	0.10
Total impurities	—	—	0.50

^a [(*S*)-1-Carboxy-3-phenylpropyl]-*l*-lysine.

^b 2-Amino-4-phenylbutanoic acid.

^c [(*R*)-1-Carboxy-3-phenylpropyl]-*l*-lysyl-*l*-proline.

^d [(*S*)-1-Carboxy-3-cyclohexylpropyl]-*l*-lysyl-*l*-proline.

^e (*S*)-2-[(*3S,8aR*)-3-(4-Aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-*a*]pyrazin-2(*1H*)-yl]-4-phenylbutanoic acid.

^f (*S*)-2-[(*3S,8aS*)-3-(4-Aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-*a*]pyrazin-2(*1H*)-yl]-4-phenylbutanoic acid.

^g *N*²,*N*⁶-Bis[(*S*)-1-Carboxy-3-phenylpropyl]-*l*-lysyl-*l*-proline.

■ 2S (USP39)

SPECIFIC TESTS

• Optical Rotation $\langle 781S \rangle$, Specific Rotation

Diluent: 0.25 M zinc acetate solution prepared as follows. Mix 600 mL of water with 150 mL of glacial acetic acid and 54.9 g of zinc acetate, and stir to dissolve the zinc acetate. While stirring, add 150 mL of ammonium hydroxide, cool to room temperature, and adjust with ammonium hydroxide to a pH of 6.4. Transfer the solution to a 1000-mL volumetric flask, and dilute with water to volume.

Sample solution: 10 mg/mL of Lisinopril in *Diluent*

Acceptance criteria: -115.3° to -122.5° ($\lambda = 405 \text{ nm}$)

• Water Determination $\langle 921 \rangle$, Method I: 8.0%–9.5%

ADDITIONAL REQUIREMENTS

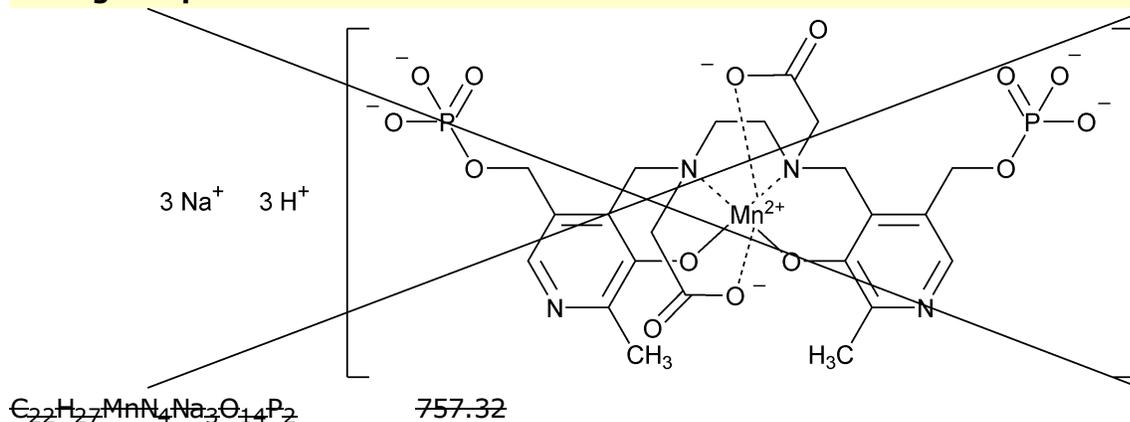
- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** (11)

USP Lisinopril RS **BRIEFING**

Mangafodipir Trisodium, USP 38 page 4195. It is proposed to omit this monograph for the following reasons:

- This drug substance is not used in any dosage form for human use in the United States.
- This drug substance is currently not used in veterinary medicine in the United States.

(SM4: R. Ravichandran.) Correspondence Number—C135177

Comment deadline: September 30, 2015**Delete the following:****■ Mangafodipir Trisodium**

Trisodium trihydrogen (OC-6-13) [[N,N'-1,2-ethanediylbis[N-[[3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinyl]methyl]glycinato]](8-)] manganate(6-).

Trisodium trihydrogen (OC-6-13) [[N,N'-ethylenebis[N-[[3-hydroxy-5-(hydroxymethyl)-2-methyl-4-pyridyl]methyl]glycine]-5,5'-bis(phosphato)](8-)] manganate(6-) [[140678-14-4]].

» Mangafodipir Trisodium contains not less than 97.0 percent and not more than 103.0 percent of $\text{C}_{22}\text{H}_{27}\text{MnN}_4\text{Na}_3\text{O}_{14}\text{P}_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers. Store in a cold place.

USP Reference standards (11)—

USP Endotoxin RS

USP Mangafodipir Related Compound A RS

Manganese(II) dipyridoxal monophosphate sodium salt.

USP Mangafodipir Related Compound B RS

~~Manganese(II) dipyridoxal diphosphate mono-overalkylated sodium salt.
USP Mangafodipir Related Compound C RS
Manganese(II) dipyridoxal diphosphate sodium salt.
USP Mangafodipir Trisodium RS
Manganese(II) dipyridoxal diphosphate.~~

Identification—

A: ~~Infrared Absorption (197K):~~

B: It meets the requirements of the tests for ~~Sodium (191)~~ and ~~Manganese (191)~~.

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—The total aerobic microbial count is not more than 500 cfu per g.

Bacterial endotoxins (85): not more than 0.13 USP Endotoxin Unit per mg.

pH (791): between 5.5 and 7.0, in a solution (1 in 100).

Water, Method I (921): not more than 20%.

Limit of residual solvents—

Internal standard solution— Transfer 600 μL of methyl ethyl ketone to a 100-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a concentration of about 5 mg per mL. Transfer 2 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.1 mg per mL.

Standard stock solution— Transfer about 1 g of dehydrated alcohol and 1 g of acetone, both accurately weighed, to a 100-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a known concentration of about 1 mg each of alcohol and acetone per mL.

Standard solutions— Transfer 10.0 mL of *Internal standard solution* to each of four 100-mL volumetric flasks. Separately add 0 mL, 1.0 mL, 5.0 mL, and 10.0 mL of *Standard stock solution* to the volumetric flasks, and dilute each with water to volume to obtain solutions having known concentrations of 0.0 μg per mL and about 10 μg per mL, 50 μg per mL, and 100 μg per mL each of alcohol and acetone, respectively. Add 7.0 mL of each *Standard solution* to separate headspace sample vials, and cap.

Test solution— Transfer about 1 g of Mangafodipir Trisodium, accurately weighed, to a sample vial, add 7.0 mL of the *Standard solution* having a concentration of 0.0 μg per mL, cap, and swirl to dissolve.

Chromatographic system (see Chromatography (621))— The gas chromatograph is equipped with a flame ionization detector, a 0.32-mm \times 30-m fused silica column coated with 1.8- μm G43 stationary phase. The carrier gas is helium, flowing at a rate of 1.5 mL per minute. The temperatures of the injection port and the oven are maintained at 150 $^{\circ}$ and 50 $^{\circ}$, respectively. The bath temperature for the headspace sample vials is maintained at 90 $^{\circ}$, the valve/loop temperature is maintained at 130 $^{\circ}$, and the sample thermostating time is 15 minutes.

Chromatograph the *Standard solutions*, and record the peak responses as directed for *Procedure*: the resolution, R , between alcohol and acetone is not less than 5; and the relative standard deviation for replicate injections of the *Standard solution* having a concentration of 100 µg per mL, determined from the peak response ratios of the analyte to the internal standard, is not more than 2.0%. Calculate the peak response ratios of the analyte to the internal standard, and plot the results. Determine the linear regression equation of the standards by the mean square method, and record the linear regression equation and the correlation coefficient. A suitable system is one that yields a line having a correlation coefficient of not less than 0.990.

Procedure— Separately inject equal volumes (about 1 mL) of the gaseous headspace of each of the *Standard solutions* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentages (w/w) of alcohol and acetone in the portion of Mangafodipir-Trisodium taken by the formula:

$$(7/10,000)(C/W)$$

in which C is the concentration, in µg per mL, of alcohol or acetone in the *Test solution*, as determined from the relevant standard response line; and W is the weight, in g, of Mangafodipir-Trisodium taken: not more than 0.1% of alcohol is found; and not more than 0.01% of acetone is found, both calculated on the anhydrous basis.

Limit of free manganese and free fodipir—

Ascorbic acid solution— Dissolve 0.5 g of ascorbic acid in 10 mL of water.

Manganese solution— Transfer about 3.6 g of manganese chloride, accurately weighed, to a 1000 mL volumetric flask, dissolve in and dilute with 0.1 N hydrochloric acid to volume, and mix. Transfer 100.0 mL of this solution to a 500 mL volumetric flask, dilute with water to volume, and mix.

Edetate titrant solution— Transfer about 37 g of edetate disodium, accurately weighed, to a 1000 mL volumetric flask, dilute with water to volume, and mix. Transfer 36 mL of this solution to a 1000 mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a concentration of 0.0036 moles per L.

standardization of 0.0036 M edetate titrant solution— Accurately weigh about 200 mg of chelometric standard calcium carbonate, previously dried at 110 ° for 2 hours and cooled in a desiccator, transfer to a 100 mL volumetric flask, and add 10 mL of water and about 4 mL of diluted hydrochloric acid. Swirl the flask to dissolve, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a beaker while stirring, preferably with a magnetic stirrer; and add about 15 mL of sodium hydroxide TS and enough hydroxynaphthol blue indicator to achieve a percent transmission of about 95%, using a suitable autotitrator at a wavelength of 620 nm, calibrated to 100% transmission with water. Add 20.0 mL of *Edetate titrant solution*, and continue to titrate until 3 mL of titrant have been added beyond the sharp break point, as determined from the titration curve obtained by plotting relative transmittance versus volume, in mL, of titrant added. Determine the endpoint volume from the titration curve. The final titration volume is the sum of the endpoint volume and the 20.0 mL of *Edetate titrant solution* initially added. Calculate the molarity of the *Edetate titrant solution* by the formula:

$$(5/100.09)(W)/(100V)$$

in which 100.09 is the molecular weight of calcium carbonate; W is the weight, in mg, of the

calcium carbonate taken; and V is the final titration volume, in mL, of *Edetate titrant solution*.

Procedure— Transfer about 1 g of Mangafodipir Trisodium, accurately weighed, to a suitable beaker, add about 100 mL of water, 1.0 mL of *Ascorbic acid solution*, 10 mL of ammonia–ammonium chloride buffer TS, 0.1 mL of eriochrome black TS, and 1.0 mL of *Manganese solution*, and record the color. If the color is yellow to green, add additional 1.0 mL increments of *Manganese solution* until the color is red. Record the volume added. Titrate with the *Edetate titrant solution*, determining the endpoint photometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Calculate the percentage of free manganese in the portion of Mangafodipir Trisodium taken by the formula:

$$5.49V(M/W)$$

in which V is the volume, in mL, of the *Edetate titrant solution*; M is the molarity of the *Edetate titrant solution*; and W is the weight, in g, of Mangafodipir Trisodium taken. Calculate the percentage of free fodipir in the portion of Mangafodipir Trisodium taken by the formula:

$$63.85V(M/W)$$

in which V , M , and W are as defined herein: not more than 0.03% of free manganese is found; and not more than 0.5% of free fodipir is found, both calculated on the anhydrous basis.

Related compounds—

Ascorbic acid solution— Dissolve 0.4 g of ascorbic acid in 100 mL of water.

Phosphate buffer— Prepare as directed in the *Assay*.

Mobile phase— Prepare as directed in the *Assay*. [Note—Increasing the proportion of acetonitrile will decrease the retention times.]

System suitability stock solution— Prepare as directed for *Standard stock preparation* in the *Assay*.

System suitability solution 1— Prepare a solution of USP Mangafodipir Trisodium RS having a known concentration of about 4.0 mg per mL. Transfer 5.0 mL of this solution to a 50 mL volumetric flask, add 5.0 mL of *System suitability stock solution*, 5.0 mL of *Phosphate buffer*, and 5.0 mL of *Ascorbic acid solution*. Dilute with nitrogen-purged water to volume, and mix to obtain a solution having a concentration of about 0.4 mg of USP Mangafodipir Trisodium RS, and about 0.01 mg each of USP Mangafodipir Related Compound A RS and USP Mangafodipir Related Compound B RS per mL. [Note—Store in a refrigerator and under nitrogen to avoid excessive exposure to heat, air, and light.]

System suitability solution 2— Transfer about 10 mg of USP Mangafodipir Related Compound C RS to a 100 mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50 mL volumetric flask, and add 5.0 mL of *Phosphate buffer*.

Test solution— Transfer an accurately weighed quantity of Mangafodipir Trisodium, equivalent to about 100 mg of mangafodipir trisodium, to a 50 mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 50 mL volumetric flask, add 5.0 mL of *Phosphate buffer*, dilute with water to volume, and mix. [Note—Store in a refrigerator and under nitrogen to avoid excessive exposure to heat, air, and light.]

Chromatographic system (see *Chromatography* (621))— Prepare as directed in the *Assay*. Chromatograph *System suitability solution 2*, and record the peak responses as directed for

Procedure: note the elution time to identify the mangafodipir related compound C peak, if present, in the chromatogram of *System suitability solution 1*. Chromatograph *System suitability solution 1*, and record the peak responses as directed for *Procedure*: the retention time for mangafodipir is between 18 and 30 minutes. The peak area for mangafodipir related compound C is less than 0.1%. [Note—If the peak area is more than 0.1% of the total of all peak areas, prepare fresh quantities of *Ascorbic acid solution* and *System suitability solution 1*, and repeat the test. If the peak area of mangafodipir related compound C is still greater than 0.1%, repeat the test using another column. A contaminated column can result in oxidation of Mn(II) to Mn(III), forming related compound C.] The tailing factor for the mangafodipir peak is not more than 2.3; the column efficiency is not less than 1000 theoretical plates; the resolution, R_s , between mangafodipir related compound B and mangafodipir is not less than 1.5; and the relative standard deviation for replicate injections is not more than 10% for each peak. [Note—If the resolution is less than 1.5, adjust the *Mobile phase* by increasing the concentration of tetrabutylammonium hydrogen sulfate.]

Procedure— Inject about 10 μL of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for all the major peaks. The relative retention times for ascorbic acid, mangafodipir related compound A, Mn(II) 5-methyl dipyridoxal monophosphate (Mn(II) 5-methyl DPMP) if present, mangafodipir related compound C, mangafodipir related compound B, and mangafodipir are about 0.1, 0.3, 0.4, 0.6, 0.8, and 1.0, respectively. Calculate the percentages of mangafodipir related compound A, mangafodipir related compound B, mangafodipir related compound C, and Mn(II) 5-methyl DPMP in the portion of Mangafodipir Trisodium taken by the formula:

$$100(r_f/r_s)$$

in which r_f is the peak area of each impurity; and r_s is the sum of the areas of all of the peaks: not more than 0.5% each of mangafodipir related compound A and mangafodipir related compound B is found; not more than 0.6% of mangafodipir related compound C is found; not more than 0.3% of Mn(II) 5-methyl DPMP is found; not more than 0.3% of any other impurity is found; not more than a total of 0.5% of other impurities is found; and not more than a total of 2.0% of impurities is found.

Assay—

Phosphate buffer— Transfer about 26.8 g of dibasic sodium phosphate to a 1000-mL volumetric flask, add 900 mL of water, and adjust with 1 N sodium hydroxide or 1 N hydrochloric acid to a pH of about 8.0. Dilute with water to volume, filter, and degas.

Mobile phase— Transfer about 0.61 g of boric acid and 9.2 g of tetrabutylammonium hydrogen sulfate to a 1000-mL volumetric flask, add 640 mL water, and mix. Adjust with 3 N sodium hydroxide to a pH of about 9.3, add 250 mL of acetonitrile, dilute with water to volume, and mix. Adjust with 3 N hydrochloric acid or 3 N sodium hydroxide to a pH of about 10.5, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard stock preparation— Transfer about 10 mg each of USP Mangafodipir Related Compound A RS and USP Mangafodipir Related Compound B RS, both accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix.

Standard preparation— Transfer about 100 mg of USP Mangafodipir Trisodium RS to a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a

50-mL volumetric flask, add 5.0 mL of *Standard stock preparation* and 5.0 mL of *Phosphate buffer*, dilute with water to volume, and mix. [Note—Store in a refrigerator and under nitrogen to avoid exposure to excessive heat, air, or light.]

Assay preparation— Transfer an accurately measured quantity of Mangafodipir Trisodium, equivalent to about 100 mg of mangafodipir trisodium, to a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of *Phosphate buffer*, dilute with water to volume, and mix. [Note—Store in a refrigerator and under nitrogen to avoid exposure to excessive heat, air, or light.]

Chromatographic system (see *Chromatography* ~~(621)~~)— The liquid chromatograph is equipped with a 310-nm detector and a 4.6-mm × 15-cm column that contains 5- μ m packing L21. The chromatograph is maintained at about 20°. The flow rate is 0.8 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, R_s , between mangafodipir related compound A and mangafodipir related compound B is not less than 1.5; the column efficiency is not less than 1000 theoretical plates; and the tailing factor is not more than 2.3.

Procedure— Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{22}H_{27}MnN_4Na_3O_{14}P_2$ in the portion of Mangafodipir Trisodium taken by the formula:

$$25,000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Mangafodipir Trisodium RS in the *Standard preparation*; W is the weight, in mg, of the Mangafodipir Trisodium taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. ■ 2S (USP39)

BRIEFING

Mangafodipir Trisodium Injection, USP 38 page 4197. It is proposed to omit this monograph for the following reasons:

- This dosage form is not for human use in the United States.
- This dosage form is currently not used in veterinary medicine in the United States.

(SM4: R. Ravichandran.) Correspondence Number—C135178

Comment deadline: September 30, 2015

Delete the following:

■ **Mangafodipir Trisodium Injection**

» Mangafodipir Trisodium Injection is a sterile solution of Mangafodipir Trisodium in Water for Injection. It contains not less than 94.0 percent and not more than 106.0 percent of the labeled amount of mangafodipir trisodium ($C_{22}H_{27}MnN_4Na_3O_{14}P_2$). It may contain stabilizers and buffers. It contains no antimicrobial agents.

Repackaging and storage. Preserve in single-dose containers of Type I glass. Store at

~~**Packaging and storage**—Preserve in single-dose containers of Type I glass. Store at controlled room temperature, with containers on their sides in the original carton.~~

~~**USP Reference standards**—~~

- ~~USP Endotoxin RS~~
- ~~USP Mangafodipir Trisodium RS~~
- ~~Manganese(II) dipyridoxal diphosphate.~~

~~**Identification**—~~

~~**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.~~

~~**B:** It meets the requirements of the tests for *Manganese*.~~

~~**Bacterial endotoxins:** not more than 0.66 USP Endotoxin Unit per mg of mangafodipir trisodium.~~

~~**pH:** between 8.4 and 9.2.~~

~~**Osmolarity:** between 244 and 330 mOsmol per kg of water.~~

~~**Other requirements**—It meets the requirements under *Injections*.~~

~~**Assay**—~~

~~*Phosphate buffer* and *Mobile phase*— Proceed as directed in the *Assay* under *Mangafodipir Trisodium*.~~

~~*Standard preparation*— Prepare a solution of USP Mangafodipir Trisodium RS in water having a known concentration of about 2 mg per mL. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of *Phosphate buffer*, dilute with water to volume, and mix. [Note—Store under nitrogen to avoid excessive exposure to air and light.]~~

~~*Assay preparation*— Transfer an accurately measured volume of *Injection*, equivalent to about 100 mg of mangafodipir trisodium, to a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of *Phosphate buffer*, dilute with water to volume, and mix. [Note—Store under nitrogen to avoid excessive exposure to air and light.]~~

~~*Chromatographic system*— Prepare as directed in the *Assay* under *Mangafodipir Trisodium*. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 1000 theoretical plates; and the tailing factor is not more than 2.3.~~

~~*Procedure*— Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of mangafodipir trisodium ($C_{22}H_{27}MnN_4Na_3O_{14}P_2$) in each mL of the *Injection* taken by the formula:~~

$$250(C/V)(r_U/r_S)$$

~~in which C is the concentration, in mg per mL, of USP Mangafodipir Trisodium RS in the~~

~~Standard preparation; V is the volume, in mL, of Injection taken to prepare the Assay preparation; and r_U and r_S are the mangafodipir peak responses obtained from the Assay preparation and the Standard preparation, respectively.~~ ■2S (USP39)

BRIEFING

Methotrexate Injection, USP 38 page 4320. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Replace the current Assay HPLC procedure with the Assay method used in the current *Methotrexate* monograph. The proposed liquid chromatographic procedure is based on analysis using the Kromasil C18 brand of L1 column. The typical retention time for methotrexate is about 12 min.
2. Add *Identification* test B using a retention time agreement based on the proposed method for the Assay.
3. Add a stability-indicating HPLC procedure for the *Organic Impurities* test with the same HPLC parameters as used in the proposed Assay method.
4. Update the *Packaging and Storage* section to be consistent with approved manufacturers' package inserts.
5. Add the following existing USP Reference Standards to the *USP Reference Standards* section to support the proposed procedures in the Assay and the test for *Organic Impurities*: USP Methotrexate Related Compound B RS, USP Methotrexate Related Compound C RS, and USP Methotrexate Related Compound E RS.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM3: D.A. Porter.)

Correspondence Number—C148572

Comment deadline: September 30, 2015

Methotrexate Injection

DEFINITION

Methotrexate Injection is a sterile solution of Methotrexate in Water for Injection prepared with the aid of Sodium Hydroxide. It contains NLT 90.0% and NMT 110.0% of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$).

IDENTIFICATION

● **A. Infrared Absorption** 〈197K〉

Sample: Dilute, if necessary, a volume of Injection, equivalent to about 25 mg of methotrexate, with water to obtain a solution with a concentration of about 2.5 mg/mL. Adjust with 0.1 N hydrochloric acid to a pH of 4.0. Place the slurry in a 50-mL centrifuge tube, and centrifuge. Decant the supernatant, add 25 mL of acetone, shake, and pass through a solvent-resistant membrane filter of 0.45- μ m pore size. Air-dry the filtered precipitate.

Acceptance criteria: Meets the requirements

~~Add the following:~~

then dilute with *Solution A* to volume.

Sample solution: Nominally 0.2 mg/mL of methotrexate from Injection prepared as follows. Transfer a sufficient amount of Injection to an appropriate volumetric flask. Add about 5% of the flask volume of DMSO and sonicate for 2 min at ambient temperature, then add 30% of the flask volume of *Solution A* and sonicate. Dilute with *Solution A* to volume.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

■ 2S (USP39)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Methotrexate RS in the *Standard solution* (μg/mL)

C_U nominal concentration of methotrexate in the *Sample solution* (μg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

■ • Organic Impurities

Solution A, Solution B, Mobile phase, Sample solution, and Chromatographic system:

Proceed as directed in the *Assay*.

Standard solution: 0.2 μg/mL each of USP Methotrexate RS, USP Methotrexate Related Compound B RS, USP Methotrexate Related Compound C RS, and USP Methotrexate Related Compound E RS in *Solution A* prepared as follows. Add a sufficient amount of each Reference Standard to a suitable volumetric flask and add DMSO equivalent to 5% of the flask volume. Sonicate to achieve dissolution, then dilute with *Solution A* to volume. Sonicate if necessary to aid dissolution.

Sample solution: Nominally 0.2 mg/mL of methotrexate from Injection prepared as directed

in the Assay

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between methotrexate related compound B and methotrexate related compound C

Relative standard deviation: NMT 5.0% each for methotrexate, methotrexate related compound B, methotrexate related compound C, and methotrexate related compound E

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methotrexate related compound B and methotrexate related compound C in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each corresponding impurity from the *Sample solution*

r_S = peak response of each corresponding Reference Standard from the *Standard solution*

C_S = concentration of each corresponding Reference Standard in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of methotrexate in the *Sample solution* ($\mu\text{g/mL}$)

Calculate the percentage of methotrexate related compound E free acid in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methotrexate Related Compound E RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of methotrexate in the *Sample solution* ($\mu\text{g/mL}$)

M_{r1} = molecular weight of methotrexate related compound E free acid, 325.33

M_{r2} = molecular weight of USP Methotrexate Related Compound E RS, 343.56

[Note—USP Methotrexate Related Compound E RS is 4-[(2,4-Diaminopteridin-6-yl)methyl] (methyl)amino}benzoic acid, hemihydrochloride.]

Calculate the percentage of any individual unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of methotrexate from the *Standard solution*

C_S = concentration of USP Methotrexate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of methotrexate in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 2*. Disregard any unspecified degradation product peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methotrexate related compound B	0.67	0.3
Methotrexate related compound C	0.73	3.0
Methotrexate	1.0	—
Methotrexate related compound E free acid ^a	1.41	0.3
Any individual unspecified degradation product	—	0.2
Total unspecified degradation products	—	1.0

^a 4-{{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino}benzoic acid.

■ 2S (USP39)

SPECIFIC TESTS

- **pH** 〈 791 〉: 7.0–9.0
- **Bacterial Endotoxins Test** 〈 85 〉: NMT 0.4 USP Endotoxin Units/mg of methotrexate sodium
- **Other Requirements**: Meets the requirements in *Injections* 〈 1 〉

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage**: Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

■ Store at controlled room temperature. ■ 2S (USP39)

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Endotoxin RS

USP Methotrexate RS

■ USP Methotrexate Related Compound B RS

(S)-2-{{4-[(2,4-Diaminopteridin-6-yl)methylamino]benzamido}pentanedioic acid.

C₁₉H₂₀N₈O₅ 440.41

USP Methotrexate Related Compound C RS

(S)-2-{{4-[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl)methyl](methyl)amino}benzamido}pentanedioic acid.

C₂₀H₂₁N₇O₆ 455.42

USP Methotrexate Related Compound E RS

4-{{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino}benzoic acid, hemihydrochloride.

C₁₅H₁₅N₇O₂·½ HCl 343.56 (anhydrous)

C₁₅H₁₅N₇O₂ 325.33 (free acid)

■ 2S (USP39)

BRIEFING

Methotrexate Tablets, *USP 38* page 4321. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Replace the current *Assay* HPLC procedure with the *Assay* method used in the current *Methotrexate* monograph. The proposed liquid chromatographic procedure is based on analysis using the Kromasil C18 brand of L1 column. The typical retention time for methotrexate is about 12 min.
2. Add *Identification* test *B* using a retention time agreement based on the proposed method for the *Assay*.
3. Add a stability-indicating HPLC procedure for the *Organic Impurities* test with the same HPLC parameters as used in the proposed *Assay* method.
4. Update the *Packaging and Storage* section to be consistent with approved manufacturers' package inserts.
5. Add the following existing USP Reference Standards to the *USP Reference Standards* section to support the proposed procedures in the *Assay* and the test for *Organic Impurities*: USP Methotrexate Related Compound B RS, USP Methotrexate Related Compound C RS, and USP Methotrexate Related Compound E RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: D.A. Porter.)

Correspondence Number—C148573

Comment deadline: September 30, 2015

Methotrexate Tablets

DEFINITION

Methotrexate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methotrexate (C₂₀H₂₂N₈O₅).

IDENTIFICATION

● **A. Ultraviolet Absorption** (197U)

Standard solution: 25 µg/mL of USP Methotrexate RS in dilute hydrochloric acid (1 in 100)

Sample solution: Dissolve 1 Tablet in 100 mL of dilute hydrochloric acid (1 in 100), and filter the solution.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*.

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■2S (USP39)

ASSAY

Change to read:

- **Procedure**

Buffer: 0.2 M dibasic sodium phosphate and 0.1 M citric acid (63:37), adjusted if necessary with 0.1 M citric acid or 0.2 M dibasic sodium phosphate to a pH of 6.0

Mobile phase: Acetonitrile and *Buffer* (10:90)

System suitability solution: 0.1 mg/mL each of USP Methotrexate RS and folic acid in *Mobile phase*

Standard solution: 100 µg/mL of USP Methotrexate RS in *Mobile phase*

Sample solution: Nominally 100 µg/mL of methotrexate from Tablets prepared as follows. Powder NLT 20 Tablets. Dissolve the powder using a mechanical shaker or ultrasonic bath.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 302 nm

Column: 4.6 mm × 25 cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for folic acid and methotrexate are 0.35 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8.0 between the folic acid and methotrexate peaks

Relative standard deviation: NMT 2.5% for the methotrexate peak

■ **Buffer:** 3.4 mg/mL of anhydrous monobasic sodium phosphate in water. Adjust with 1 N sodium hydroxide to a pH of 6.0.

Solution A: Acetonitrile and *Buffer* (5:95)

Solution B: Acetonitrile and *Buffer* (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
30	50	50
34	50	50
35	100	0
40	100	0

Standard solution: 0.2 mg/mL of USP Methotrexate RS in *Solution A* prepared as follows. Add a sufficient amount of USP Methotrexate RS to a suitable volumetric flask and add DMSO equivalent to 5% of the flask volume. Sonicate to achieve dissolution, then dilute with *Solution A* to volume.

Sample solution: Nominally 0.2 mg/mL of methotrexate from NLT 20 Tablets prepared as follows. Powder the Tablets and transfer a sufficient quantity to an appropriate volumetric flask. Add about 1% of the flask volume of DMSO and sonicate for 2 min at ambient temperature, then add 75% of the flask volume of *Solution A* and sonicate for

an additional 30 min at ambient temperature. Cool, then dilute with *Solution A* to volume. Centrifuge at 3000 rpm and use the clear upper layer for analysis.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

■ 2S (USP39)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Methotrexate RS in the *Standard solution* (μg/mL)

C_U nominal concentration of methotrexate in the *Sample solution* (μg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

● Dissolution 〈 711 〉

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: USP Methotrexate RS in *Medium*

Detector: UV 306 nm (maximum absorbance)

Analysis: Determine the amount of methotrexate ($C_{20}H_{22}N_8O_5$) dissolved from UV absorbances on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$) is dissolved.

● Uniformity of Dosage Units 〈 905 〉: Meet the requirements

IMPURITIES

Add the following:**Organic Impurities**

Solution A, Solution B, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.2 µg/mL each of USP Methotrexate RS, USP Methotrexate Related Compound B RS, USP Methotrexate Related Compound C RS, and USP Methotrexate Related Compound E RS in *Solution A* prepared as follows. Add a sufficient amount of each Reference Standard to a suitable volumetric flask and add DMSO equivalent to 5% of the flask volume. Sonicate to achieve dissolution, then dilute with *Solution A* to volume. Sonicate if necessary to aid dissolution.

Sample solution: Nominally 0.2 mg/mL of methotrexate from Tablets prepared as directed in the *Assay*

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between methotrexate related compound B and methotrexate related compound C

Relative standard deviation: NMT 2.5% each for methotrexate, methotrexate related compound B, methotrexate related compound C, and methotrexate related compound E

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methotrexate related compound B and methotrexate related compound C in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each corresponding impurity from the *Sample solution*

r_S = peak response of each corresponding Reference Standard from the *Standard solution*

C_S = concentration of each corresponding Reference Standard in the *Standard solution* (µg/mL)

C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)

Calculate the percentage of methotrexate related compound E free acid in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methotrexate Related Compound E RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of methotrexate related compound E free acid, 325.33

M_{r2} = molecular weight of USP Methotrexate Related Compound E RS, 343.56

[Note—USP Methotrexate Related Compound E RS is 4- {[(2,4-Diaminopteridin-6-yl)methyl] (methyl)amino}benzoic acid, hemihydrochloride.]

Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each unspecified degradation product from the *Sample solution*

r_S peak response of methotrexate from the *Standard solution*

C_S concentration of USP Methotrexate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U nominal concentration of methotrexate in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 2*. Disregard any unspecified degradation product peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methotrexate related compound B	0.67	0.5
Methotrexate related compound C	0.73	3.0
Methotrexate	1.0	—
Methotrexate related compound E free acid ^a	1.43	0.3
Any individual unspecified degradation product	—	0.2
Total unspecified degradation products	—	1.0

^a 4-[[[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino]benzamido]pentanedioic acid.

■ 2S (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in well-closed containers. A unit-of-use container contains a quantity of Tablets sufficient to provide one week's therapy as indicated in the labeling.

■ Store at controlled room temperature. Protect from light. ■ 2S (USP39)

- **Labeling:** When packaged in a unit-of-use container, the label indicates the total amount of methotrexate present as one week's supply.

Change to read:

- **USP Reference Standards** { 11 }

USP Methotrexate RS

■ USP Methotrexate Related Compound B RS

(S)-2-{4-[(2,4-Diaminopteridin-6-yl)methylamino]benzamido}pentanedioic acid.

$\text{C}_{19}\text{H}_{20}\text{N}_8\text{O}_5$ 440.41

USP Methotrexate Related Compound C RS

(S)-2-(4-[[[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]

(methyl)amino}benzamido)pentanedioic acid.

$C_{20}H_{21}N_7O_6$ 455.42

USP Methotrexate Related Compound E RS

4- {[(2,4-Diaminopteridin-6-yl)methyl] (methyl)amino}benzoic acid, hemihydrochloride.

$C_{15}H_{15}N_7O_2 \cdot \frac{1}{2} HCl$ 343.56 (anhydrous)

$C_{15}H_{15}N_7O_2$ 325.33 (free acid)

■ 2S (USP39)

BRIEFING

Methylene Blue, *USP 38* page 4341. On the basis of comments received, it is proposed to modernize the monograph as follows:

1. The chemical information is revised to include different hydrated forms of the drug substance.
2. The acceptance criteria in the *Definition* and *Assay* are revised from 98.0%–103.0% to 97.0%–103.0%.
3. An orthogonal *Identification* test *B* is added based on the retention time agreement from the proposed *Assay*.
4. *Identification* test *C* is included to test for the chloride counter ion.
5. The UV spectrophotometric *Assay* is replaced with a stability-indicating liquid chromatographic procedure. The liquid chromatographic procedure is validated using the XBridge phenyl column with L11 packing. The typical retention time for methylene blue under the conditions specified is about 11 min.
6. A liquid chromatographic *Organic Impurities* test is included. The proposed procedure is validated using the XBridge phenyl column with L11 packing. The typical retention time for methylene blue under the conditions specified is about 11 min.
7. The tests for *Arsenic* and *Copper or Zinc* are revised to be consistent with the FDA-approved procedures and specifications.
8. The *Loss on Drying* test conditions and *Acceptance criteria* are revised to accommodate the different hydrated forms.
9. The *Bacterial Endotoxins Test* and *Microbial Enumeration Tests* are included to be consistent with the FDA-approved procedures and specifications.
10. New Reference Standards are added to the *USP Reference Standards* section in support of the proposed revisions.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

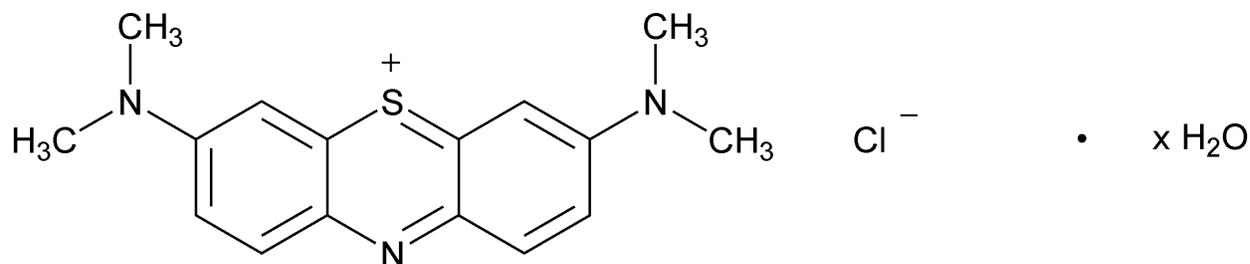
(SM2: S. Ramakrishna.)

Correspondence Number—C150040

Comment deadline: September 30, 2015

Methylene Blue

Change to read:



$C_{16}H_{18}ClN_3S \cdot$

■ xH_2O ■ 2S (USP39)

Phenothiazin-5-ium, 3,7-bis(dimethylamino)-, chloride; trihydrate.

■
3,7-Bis(dimethylamino)phenothiazin-5-ium chloride;

Pentahydrate 409.93

[32680-41-4]. ■ 2S (USP39)

C.I. Basic Blue 9

■ ■ 2S (USP39)

Trihydrate

■ 373.90

■ 2S (USP39)

[7220-79-3].

■ Monohydrate 337.90

[122965-43-9].

■ 2S (USP39)

Anhydrous 319.85

[61-73-4].

DEFINITION

Change to read:

Methylene Blue contains ~~NLT 98.0%~~

■ NLT 97.0% ■ 2S (USP39)

and NMT 103.0% of methylene blue ($C_{16}H_{18}ClN_3S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

•

■ **A.** ■ 2S (USP39)

Infrared Absorption (197K)

Add the following:

■ • **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

Add the following:

■ ● **C. Identification Tests—General** (191), *Chloride*

Sample solution: Ignite 50 mg of Methylene Blue with 0.5 g of anhydrous sodium carbonate. Cool and dissolve the residue in 10 mL of 2 N nitric acid. Filter and use 2 mL of the filtrate for performing the test.

Acceptance criteria: Meets the requirements ■_{2S} (USP39)

ASSAY

Change to read:

● **Procedure**

~~**Standard solution:** about 2 µg/mL of USP Methylene Blue RS in diluted alcohol~~

~~**Sample solution:** about 2 µg/mL of methylene blue in diluted alcohol~~

~~**Spectrophotometric system**~~

~~**Mode:** UV-Vis~~

~~**Detector:** maximum absorbance at about 663 nm~~

~~**Cell:** 1 cm~~

~~**Blank:** Diluted alcohol~~

~~**Analysis**~~

~~**Samples:** Standard solution and Sample solution~~

Calculate the percentage of methylene blue ($C_{16}H_{18}ClN_3S$) in the portion of methylene blue taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

~~A_U absorbance of the Sample solution~~

~~A_S absorbance of the Standard solution~~

~~C_S concentration of USP Methylene Blue RS from Standard solution (µg/mL)~~

~~C_U concentration of methylene blue in the Sample solution (µg/mL)~~

~~**Acceptance criteria:** 98.0%–103.0% on the anhydrous basis~~

■ [

Note—All solutions containing methylene blue are recommended to be prepared fresh before analysis.]

Solution A: 0.1% of trifluoroacetic acid in water

Solution B: Acetonitrile

Diluent: Solution A and Solution B (70:30)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
25	30	70
32	30	70

Time (min)	Solution A (%)	Solution B (%)
33	80	20

Standard solution: 1 mg/mL of USP Methylene Blue RS in *Diluent*. Stirring and sonication may be necessary for complete dissolution.

Sample solution: 1 mg/mL of Methylene Blue in *Diluent*. Stirring and sonication may be necessary for complete dissolution.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 246 nm

Column: 4.6-mm × 10-cm; 3.5-μm packing L11

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 3.0

Relative standard deviation: NMT 1.10%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylene blue (C₁₆H₁₈ClN₃S) in the portion of Methylene Blue taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of methylene blue from the *Sample solution*

r_S

= peak response of methylene blue from the *Standard solution*

C_S

= concentration of USP Methylene Blue RS in the *Standard solution* (mg/mL)

C_U

= concentration of Methylene Blue in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis ■2S (USP39)

IMPURITIES

Change to read:

- **Residue on Ignition** ~~(281)~~: NMT 1.2%

- NMT 0.15% ■ 2S (USP39)

Change to read:

- ~~Arsenic, Method I (211)~~

- **Arsenic** ■ 2S (USP39)

Standard solution: ~~1 mg/ml of USP Methylene Blue RS in Diluent~~

Sample solution: ~~Mix 0.375 g with 10 mL of water in the arsine generator flask. Add 15 mL of nitric acid and 5 mL of perchloric acid, mix, and heat cautiously to the production of strong fumes of perchloric acid. Cool, wash down the sides of the flask with water, and again heat to strong fumes. Again cool, wash down the sides of the flask, and heat to fumes. Cool, dilute with water to 52 mL, and add 3 mL of hydrochloric acid.~~

Analysis: ~~The resulting solution meets the requirements of the test, the addition of 20 mL of 7 N sulfuric acid specified for Procedure being omitted~~

- **Analysis:** Proceed as directed in *Elemental Impurities—Procedures (233)*, Procedure 2: ICP-MS. ■ 2S (USP39)

Acceptance criteria: 8 ppm

Change to read:

- **Copper or Zinc**

Sample solution: ~~Ignite 1.0 g in a porcelain crucible, using as low a temperature as practicable, until all of the carbon is oxidized. Cool the residue, add 15 mL of 2 N nitric acid, and boil for 5 minutes. Filter the cooled solution, and wash any residue with 10 mL of water. To the combined filtrate and washing add an excess of 6 N ammonium hydroxide, and filter the solution into a 50 mL volumetric flask. Wash the precipitate with small portions of water, adding the washings to the filtrate, dilute the solution with water to volume, and mix.~~

Control solution: ~~Boil a quantity of cupric sulfate, equivalent to 200 µg of copper, with 15 mL of 2 N nitric acid for 5 minutes and treat this solution as directed above under Sample solution, beginning with "Filter the cooled solution"~~

Analysis:—

Samples: ~~Control solution and Sample solution~~

~~To 25 mL each of the Control solution and Sample solution add 10 mL of hydrogen sulfide TS~~

Acceptance criteria for Zinc: ~~No turbidity is produced within 5 minutes (absence of zinc).~~

Acceptance criteria for Copper: ~~Any dark color produced does not exceed that of the Control solution (0.02% of copper).~~

- **Analysis:** Proceed as directed in *Elemental Impurities—Procedures (233)*, Procedure 2: ICP-MS.

- **Acceptance criteria:** NMT 100 ppm of zinc and NMT 200 ppm of copper ■ 2S (USP39)

Change to read:

- **Organic Impurities**

Standard stock solution: 100 µg/mL of USP Methylene Blue RS in methanol

Standard solution: 10 µg/mL from *Standard stock solution* in methanol

Sample solution: 1.0 mg/mL of Methylene Blue in methanol

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of octadecylsilylanized chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Upper layer separated from a well-shaken mixture of *n*-butanol, glacial acetic acid, and water (4:1:5)

Analysis

Samples: *Standard stock solution*, *Standard solution*, and *Sample solution*

Proceed as directed in the chapter.

Acceptance criteria: The R_f value of the principal spot from the *Sample solution* corresponds to that from the *Standard stock solution*, and other spots, if present from the *Sample solution*, consist of a secondary spot that in size or intensity is NMT the principal spot from the *Standard stock solution* (10%), and NMT two additional spots, neither of which exceeds in size or intensity the principal spot from the *Standard solution* (1%).

■ **Diluent, Mobile phase, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

System suitability solution: 1 mg/mL of USP Methylene Blue RS and 0.025 mg/mL of USP Azure B RS in *Diluent*

Sensitivity solution: 0.5 µg/mL of USP Methylene Blue RS in *Diluent* from the *Standard solution*

System suitability

Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 3.5 between methylene blue and azure B peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 3.0, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of azure B or any unspecified impurity in the portion of Methylene Blue taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U

= peak response of azure B or any unspecified impurity from the *Sample solution*

r_T

= sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azure B ^a	0.8	2.5
Methylene blue	1.0	—
Any unspecified impurity	—	0.10
Total impurities ^b	—	0.5
^a 3-(Dimethylamino)-7-(methylamino)-phenothiazine-5-ium chloride.		

^b Total impurities does not include azure B.

■ 2S (USP39)

SPECIFIC TESTS

Change to read:

● **Loss on Drying** 〈 731 〉

Analysis: Dry at 75° and at a pressure not exceeding 5 mm of mercury for 4 h

■ 105° for 5 h. ■ 2S (USP39)

Acceptance criteria: 8.0%—18.0%

■ 8.0%—22.0% ■ 2S (USP39)

Add the following:

■ ● **Bacterial Endotoxins Test** 〈 85 〉: NMT 2.5 USP Endotoxin Units/mL ■ 2S (USP39)

Add the following:

■ ● **Microbial Enumeration Tests** 〈 61 〉: The total aerobic microbial count is NMT 10² cfu/g.
■ 2S (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

● **Packaging and Storage:** Preserve in well-closed containers and protect from light. Store at 25°, excursions permitted between 15° and 30°

■ below 30° . ■ 2S (USP39)

Change to read:

- **USP Reference Standards** < 11 >

- USP Azure B RS

3-(Dimethylamino)-7-(methylamino)-phenothiazine-5-ium chloride.

$C_{15}H_{16}ClN_3S$ 305.82

USP Endotoxin RS

- 2S (USP39)

USP Methylene Blue RS

BRIEFING

Metronidazole Gel, USP 38 page 4384 and PF 40(4) [July–Aug. 2014]. Based on comments received on the PF 40(4) proposal, those revisions are canceled. It is proposed to modernize the monograph with the following changes:

1. Add a validated stability-indicating HPLC procedure for the test for *Organic Impurities*. This procedure uses the Inertsil C8 brand of L7 packing column manufactured by GL Sciences. The typical retention time for metronidazole is about 5.6 min.
2. Replace the *Assay* by HPLC with a new HPLC procedure that uses the same HPLC parameters in the proposed test for *Organic Impurities*.
3. Replace *Identification* test A by TLC with the UV spectrum matching in the proposed *Assay*.
4. Introduce USP Tinidazole Related Compound A RS for system suitability and quantitation in the *Assay* and the test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM1: D. Min.)

Correspondence Number—C136689; C151848

Comment deadline: September 30, 2015

Metronidazole Gel**DEFINITION**

Metronidazole Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$).

IDENTIFICATION**Delete the following:**

- ~~A. Thin-Layer Chromatographic Identification Test~~ < 201 >

~~**Standard solution:** 0.5 mg/mL of USP Metronidazole RS~~

~~**Sample solution:** Transfer a quantity of Gel, equivalent to 7.5 mg of metronidazole, to a suitable flask. Add 15 mL of water, shake to disperse, and sonicate for about 10 min. Elute a portion of this solution through a 10-mm × 15-cm chromatographic column containing a~~

~~r_S~~ peak response from the ~~Sample solution~~

~~r_S~~ peak response from the ~~Standard solution~~

~~C_S~~ concentration of USP Metronidazole RS in the ~~Standard solution~~ ($\mu\text{g/mL}$)

~~C_S~~ nominal concentration of the ~~Sample solution~~ ($\mu\text{g/mL}$)

■ Solution A:

Methanol and water (20:80)

Solution B: Methanol

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10.0	100	0
15.0	10	90
15.1	100	0
20.0	100	0

System suitability solution: 0.6 $\mu\text{g/mL}$ of USP Metronidazole RS and 0.6 $\mu\text{g/mL}$ of USP Tinidazole Related Compound A RS in *Solution A*

Standard solution: 30 $\mu\text{g/mL}$ of USP Metronidazole RS in *Solution A*

Sample stock solution: Nominally 300 $\mu\text{g/mL}$ of metronidazole in *Solution A* prepared as follows. Transfer a portion of Gel to a suitable volumetric flask. Add *Solution A* equivalent to 50% of the flask volume and sonicate or vortex until dissolved. Dilute with *Solution A* to volume. [Note—On the basis of formulation, if necessary, centrifuge a portion of the solution at 3000 rpm for 10 min and pass a portion of the supernatant through a filter of 0.45- μm pore size. Use the filtrate.]

Sample solution: Nominally 30 $\mu\text{g/mL}$ of metronidazole in *Solution A* prepared from the *Sample stock solution*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 319 nm. For *Identification test A*, use a diode-array detector in the range of 210–500 nm.

Column: 4.6-mm \times 15-cm; 5- μm packing L7

Column temperature: 30 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 30 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See Table 2 for the relative retention times.]

System suitability requirements

Resolution: NLT 2.0 between metronidazole and tinidazole related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of metronidazole from the *Sample solution*

r_S

= peak response of metronidazole from the *Standard solution*

C_S

= concentration of USP Metronidazole RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= nominal concentration of metronidazole in the *Sample solution* ($\mu\text{g/mL}$)

■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

■ • **Organic Impurities**

Solution A, Solution B, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: Use the *System suitability solution* from the *Assay*.

Sample solution: Use the *Sample stock solution* from the *Assay*.

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for the relative retention times.]

System suitability requirements

Resolution: NLT 2.0 between metronidazole and tinidazole related compound A

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tinidazole related compound A in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of tinidazole related compound A from the *Sample solution*

r_S peak response of tinidazole related compound A from the *Standard solution*

C_S concentration of USP Tinidazole Related Compound A RS in the *Standard solution* ($\mu\text{g/mL}$)

$C_{\bar{F}}$ nominal concentration of metronidazole in the *Sample solution* ($\mu\text{g/mL}$)

Calculate the percentage of each individual unspecified impurity in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_{\bar{F}}$ peak response of each unspecified impurity from the *Sample solution*

$r_{\bar{S}}$ peak response of metronidazole from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Metronidazole RS in the *Standard solution* ($\mu\text{g/mL}$)

$C_{\bar{F}}$ nominal concentration of metronidazole in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.76	0.2
Metronidazole	1.0	—
Any individual unspecified impurity	—	0.3
Total impurities	—	1.0

■ 2S (USP39)

PERFORMANCE TESTS

- **Minimum Fill** 〈 755 〉: Meets the requirements

SPECIFIC TESTS

- **pH** 〈 791 〉: The apparent pH determined potentiometrically is between 4.0 and 6.5.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in laminated collapsible tubes at controlled room temperature.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Metronidazole RS

- USP Tinidazole Related Compound A RS

2-Methyl-5-nitroimidazole.

$\text{C}_4\text{H}_5\text{N}_3\text{O}_2$ 127.10 ■ 2S (USP39)

BRIEFING

Miconazole Nitrate, USP 38 page 4394. As part of the monograph modernization effort, it is proposed to make the following changes:

1. Replace the existing isocratic HPLC *Organic Impurities* test with an HPLC procedure containing a gradient elution for separation of more specified impurities. The HPLC procedure in the test for *Organic Impurities* is based on analyses performed with the Phenomenex Kinetex Phenyl-Hexyl brand of L11 column. The typical retention time for the miconazole peak in the *Organic Impurities* test is 25 min.
2. Replace the titration *Assay* with an HPLC procedure similar to that proposed in the test for *Organic Impurities*.
3. Replace the UV-based *Identification* test *B* with retention time agreement in the proposed HPLC *Assay* method.
4. Update the *USP Reference Standards* section with new Reference Standards to support the test for *Organic Impurities*.

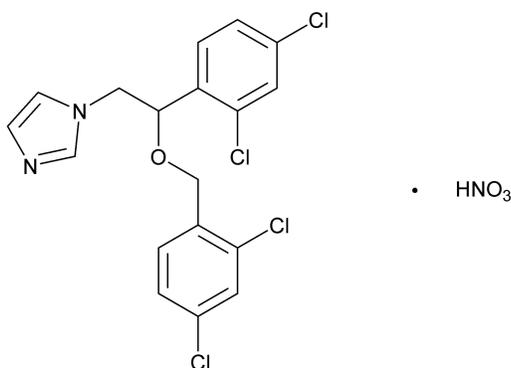
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM1: D. Min.)

Correspondence Number—C131426

Comment deadline: September 30, 2015

Miconazole Nitrate



$C_{18}H_{14}Cl_4N_2O \cdot HNO_3$ 479.14

1*H*-Imidazole, 1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]-, mononitrate;

1-[2,4-Dichloro- β -[(2,4-dichlorobenzyl)oxy]phenethyl]imidazole mononitrate [22832-87-7].

DEFINITION

Miconazole Nitrate contains NLT 98.0% and NMT 102.0% of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** $\langle 197K \rangle$

Delete the following:

- **B. Ultraviolet Absorption** $\langle 197U \rangle$

Diluent: 0.1 N hydrochloric acid in isopropyl alcohol (1 in 10)

Sample solution: 400 μ g/mL sample in *Diluent*

Acceptance criteria: Meets the requirements ■ 2S (USP39)

Add the following:

- • **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

ASSAY

Change to read:

• **Procedure**

~~**Sample solution:** 7 mg/mL of Miconazole Nitrate in glacial acetic acid~~

~~**Titrimetric system**~~

~~**Mode:** Direct titration~~

~~**Titrant:** 0.1 N perchloric acid VS~~

~~**Endpoint detection:** Potentiometric~~

~~**Analysis:** Titrate the *Sample solution* with *Titrant* using a glass-calomel electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 47.92 mg of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$).~~

~~**Acceptance criteria:** 98.0%–102.0% on the dried basis~~

■ **Solution A:** Methanol, water, and 1 M triethylammonium acetate (300:700:10)

Solution B: Acetonitrile, methanol, and 1 M triethylammonium acetate (250:750:2)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
5	70	30
10	44	56
27	44	56
30	25	75
35	25	75
36	70	30
40	70	30

Diluent: Methanol and water (70:30)

System suitability solution: 0.1 mg/mL of USP Miconazole Nitrate RS and 6 µg/mL of USP Miconazole Related Compound F RS in *Diluent*. Sonication may be needed to aid dissolution.

Standard solution: 0.1 mg/mL of USP Miconazole Nitrate RS in *Diluent*. Sonication may be needed to aid dissolution.

Sample solution: 0.1 mg/mL of Miconazole Nitrate in *Diluent*. Sonication may be needed to aid dissolution.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 10-cm; 2.6-µm packing L11

Column temperature: 40°

Flow rate: 0.8 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for miconazole related compound F and miconazole are 0.96 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between miconazole related compound F and miconazole, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$) in the portion of Miconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of miconazole from the *Sample solution*

r_S

= peak response of miconazole from the *Standard solution*

C_S

= concentration of USP Miconazole Nitrate RS in the *Standard solution* (mg/mL)

C_U

= concentration of Miconazole Nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ■2S (USP39)

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.2%

Change to read:

- **Organic Impurities**

Mobile phase: ~~Methanol, acetonitrile, and 0.2 M ammonium acetate (32:30:38)~~

System suitability solution: ~~25 µg/mL each of USP Miconazole Nitrate RS and USP Econazole Nitrate RS in Mobile phase~~

Sample stock solution: ~~10 mg/mL of Miconazole Nitrate in Mobile phase~~

~~**Sample solution:** 25 µg/mL of Miconazole Nitrate, from *Sample stock solution*, in *Mobile phase*~~

~~**Chromatographic system-**~~

~~(See *Chromatography* ⁶²¹, *System Suitability*.)~~

~~**Mode:** LC~~

~~**Detector:** UV 235 nm~~

~~**Column:** 4.6 mm × 10 cm; 3 µm packing L1~~

~~**Flow rate:** 2 mL/min~~

~~**Injection volume:** 10 µL~~

~~**Run time:** 1.2 times the retention time of the main peak~~

~~**System suitability-**~~

~~**Sample:** *System suitability solution*~~

~~[Note—The relative retention times for econazole and miconazole are 0.5 and 1.0, respectively.]~~

~~**Suitability requirements-**~~

~~**Resolution:** NLT 10 between econazole and miconazole~~

~~**Relative standard deviation:** NMT 2.0%~~

~~**Analysis-**~~

~~**Samples:** *Sample stock solution* and *Sample solution*~~

~~Measure the responses of all peaks, excluding the peak representing nitrate ion and any peak producing a response less than 0.2 times the response of the main peak.~~

~~**Acceptance criteria:** The response of any individual peak, other than the main peak of the *Sample stock solution*, is NMT that of the main peak of the *Sample solution* (0.25%), and the sum of the responses of all peaks, other than the main peak of the *Sample stock solution*, is NMT twice the response of the main peak of the *Sample solution* (0.5%).~~

■ Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system:

Proceed as directed in the Assay.

Standard solution: 1.2 µg/mL each of USP Miconazole Nitrate RS, USP Econazole Nitrate RS, USP Miconazole Related Compound C RS, USP Miconazole Related Compound F RS, and USP Miconazole Related Compound I RS in *Diluent*

Sample solution: 600 µg/mL of Miconazole Nitrate in *Diluent*. Sonication may be needed to aid dissolution.

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between miconazole related compound C and miconazole related compound I, NLT 1.5 between miconazole related compound I and econazole, and NLT 1.5 between miconazole related compound F and miconazole

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of miconazole related compound C, miconazole related compound F, miconazole related compound I, or econazole nitrate in the portion of Miconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of miconazole related compound C, miconazole related compound F, miconazole related compound I, or econazole nitrate from the *Sample solution*

r_S

= peak response of miconazole related compound C, miconazole related compound F, miconazole related compound I, or econazole nitrate from the *Standard solution*

C_S

= concentration of USP Miconazole Related Compound C RS, USP Miconazole Related Compound F RS, USP Miconazole Related Compound I RS, or USP Econazole Nitrate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= concentration of Miconazole Nitrate in the *Sample solution* ($\mu\text{g/mL}$)

Calculate the percentage of any individual specified or unspecified impurity in the portion of Miconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of each impurity from the *Sample solution*

r_S

= peak response of miconazole from the *Standard solution*

C_S

= concentration of USP Miconazole Nitrate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U

= concentration of Miconazole Nitrate in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 2*. Disregard limit: 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deschlorobenzyl econazole ^a	0.22	0.25
^a 1-(2,4-Dichlorophenyl)-2-(1 <i>H</i> -imidazol-1-yl)ethanol.		

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Miconazole quarternary salt ^b	0.57	0.25
Miconazole benzyl analog ^c	0.65	0.25
Miconazole related compound C	0.74	0.25
Miconazole related compound I	0.76	0.25
Econazole nitrate	0.78	0.25
Miconazole 2,6-isomer ^d	0.87	0.25
Miconazole 2,5-isomer ^e	0.94	0.25
Miconazole related compound F	0.96	0.25
Miconazole	1.0	—
Any individual unspecified impurity	—	0.25
Total impurities	—	0.5
a	1-(2,4-Dichlorophenyl)-2-(1 <i>H</i> -imidazol-1-yl)ethanol.	

^b 2-(3-{2-[(2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazol-3-ium-1-yl)-2-methylpropanoate.

^c 1-[2-(Benzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.

^d 1-{2-[(2,6-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.

^e 1-{2-[(2,5-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.

■ 2S (USP39)

SPECIFIC TESTS

● **Loss on Drying** 〈 731 〉

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light.

Change to read:

● **USP Reference Standards** 〈 11 〉

USP Econazole Nitrate RS

USP Miconazole Nitrate RS

■ USP Miconazole Related Compound C RS

2-[(2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethan-1-amine hydrochloride.

C₁₅H₁₃Cl₄NO·HCl 401.53

USP Miconazole Related Compound F RS

1- {2- [(3,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.

C₁₈H₁₄Cl₄N₂O 416.13

USP Miconazole Related Compound I RS

1- {2- [(2-Chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole mononitrate.

C₁₈H₁₅Cl₃N₂O·HNO₃ 444.69

■ 2S (USP39)

BRIEFING

Octreotide Acetate, PF 36(6) [Nov.–Dec. 2010]. On the basis of comments received, the following revisions are proposed:

1. *Identification* test A is deleted, and the test by HPLC retention time agreement is moved from *Identification* test B to *Identification* test A. The *Analysis* is revised to add an *Identification sample solution* and the requirement of sample and Standard peak coelution.
2. The test for *Amino Acid Analysis* is moved from *Specific Tests* to *Identification* test B. The *Acceptance criteria* for *Trp* is revised and the requirement for total peptide content is removed.
3. The liquid chromatographic procedure in the *Assay* and *Octreotide Acetate Related Compounds* is revised by modifying the gradient and adding the injection repeatability requirement. The note for the *Standard solution* is deleted. The test procedure is based on analyses performed with the Phenomenex Synergi Max–RP C12 brand of L87 column. The particle size of the column is added. The typical retention time for the octreotide peak is about 16.5 min.
4. The test procedure for *Trifluoroacetic Acid (TFA)* is revised to use *Trifluoroacetic Acid (TFA) in Peptides* 〈 503.1 〉. A note is added to indicate the test is only required if trifluoroacetic acid is used in the manufacturing process.
5. The gas chromatographic procedure in the *Limit of Triethylamine* is deleted because triethylamine is tested by the manufacturer as in-process control and is not tested on the finished goods.
6. The requirements for *pH* and *Optical Rotation* in the *Specific Tests* section are deleted; the rest of the monograph tests are sufficient.
7. The *Microbial Enumeration Tests* and *Tests for Specified Microorganisms* are added to the *Specific Tests* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

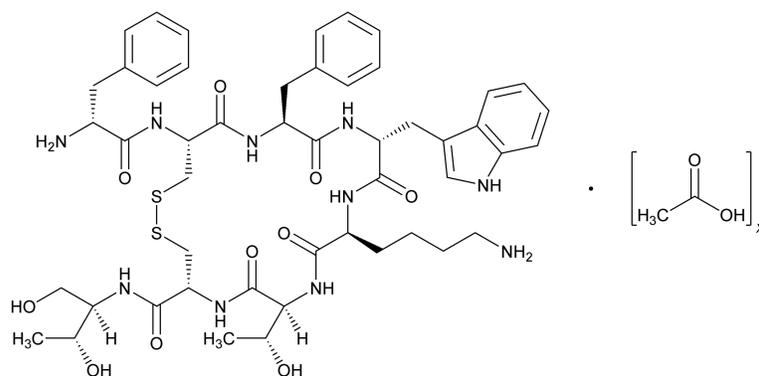
(BIO1: C. Li.)

Correspondence Number—C153058

Comment deadline: September 30, 2015

Add the following:

■ **Octreotide Acetate**



$C_{49}H_{66}N_{10}O_{10}S_2 \cdot xC_2H_4O_2$ 1019 (as free base)

L-Cysteinamide, d-phenylalanyl-L-cysteinyl-L-phenylalanyl-d-tryptophyl-L-lysyl-L-threonyl-N-[2-hydroxy-1-(hydroxymethyl)propyl]-, cyclic (2→7)-disulfide, [R-(R*,R*)]-, acetate (salt); d-Phenylalanyl-L-cysteinyl-L-phenylalanyl-d-tryptophyl-L-lysyl-L-threonyl-N-[(1R,2R)-2-hydroxy-1-(hydroxymethyl)propyl]-L-cysteinamide cyclic (2→7)-disulfide acetate (salt); d-Phenylalanyl-L-hemicystyl-L-phenylalanyl-d-tryptophyl-L-lysyl-L-threonyl-L-hemicystyl-L-threoninol cyclic (2→7)-disulfide acetate (salt) [79517-01-4].

DEFINITION

Octreotide Acetate is a long-acting synthetic octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin. It contains NLT 95.0% and NMT 105.0% of octreotide ($C_{49}H_{66}N_{10}O_{10}S_2$), calculated on the anhydrous, acetic acid-free basis.

IDENTIFICATION

Delete the following:

- ~~A. Infrared Absorption~~ ~~(197K)~~ ■ 2S (USP39)

Change to read:

- B-
- A. HPLC ■ 2S (USP39)

~~The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.~~

■ **Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

Identification sample solution: Mix an equal volume of the *Standard solution* and the *Sample solution*.

Analysis

Samples: *Identification sample solution, Standard solution, and Sample solution*

Examine the chromatograms of the *Identification sample solution, Standard solution, and Sample solution*

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, and the major peak of the *Identification*

sample solution elutes as a single peak. ■ 2S (USP39)

Change to read:

● **B. Amino Acid Analysis**

~~[Note—The following method is given for informational purposes; any validated amino acid analysis method can be used. See *Biotechnology-Derived Articles—Amino Acid Analysis* 1052.]~~

■ 2S (USP39)

Diluent: 0.1 M hydrochloric acid

Standard amino acid mixture: 2500 nmol/mL of *Lys, His, NH₃, Arg, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe*, and 1250 nmol/mL of *Cys-Cys* in *Diluent*

Standard tryptophan solution: 0.2042 mg/mL of tryptophan in *Diluent*. This solution contains 1000 nmol/mL of *Trp*.

Standard threonin-ol solution: 0.1052 mg/mL of *Thr-ol* in *Diluent*. This solution contains 1000 nmol/mL *Thr-ol*. [Note—1000 nmol/mL = 1 mM]

Standard solution: *Diluent, Standard amino acid mixture, Standard tryptophan solution, and Standard threonin-ol solution* (76:4:10:10). The concentration is 100.0 nmol/mL for each amino acid.

Sample solution A: Weigh 1.00–1.50 mg of Octreotide Acetate into a 5-mL ampule. Add 1000 μ L of *Diluent* and 1.2 mL of 30% (w/w) hydrochloric acid, cool the ampule in dry ice, evacuate, and seal by melting. Heat the ampule for 16 h at 115°. Allow to cool and transfer the contents of the ampule quantitatively into a 25-mL round-bottomed flask, rinse with *Diluent*, and evaporate to dryness on a rotary evaporator. Dissolve the residue in 10.00 mL of *Diluent*.

Sample solution B: Weigh 1.00–1.50 mg of Octreotide Acetate into a 5-mL ampule. Add 1000 μ L of *Diluent* and 1.2 mL of 30% (w/w) hydrochloric acid with 1% (v/v) thioglycolic acid, cool the ampule in dry ice, evacuate, and seal by melting. Heat the ampule for 16 h in a heating block at 115°. Allow to cool and transfer the contents of the ampule quantitatively into a 25-mL round-bottomed flask, rinse with *Diluent*, and evaporate to dryness on a rotary evaporator. Dissolve the residue in 10.00 mL of *Diluent*.

Analysis

Samples: *Standard solution, Sample solution A, and Sample solution B*

Standardize the instrument with the *Standard solution*. Inject a suitable volume of *Standard solution, Sample solution A, and Sample solution B*. Evaluate the peak areas of each amino acid found in relation to the peak areas of the respective amino acids in the *Standard solution*, express the content of each amino acid in nmoles.

Calculate A , the average number of nmoles of the amino acids found to be stable under hydrolysis conditions (three stable amino acids—2 *Phe* and 1 *Lys*) taken:

$$A = N_T/3$$

N_T total nmoles of the stable amino acids

Calculate the ratio of the amino acids taken:

$$\text{Result} = N_E/A$$

$N_{\bar{E}}$ nmoles of each amino acid

[Note—For *Trp* use only data obtained with *Sample solution B*. For *Cys* use only data obtained with *Sample solution A*.]

Acceptance criteria: See *Table 1*.

Table 1

Name	Acceptance Criteria
<i>Thr</i>	0.7–1.1
<i>Lys</i>	0.9–1.3
<i>Phe</i>	1.8–2.2
<i>Trp</i>	0.6 ■0.4–■2S (USP39) 1.1
<i>Cys</i>	1.0–2.2
<i>Thr-ol</i>	0.6–1.3

ASSAY

Change to read:

• **Procedure**

Solution A: 0.02% (v/v) of trifluoroacetic acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20	70	30
■25 ■2S (USP39)	65 ■2S (USP39)	35 ■2S (USP39)
25		
■30 ■2S (USP39)	10	90
30		
■35 ■2S (USP39)	10	90
33		
■40 ■2S (USP39)	90	10
45	90	10 ■2S (USP39)

System suitability solution: 0.8

■5 ■2S (USP39)

mg/mL of USP Octreotide Acetate RS and 0.2 mg/mL of USP Octreotide Non-Cyclic System Suitability Marker RS in *Solution A*

Standard solution: 0.5 mg/mL of USP Octreotide Acetate RS in *Solution A* [Note—Place USP

~~Octreotide Acetate RS in a desiccator containing saturated NaCl solution for at least 30 min before weighing.]~~

■ ~~2S (USP39)~~

Sample solution: 0.5 mg/mL of Octreotide Acetate in *Solution A*. [Note—Place Octreotide Acetate in a desiccator containing saturated sodium chloride solution for at least 30 min before weighing. Determine the water content by suitable analysis.]

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm;

■ 4- μ m ■ 2S (USP39)

packing L87

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution*

■ and *Standard solution* ■ 2S (USP39)

[Note—The retention times for octreotide and non-cyclic octreotide in the *System suitability solution* are about 16.5 and 18.5 min, respectively.]

Suitability requirements

Resolution: NLT 2.0 between octreotide and non-cyclic octreotide, *System suitability solution*

■ **Relative standard deviation:** NMT 2.0%, *Standard solution* ■ 2S (USP39)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of octreotide ($C_{49}H_{66}N_{10}O_{10}S_2$) in the portion of Octreotide Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [100/(100 - W - Ac)] \times 100$$

r_U = peak response of octreotide from the *Sample solution*

r_S = peak response of octreotide from the *Standard solution*

C_S = concentration of USP Octreotide Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Octreotide Acetate in the *Sample solution* (mg/mL)

W = water content in the Octreotide Acetate sample (%)

Ac = acetic acid content in the Octreotide Acetate sample (%)

Acceptance criteria: 95.0%–105.0% on the anhydrous, acetic acid-free basis

OTHER COMPONENTS

Add the following:

- ● **Acetic Acid In Peptides** 〈 503 〉: 5.0%–12.8% ■ 2S (USP39)

PRODUCT RELATED SUBSTANCES IMPURITIES

Change to read:

- **Octreotide Acetate Related Compounds**

■ [

Note—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]

■ 2S (USP39)

Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity taken, disregarding any peak with a retention time of less than 5 min and any peak with an area less than 0.1% of the main peak:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_T sum of the peak responses from the *Sample solution*, excluding those of the solvent peaks

Acceptance criteria:

~~**Any individual impurity:** NMT 0.5%~~

~~**Total impurities:** NMT 2.0%~~

■ See Table 3.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acetyl-Lys ⁵ -octreotide ^a	1.4	0.5
Acetyl-Phe ¹ -octreotide ^b	1.5	0.5
Unspecified impurity	—	0.5
Total impurities	—	2.0

^a d-Phenylalanyl-l-hemicystyl-l-phenylalanyl-d-tryptophyl-(N-acetyl)-l-lysyl-l-threonyl-l-hemicystyl-l-threoninol cyclic (2→7)-disulfide.

^b (N-Acetyl)-d-Phenylalanyl-l-hemicystyl-l-phenylalanyl-d-tryptophyl-l-lysyl-l-threonyl-l-hemicystyl-l-threoninol cyclic (2→7)-disulfide.

■ 2S (USP39)

PROCESS RELATED IMPURITIES**Change to read:**

- **Trifluoroacetic Acid (TFA)**

- **Trifluoroacetic Acid (TFA) in Peptides** (503.1): ■_{2S} (USP39)

Solution A: Phosphoric acid 0.07% (v/v). Adjust with 1 N sodium hydroxide to a pH of 3.0 ± 0.1.

Solution B: Methanol

Diluent: *Solution A* and *Solution B* (95:5)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	95	5
10	50	50
20	50	50
21	95	5
30	95	5

Acetic acid stock solution: 4 mg/mL of USP Glacial Acetic Acid RS in *Diluent*

TFA stock solution: 0.08 mg/mL of TFA in *Diluent*

Standard solution: 0.4 mg/mL of USP Glacial Acetic Acid RS, 0.016 mg/mL of TFA in *Diluent* prepared with *Acetic acid stock solution*, *TFA stock solution*, and *Diluent* (5:10:35)

Sample solution: 8 mg/mL of Octreotide Acetate in *Diluent*. Sonicate until solid dissolves completely.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6 mm × 25 cm;

- 5 μm ■_{2S} (USP39)

packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

[Note—The retention times for the TFA and acetic acid are about 2.6 and 3.9 min, respectively.]

Suitability requirements

Resolution: NLT 1.5 between acetic acid and TFA

Relative standard deviation: NMT 15%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of TFA in the portion of Octreotide Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of TFA in the *Standard solution* (mg/mL)

C_U = concentration of Octreotide Acetate in the *Sample solution* (mg/mL)

P = purity factor of TFA standard

Acceptance criteria: NMT 0.25%

■ NMT 0.25%.

[Note—Perform this test if trifluoroacetic acid is used in the manufacturing process.]

■ 2S (USP39)

Delete the following:

■ • **Limit of Triethylamine**

[Note—Perform if triethylamine is used in the manufacturing process.]

Internal standard solution: 0.1% of *n*-propanol in 10% sodium hydroxide

Solution A: 1 mg/mL of triethylamine (1000 ppm)

Standard stock solution 1: *Solution A* and water (5:95) (50 ppm)

Standard stock solution 2: *Standard stock solution 1* and water (5:45) (5 ppm)

Standard solution: Pipette 5 mL of *Standard stock solution 2* into a headspace sampler vial, add 1 mL of *Internal standard solution*, and seal the vial.

Sample solution: Transfer 10 mg of Octreotide Acetate into a headspace sampler vial, add 5 mL of water and 1 mL of *Internal standard solution*, and seal the vial.

Blank: Pipette 5 mL of water into a headspace vial, add 1 mL of *Internal standard solution*, and seal the vial.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC, system equipped with headspace sampler

Detector: Flame ionization

Column: 0.32 mm × 30 m fused silica capillary column containing a 1.5 μm film of phase G## (See *Reagents, Indicators, and Solutions, Chromatographic Columns*)

Temperature

Injector: 180°

Detector: 250°

Column See the temperature program below:

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	2
40	5	80	0
80	20	250	6.5

Carrier gas: Nitrogen

Head pressure: 30 kPa

Injection type: Split (ratio 1:10)

Run time: 25 min

Head-space sampler conditions

Vial temperature: 70 °

Syringe temperature: 100 °

Injection volume: 1 mL of gaseous phase

Conditioning time: 30 min (10 min with agitation)

System suitability

Sample: *Standard solution*

[Note—The retention time for *n*-propanol is about 7.35 min; and the retention time of triethylamine in relation to *n*-propanol is 1.6 min.]

Suitability requirements

Relative standard deviation: NMT 15% for the ratio of the detector response of triethylamine to the detector response of *n*-propanol, six injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the content of triethylamine, in ppm, in the portion of Octreotide Acetate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times P \times 10^6$$

A_U ratio of the detector response of triethylamine to the detector response of *n*-propanol in the *Sample solution*

A_S ratio of the detector response of triethylamine to the detector response of *n*-propanol in the *Standard solution*

C_S concentration of triethylamine in the *Standard solution* (ppm)

C_U concentration of Octreotide Acetate in the *Sample solution* (ppm)

P = purity factor of triethylamine standard

Acceptance criteria: NMT 2500 ppm ■_{2S} (USP39)

SPECIFIC TESTS

Delete the following:

■ • Amino Acid Content

Procedure

HPLC [Note—The following method is given for informational purposes; any validated amino acid analysis method can be used. See *Biotechnology Derived Articles—Amino Acid Analysis* (1052)]

Diluent: 0.1 M hydrochloric acid

Standard amino acid mixture: 2500 nmol/mL of *Lys, His, NH₃, Arg, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe*, and 1250 nmol/mL of *Cys-Cys* in *Diluent*.

Standard tryptophan solution: 0.2042 mg/mL of tryptophan in *Diluent*. This solution contains 1000 nmol/mL of *Trp*.

Standard threonin-ol solution: 0.1052 mg/mL of threonin-ol in *Diluent*. This solution contains 1000 nmol/mL *Thr-ol*.

Internal standard solution: 0.1031 mg/mL of γ -aminobutyric acid (Gaba) in *Diluent*. This solution contains 1000 nmol/mL of Gaba.

Standard solution: *Diluent*, *Standard amino acid mixture*, *Standard tryptophan solution*, *Standard threonin-ol solution*, and *Internal standard solution* (66:4:10:10:10). The concentration is 100.0 nmol/mL for each amino acid.

Sample solution A: Weigh 1.00–1.50 mg of Octreotide Acetate into a 5-mL ampule. Add 1000 μ L of *Internal standard solution* and 1.2 mL of 30% (w/w) hydrochloric acid, cool the ampule in dry ice, evacuate, and seal by melting. Heat the ampule for 16 h in a heating block at 115 $^{\circ}$. Allow to cool and transfer the contents of the ampule quantitatively into a 25-mL round-bottomed flask, rinse with *Diluent*, and evaporate to dryness on a rotary evaporator. Dissolve the residue in 10.00 mL of *Diluent*.

Sample solution B: Weigh 1.00–1.50 mg of Octreotide Acetate into a 5-mL ampule. Add 1000 μ L of *Internal standard solution* and 1.2 mL of 30% (w/w) hydrochloric acid with 1% (v/v) thioglycolic acid, cool the ampule in dry ice, evacuate, and seal by melting. Heat the ampule for 16 h in a heating block at 115 $^{\circ}$. Allow to cool and transfer the contents of the ampule quantitatively into a 25-mL round-bottomed flask, rinse with *Diluent*, and evaporate to dryness on a rotary evaporator. Dissolve the residue in 10.00 mL of *Diluent*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC, system equipped with amino acid analyzer

Detector: Vis 440 nm and 570 nm

Precolumn: 4.6-mm \times 1-cm cation-exchanger precolumn

Column: 4.6-mm \times 2-cm cation-exchanger precolumn

Flow rate: 25 mL/h

Injection volume: 50 μ L

Analysis

Samples: *Standard solution*, *Sample solution A*, and *Sample solution B*

Standardize the instrument with the *Standard solution*. Inject suitable volume of *Standard solution*, *Sample solution A*, and *Sample solution B* into the amino acid analyzer. Evaluate the peak areas of each amino acid found in relation to the peak areas of the respective amino acids in the *Standard solution*, express the content of each amino acid in nmoles. Calculate A , the average number of nmoles of the amino acids found to be stable under hydrolysis conditions (three stable amino acids—2 *Phe* and 1 *Lys*) taken:

$$A = N_t / 3$$

N_t = total nmoles of the stable amino acids

Calculate the ratio of the amino acids taken:

$$\text{Result} = N_i / A$$

N_i = nmoles of each amino acid

[Note—For *Trp* use only data obtained with *Sample solution B*. For *Cys* use only data obtained with *Sample solution A*]

Calculate the percent content of amino acids (peptide content) taken:

$$\text{Result} = (A/W_r) \times (1019/50)$$

W_r = Weight of Octreotide Acetate sample (mg)

Acceptance criteria: See Table 3.

Table 3

Name	Acceptance Criteria
Thr	0.7–1.1
Lys	0.9–1.3
Phe	1.8–2.2
Trp	0.6–1.1
Cys	1.0–2.2
Thr-ol	0.6–1.3
Total peptide content	NLT 78%

■ 2S (USP39)

- **Water Determination** (921), Method I: NMT 10.0%

Delete the following:

- **pH** (791): 5–7, in a 1% solution in carbon dioxide-free water ■ 2S (USP39)

Delete the following:

- **Optical Rotation** (781), Specific Rotation

Sample solution: 2 mg/mL of Octreotide Acetate in 1% glacial acetic acid

Acceptance criteria: -14.5° to -23° ■ 2S (USP39)

- **Bacterial Endotoxins Test** (85): NMT 466 USP Endotoxin Units/mg of octreotide acetate

Add the following:

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62):
The total aerobic microbial count is NMT 100 cfu/g. The total yeast and mold count is NMT 100 cfu/g. ■ 2S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in air-tight containers. Store at a temperature of 2° – 8° , protected from light.
- **USP Reference Standards** (11)
 USP Glacial Acetic Acid RS
 USP Octreotide Acetate RS
 USP Octreotide Non-Cyclic System Suitability Marker RS

■ 2S (USP39)

Oxymorphone Hydrochloride Injection, *USP 38* page 4721. On the basis of comments received, it is proposed to make the following revisions:

1. Replace the UV procedure for the *Assay* with an HPLC procedure. The proposed liquid chromatographic procedure is based on analysis performed with the Zorbax Eclipse XDB-C18 brand of L1 column. The typical retention time is about 10.7 min for oxymorphone.
2. Revise *Identification* test *A* with the procedure based on the UV spectra as obtained in the proposed *Assay*.
3. Add *Identification* test *B* based on the retention time agreement as obtained in the proposed *Assay*.
4. Add a test for *Organic Impurities* based on the HPLC procedure similar to that in the proposed *Assay*. The proposed liquid chromatographic procedure is based on analysis performed with the Zorbax Eclipse XDB-C18 brand of L1 column. The typical retention time is about 10.7 min for oxymorphone.
5. Update the *Package and Storage* section based on the package insert.
6. Add USP Oxymorphone Related Compound A RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: H. Cai.)

Correspondence Number—C124548

Comment deadline: September 30, 2015

Oxymorphone Hydrochloride Injection

DEFINITION

Oxymorphone Hydrochloride Injection is a sterile solution of Oxymorphone Hydrochloride in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$).

IDENTIFICATION

Delete the following:

- ● ~~A. The *Sample solution* and *Standard solution* as directed in the *Assay* exhibit maxima and minima at the same wavelengths. ■2S (*USP39*)~~

Add the following:

- ● A. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■2S (*USP39*)

Add the following:

- ● B. The UV absorption spectra of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*. ■2S (*USP39*)

ASSAY**Change to read:**● **Procedure**

Standard stock solution: 0.9 mg/mL of USP Oxymorphone RS in chloroform

Standard solution: 135 µg/mL of USP Oxymorphone RS in chloroform from the *Standard stock solution*

Sample solution: Transfer a volume of Injection nominally equivalent to about 15 mg of oxymorphone hydrochloride to a 125-mL separator, and add water, if necessary, to bring the volume to 15 mL. Adjust with hydrochloric acid to a pH of less than 2, extract with five 15-mL portions of chloroform, and discard the chloroform extracts. Adjust the aqueous phase with ammonium hydroxide to a pH of 9.5, and extract with four 20-mL portions of chloroform. Filter the chloroform extracts through a chloroform-moistened pledget of cotton into a 100-mL volumetric flask, dilute with chloroform to volume, and mix.

Instrumental conditions-

Analytical wavelength: Maximum absorbance at about 282 nm

Cell: 1 cm

Blank: Chloroform

Analysis-

Samples: *Standard solution, Sample solution, and Blank*

Concomitantly determine the absorbances of the solutions.

Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Oxymorphone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of oxymorphone hydrochloride in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80

M_{r2} = molecular weight of oxymorphone, 301.34

- Protect all solutions containing oxymorphone from light and use clear glass HPLC vials.

Solution A: Dissolve 2.02 g of sodium 1-heptanesulfonate in 900 mL of water. Add 100 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.

Solution B: Dissolve 2.02 g of sodium 1-heptanesulfonate in 750 mL of water. Add 250 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0

Time (min)	Solution A (%)	Solution B (%)
3	100	0
35	0	100
40	0	100
40.1	100	0
50.1	100	0

Diluent: Dissolve 2.02 g of anhydrous sodium 1-heptanesulfonate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.1.

Standard solution: 0.14 mg/mL of USP Oxymorphone RS prepared as follows. Transfer a suitable amount of USP Oxymorphone RS to a suitable volumetric flask. Add 50% of the flask volume of *Diluent*. Sonicate to dissolve, if necessary. Add 9% of the flask volume of acetonitrile. Cool to room temperature and dilute with *Diluent* to volume.

Sample solution: Nominally 0.15 mg/mL of oxymorphone hydrochloride from Injection prepared as follows. Transfer a suitable volume of the composite sample from NLT 20 ampules to a suitable volumetric flask. Dilute with *Solution A* to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector

Assay: UV 230 nm

Identification test B: Diode array UV 200–360 nm

Column: 4.6-mm × 7.5-cm; 3.5-μm packing L1

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 30 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxymorphone hydrochloride (C₁₇H₁₉NO₄·HCl) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U

= peak response of oxymorphone from the *Sample solution*

r_S

= peak response of oxymorphone from the *Standard solution*

C_S

= concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of oxymorphone hydrochloride in the *Sample solution* (mg/mL)

M_{r1}

= molecular weight of oxymorphone hydrochloride, 337.80

M_{r2}

= molecular weight of oxymorphone, 301.34

■ 2S (USP39)

Acceptance criteria: 93.0%–107.0%

IMPURITIES

Add the following:

■ ● Organic Impurities

Protect all solutions containing oxymorphone from light and use clear glass HPLC vials.

Solution A, Solution B, Mobile phase, Diluent, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability stock solution A: 0.2 mg/mL of USP Oxymorphone Related Compound A RS prepared as follows. Transfer a suitable amount of USP Oxymorphone Related Compound A RS to a suitable volumetric flask. Dissolve with 24% of the flask volume of 0.1 N hydrochloric acid and dilute with acetonitrile to volume.

System suitability stock solution B: 0.02 mg/mL of USP Oxymorphone Related Compound A RS in acetonitrile from *System suitability stock solution A*

System suitability stock solution C: 0.14 mg/mL of USP Oxymorphone RS prepared as follows. Transfer a suitable amount of USP Oxymorphone RS to a suitable volumetric flask. Add 50% of the flask volume of *Diluent*. Sonicate to dissolve if necessary. Add 9% of the flask volume of acetonitrile. Cool to room temperature and dilute with *Diluent* to volume.

System suitability solution: 0.0008 mg/mL of USP Oxymorphone Related Compound A RS in *System suitability stock solution C* from *System suitability stock solution B*

Standard solution: 0.00014 mg/mL of USP Oxymorphone RS prepared as follows. Dilute *System suitability stock solution C* with *Solution A*.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between oxymorphone related compound A and oxymorphone, *System suitability solution*

Relative standard deviation: NMT 10%, *Standard solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual degradation product in the portion of Injection taken:

$$\text{Result} = \left\{ \frac{r_U/F}{r_S + \Sigma(r_U/F)} \right\} \times 100$$

$r_{\bar{U}}$ peak response of each individual degradation product from the *Sample solution*

F = relative response factor of each individual degradation product (see *Table 2*)

$r_{\bar{S}}$ peak response of oxymorphone from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
10-Hydroxyoxymorphone ^a	0.59	1.0	0.20
Oxymorphone related compound A (oxymorphone <i>N</i> -oxide)	0.82	1.1	0.30
Oxymorphone	1.00	1.0	—
10-Ketooxymorphone ^b	1.37	0.83	0.30
Oxycodone ^c	1.97	1.0	—
1-Bromooxymorphone ^{c,d}	2.05	1.0	—
2,2'-Bisoxymorphone ^e	2.08	1.7	1.00
Any individual unspecified degradation product	—	1.0	0.50
Total degradation products	—	—	2.00

^a 4,5 α -Epoxy-3,10,14-trihydroxy-17-methylmorphinan-6-one.

^b 4,5 α -Epoxy-3,14-dihydroxy-17-methylmorphinan-6,10-dione.

^c Process impurities, not included in the total degradation products.

^d 1-Bromo-4,5 α -epoxy-3,14-dihydroxy-17-methylmorphinan-6-one.

^e 2,2'-Bisoxymorphone.

■ 2S (USP39)

SPECIFIC TESTS

- **Bacterial Endotoxins Test** $\langle 85 \rangle$: NMT 238.1 USP Endotoxin Units/mg of oxymorphone hydrochloride
- **pH** $\langle 791 \rangle$: 2.7–4.5
- **Other Requirements:** It meets the requirements in *Injections* $\langle 1 \rangle$.

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in single-dose or in multiple-dose containers of Type I glass.

■ Store at 25°, excursions permitted between 15° and 30°, ■ 2S (USP39)

and protected from light.

Change to read:

● **USP Reference Standards** 〈 11 〉

USP Endotoxin RS

USP Oxymorphone RS

■ **USP Oxymorphone Related Compound A RS**

4,5 α -Epoxy-3,14-dihydroxy-17-methylmorphinan-6-one *N*-oxide.

C₁₇H₁₉NO₅ 317.34

■ 2S (USP39)

BRIEFING

Oxymorphone Hydrochloride Suppositories, USP 38 page 4721. It is proposed to omit this monograph, because the drug product as defined in the monograph is no longer marketed for either human or animal use.

(SM2: H. Cai.)

Correspondence Number—C148680

Comment deadline: September 30, 2015

Delete the following:

■ **Oxymorphone Hydrochloride Suppositories**

DEFINITION

~~Oxymorphone Hydrochloride Suppositories contain NLT 93.0% and NMT 107.0% of the labeled amount of oxymorphone hydrochloride (C₁₇H₁₉NO₄·HCl).~~

IDENTIFICATION

● **A:**

~~**Standard solution:** Similarly prepared as directed for the *Sample solution* by using USP Oxymorphone RS~~

~~**Sample solution:** Place a number of Suppositories, nominally equivalent to 5 mg of oxymorphone hydrochloride, in a 125-mL separator. Add 25 mL of 0.1 N hydrochloric acid, and shake without heating until the specimen is dissolved. Wash the solution with five 25-mL portions of chloroform, shaking the separator gently to avoid forming emulsions, and discard the chloroform washings. Adjust with 6 N ammonium hydroxide to a pH of 9.5, using short-range pH indicator paper, and extract with three 25-mL portions of chloroform, filtering the extracts through chloroform-moistened glass wool into a 200-mL round-bottom flask. Evaporate the combined extracts to dryness, using a rotary evaporator. Add 25 mL of 0.1 N hydrochloric acid, insert the stopper, and swirl to dissolve the residue.~~

~~**Acceptance criteria:** The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, concomitantly measured.~~

ASSAY● **Procedure**

Mobile phase: 0.05 M sodium borate adjusted to a pH of 9.1

Internal standard solution: 3 mg/mL of procaine hydrochloride in 0.01 N hydrochloric acid

Standard stock solution: 4.5 mg/mL of USP Oxymorphone RS in 0.01 N hydrochloric acid. Sonicate, if necessary, to effect solution.

Standard solution: 1.8 mg/mL of USP Oxymorphone RS prepared as follows. Transfer 10.0 mL of *Standard stock solution*, 10.0 mL of the *Internal Standard solution*, and 5.0 mL of 0.01 N hydrochloric acid to a 125 mL separator. Extract with four 25 mL portions of chloroform, discarding the chloroform layer each time. Transfer the aqueous layer to a suitable flask, and bubble filtered air through the solution for 10 min to remove final traces of chloroform.

Sample solution: Transfer a number of Suppositories nominally equivalent to about 50 mg of oxymorphone hydrochloride to a 125 mL separator. Add 15.0 mL of 0.01 N hydrochloric acid, 10.0 mL of *Internal Standard solution*, and 25 mL of chloroform. Shake until the Suppositories dissolve. Discard the chloroform layer. Extract the aqueous layer with three 25 mL portions of chloroform, discarding the chloroform each time. Transfer the aqueous layer to a suitable flask, and bubble filtered air through the solution for 10 min to remove final traces of chloroform.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 2.1 mm × 100 cm; packing L12

Flow rate: 1 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

[Note—The retention times for oxymorphone hydrochloride and procaine hydrochloride are about 5 and 7.5 min, respectively.]

Suitability requirements

Resolution: NLT 1.5 between oxymorphone hydrochloride and procaine hydrochloride

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) in the portion of Suppositories taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of oxymorphone hydrochloride to procaine hydrochloride from the *Sample solution*

R_S = peak response ratio of oxymorphone hydrochloride to procaine hydrochloride from the *Standard solution*

C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxymorphone hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80

~~M_r molecular weight of oxymorphone, 301.34~~

~~**Acceptance criteria:** 93.0%–107.0%~~

ADDITIONAL REQUIREMENTS

- ~~**Packaging and Storage:** Preserve in well-closed containers, and store in a refrigerator.~~
- ~~**USP Reference Standards** (11)~~
 - ~~USP Oxymorphone RS~~
 - 2S (USP39)

BRIEFING

Oxymorphone Hydrochloride Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated test methods, is proposed.

1. The stability-indicating liquid chromatographic procedures in the *Assay* and the test for *Organic Impurities* are based on analyses performed with the Zorbax Eclipse XDB C18 brand of L1 column. The typical retention time for oxymorphone is about 8.3 min in the *Assay* and the test for *Organic Impurities*.
2. The liquid chromatographic procedure in the *Dissolution* test is based on analyses also performed with the Zorbax Eclipse XDB C18 brand of L1 column. The typical retention time for oxymorphone is about 1.5 min.

(SM2: H. Cai.)

Correspondence Number—C127216

Comment deadline: September 30, 2015

Add the following:

■ Oxymorphone Hydrochloride Tablets

DEFINITION

Oxymorphone Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the oxymorphone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the oxymorphone peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY

● Procedure

Protect all solutions containing oxymorphone from light and to use clear glass HPLC vials.

Solution A: Dissolve 2.02 g of sodium 1-heptanesulfonate in 900 mL of water and add 100 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.

Solution B: Dissolve 2.02 g of sodium 1-heptanesulfonate in 750 mL of water and add 250

mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
35	0	100
40	0	100
40.1	100	0
50.1	100	0

Standard solution: 0.14 mg/mL of USP Oxymorphone RS in *Solution A*. Sonicate to dissolve if necessary.

Sample solution: Nominally 0.16 mg/mL of oxymorphone hydrochloride in *Solution A* prepared as follows. Transfer NLT 8 Tablets to a suitable volumetric flask and add about 50% of the final volume of *Solution A*. Sonicate for at least 15 min with occasional vigorous shaking until the Tablets disintegrate completely. Then shake for at least 20 min. Immediately dilute with *Solution A* to volume, and mix well. Immediately pass the solution through a suitable filter of 0.45- μ m pore size, discard the first 5 mL of the filtrate, and use the filtrate for analysis.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector

Assay: UV 230 nm

Identification test B: Diode array UV 200–360 nm

Column: 4.6-mm \times 7.5-cm; 3.5- μ m packing L1

Column temperature: 40 $^{\circ}$

Flow rate: 1.0 mL/min

Injection volume: 30 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of oxymorphone from the *Sample solution*

r_S = peak response of oxymorphone from the *Standard solution*

C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxymorphone hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80

M_{r2} = molecular weight of oxymorphone, 301.34

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution 〈 711 〉

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Mobile phase: Dissolve 2.02 g of sodium 1-heptanesulfonate in 800 mL of water and add 200 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.

Standard stock solution: 0.1 mg/mL of USP Oxymorphone RS in 0.1 N hydrochloric acid

Standard solution: ($L/1000$) mg/mL of USP Oxymorphone RS in water from the *Standard stock solution*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 7.5-cm; 3.5- μ m packing L1

Column temperature: 40 $^{\circ}$

Flow rate: 1.0 mL/min

Injection volume: 60 μ L

Run time: NLT 2.7 times the retention time of oxymorphone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response of oxymorphone from the *Sample solution*

r_S = peak response of oxymorphone from the *Standard solution*

C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80

M_{r2} = molecular weight of oxymorphone, 301.34

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) is dissolved.

- **Uniformity of Dosage Units** (905): Meet the requirements

IMPURITIES

- **Organic Impurities**

Protect all solutions containing oxymorphone from light and to use clear glass HPLC vials.

Solution A, Solution B, Mobile phase, Sample solution, and Chromatographic system:

Proceed as directed in the Assay.

System suitability stock solution A: 0.2 mg/mL of USP Oxymorphone Related Compound A RS prepared as follows. Transfer an amount of USP Oxymorphone Related Compound A RS to a suitable volumetric flask. Dissolve with 24% of the flask volume of 0.1 N hydrochloric acid and dilute with acetonitrile to volume.

System suitability stock solution B: 0.02 mg/mL of USP Oxymorphone Related Compound A RS in acetonitrile from *System suitability stock solution A*

System suitability stock solution C: 0.14 mg/mL of USP Oxymorphone RS in *Solution A*

System suitability solution: 0.0008 mg/mL of USP Oxymorphone Related Compound A RS in *System suitability stock solution C* from *System suitability stock solution B*

Standard solution: 0.00014 mg/mL of USP Oxymorphone RS in *Solution A* from *System suitability stock solution C*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between oxymorphone related compound A and oxymorphone, *System suitability solution*

Relative standard deviation: NMT 10%, *Standard solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U peak response of each individual degradation product from the *Sample solution*

r_T sum of peak responses from the *Sample solution*

F relative response factor of each individual degradation product (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
^a 4,5 α -Epoxy-3,10,14-trihydroxy-17-methylmorphinan-6-one.			

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
10-Hydroxyoxymorphone ^a	0.58	1.00	0.2
Oxymorphone related compound A (oxymorphone <i>N</i> -oxide)	0.81	1.09	0.2
Oxymorphone	1.00	1.00	—
10-Ketooxymorphone ^b	1.42	0.93	0.2
Oxycodone ^c	2.11	—	—
1-Bromooxymorphone ^{c,d}	2.22	—	—
2,2'-Bisoxymorphone ^e	2.36	1.61	0.2
Any individual unspecified degradation product	—	1.00	0.2
Total degradation products	—	—	1.5

^a 4,5 α -Epoxy-3,10,14-trihydroxy-17-methylmorphinan-6-one.

^b 4,5 α -Epoxy-3,14-dihydroxy-17-methylmorphinan-6,10-dione.

^c Process impurities, not included in the total degradation products.

^d 1-Bromo-4,5 α -epoxy-3,14-dihydroxy-17-methylmorphinan-6-one.

^e 2,2'-Bisoxymorphone.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

- **USP Reference Standards** { 11 }

USP Oxymorphone RS

USP Oxymorphone Related Compound A RS

4,5 α -Epoxy-3,14-dihydroxy-17-methylmorphinan-6-one *N*-oxide.

C₁₇H₁₉NO₅ 317.34

■ 2S (USP39)

BRIEFING

Oxymorphone Hydrochloride Extended-Release Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated test methods, is proposed.

1. The stability-indicating liquid chromatographic procedures in the *Assay* and the test for *Organic Impurities* are based on analyses performed with the Zorbax Eclipse XDB C18 brand of L1 column. The typical retention time for oxymorphone is about 5.8 min in the *Assay* and in the test for *Organic Impurities*.
2. The liquid chromatographic procedure in the *Dissolution* test is based on analyses

performed with the Synergi Polar-RP brand of L11 column. The typical retention time for oxymorphone is about 1.5 min.

(SM2: H. Cai.)

Correspondence Number—C116068

Comment deadline: September 30, 2015

Add the following:

■ Oxymorphone Hydrochloride Extended-Release Tablets

DEFINITION

Oxymorphone Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY

• **Procedure**

Solution A: Dissolve 2.34 g of sodium 1-octanesulfonate monohydrate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.80.

Solution B: Acetonitrile and methanol (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.00	77.0	23.0
2.50	77.0	23.0
17.50	54.0	46.0
25.00	31.0	69.0
25.05	1.5	98.5
32.50	1.5	98.5
32.55	77.0	23.0
38.00	77.0	23.0

Diluent: Methanol and phosphoric acid (1000:1)

Standard stock solution: 1.78 mg/mL of USP Oxymorphone RS in *Diluent*

Standard solution: 0.357 mg/mL of USP Oxymorphone RS in *Solution A* from the *Standard stock solution*

Sample stock solution: Nominally 2 mg/mL of oxymorphone hydrochloride in *Diluent* prepared as follows. Take NLT 8 Tablets, cut each into small pieces, and transfer to a suitable flask. Add a suitable volume of *Diluent* and shake for at least 16 h. Centrifuge at

3500 rpm for 5 min or until a clear supernatant is obtained.

Sample solution: Nominally 0.4 mg/mL of oxymorphone hydrochloride in *Solution A* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector

Assay: UV 230 nm

Identification test B: Diode array UV 200–360 nm

Column: 4.6-mm × 7.5-cm; 3.5-μm packing L1

Column temperature: 50°

Flow rate: 1.0 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxymorphone hydrochloride (C₁₇H₁₉NO₄·HCl) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of oxymorphone from the *Sample solution*

r_S = peak response of oxymorphone from the *Standard solution*

C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxymorphone hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80

M_{r2} = molecular weight of oxymorphone, 301.34

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution { 711 }

Medium: 45 mM phosphate buffer pH 4.50 (Dissolve 6.16 g of monobasic potassium phosphate in 1 L of water. Adjust with 1 N sodium hydroxide or phosphoric acid to a pH of 4.50); 900 mL

Apparatus 2: 50 rpm, with sinker. [Note—The Sotax Helix sinker can be used.]

Time: 1, 2, and 8 h

Mobile phase: Dissolve 1.54 g of ammonium acetate in 925 mL of water and mix well. Add 75 mL of acetonitrile and adjust with trifluoroacetic acid to a pH of 4.50.

Standard stock solution: 0.2 mg/mL of USP Oxymorphone RS in *Medium*

Standard solution: [(L/900) × (301.34/337.80)] mg/mL of USP Oxymorphone RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet

Sample solution: Withdraw 1.5 mL of the solution under test

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 7.5-cm; 4-µm packing L11

Column temperature: 60°

Flow rate: 2.0 mL/min

Injection volume: 50 µL

Run time: NLT 2 times the retention time of oxymorphone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxymorphone hydrochloride (C₁₇H₁₉NO₄·HCl) dissolved at each time point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

r_U = peak response of oxymorphone from the *Sample solution*

r_S = peak response of oxymorphone from the *Standard solution*

C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80

M_{r2} = molecular weight of oxymorphone, 301.34

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: See *Table 2*.

Table 2

Time point (<i>i</i>)	Time (h)	Amount Released (%)
1	1	20–40
2	2	35–55
3	8	NLT 80

- **Uniformity of Dosage Units** { 905 } : Meet the requirements

IMPURITIES

- **Organic Impurities**

Solution A, Solution B, Mobile phase, Diluent, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sensitivity solution: 0.357 µg/mL of USP Oxymorphone RS from the *Standard solution*

prepared as follows. Add 20% of the total volume of *Diluent* and dilute with *Solution A* to volume.

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U peak response of each individual degradation product from the *Sample solution*

r_T sum of peak responses from the *Sample solution*

F relative response factor of each individual degradation product (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any peaks less than 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxymorphone related compound A ^a (oxymorphone <i>N</i> -oxide)	0.57	1.11	0.2
10-Hydroxyoxymorphone ^b	0.70	1.14	0.2
Oxymorphone	1.00	—	—
10-Ketooxymorphone ^c	1.33	0.97	0.2
Oxycodone ^d	1.82	—	—
14-Hydroxycodeinone ^{d,e}	1.89	—	—
1-Bromooxymorphone ^{d,f}	1.89	—	—
2,2'-Bisoxymorphone ^g	2.28	2.36	0.2
Any individual unspecified degradation product	—	1.00	0.2
Total degradation products	—	—	1.0

^a 4,5 α -Epoxy-3,14-dihydroxy-17-methylmorphinan-6-one *N*-oxide.

^b 4,5 α -Epoxy-3,10,14-trihydroxy-17-methylmorphinan-6-one.

^c 4,5 α -Epoxy-3,14-dihydroxy-17-methylmorphinan-6,10-dione.

^d Process impurities, not included in the total degradation products.

^e 4,5 α -Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-7-ene-6-one.

f 1-Bromo-4,5 α -epoxy-3,14-dihydroxy-17-methylmorphinan-6-one.

g 2,2'-Bioxymorphone.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP Reference Standards** 〈 11 〉
USP Oxymorphone RS
- 2S (USP39)

BRIEFING

Polyethylene Glycol 3350, PF 39(6) [Nov.–Dec. 2013]. A new monograph proposal was presented in PF 39(6). The procedure in the *Assay* is based on a size exclusion chromatographic (SEC) method of analysis performed using the Waters Ultrahydrogel DP brand of L25 column. The procedure in the test for *Limit of Ethylene Glycol and Diethylene Glycol* is based on an HPLC method of analysis using the Tosoh Bioscience Tskgel G-Oligo-PW aqueous brand of L89 column. The procedure in the test for *Limit of Formaldehyde and Acetaldehyde* is based on an HPLC method of analysis using the Agilent Zorbax Eclipse XDB C8 brand of L7 column.

On the basis of comments and data received, it is proposed to make the following revisions to the proposal in PF 39(6):

1. In *Identification* test A, add *Infrared Absorption* 〈 197A 〉 as an alternative procedure.
2. In the *Assay*, change the *Mobile phase* from water to 50 μ g/mL of sodium azide in water, and modify the column temperature to match the detector temperature of 35°.
3. The implementation date for the deletion of *Heavy Metals* 〈 231 〉 will be updated to January 1, 2018, with the rest of the references to this chapter in USP 39.
4. In the test for *Limit of Formaldehyde and Acetaldehyde*, change the *Acceptance criteria* for formaldehyde from NMT 30 μ g/g to NMT 15 μ g/g.
5. Replace the procedure for *Apparent Weight-Average Molecular Weight and Polydispersity* with a specific test procedure that is based on an SEC method of analysis using the Ultrahydrogel 250 brand of L37 column. The retention time for polyethylene glycol 3350 is about 10 min.
6. Add a specification for *Hydroxyl Value*.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(EXC: H. Wang.)

Correspondence Number—C142682; C142763; C142772; C144465

Comment deadline: September 30, 2015

Add the following:**■ Polyethylene Glycol 3350**

Poly(oxy-1,2-ethanediyl), α -hydro- ω -hydroxy-;
1,2-Ethanediol, homopolymer [25322-68-3].

DEFINITION

Polyethylene Glycol is an addition polymer of ethylene oxide and water, represented by the formula $H(OCH_2CH_2)_nOH$, in which n represents the average number of oxyethylene groups. The apparent weight-average molecular weight is 3015–3685 g/mol (Da). It contains NLT 97.0% and NMT 103.0% of polyethylene glycol 3350, calculated on the anhydrous basis. It may contain a suitable antioxidant.

IDENTIFICATION**Change to read:**

- **A. Infrared Absorption** 〈 197F 〉

- or 〈 197A 〉: ■_{2S} (USP39)

Use a thin film of test specimen, melted if necessary, in the range from 4000 to 650 cm^{-1} ,

- when the measurement is performed by using 〈 197F 〉. ■_{2S} (USP39)

- **B. Chromatographic Identity**

- Analysis:** Proceed as directed in the *Assay*.

- Acceptance criteria:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY**Change to read:**

- **Procedure**

- Mobile phase:** Water

- 50 $\mu g/mL$ of sodium azide in water ■_{2S} (USP39)

Standard solution: 20 mg/mL of USP Polyethylene Glycol 3350 RS in *Mobile phase*

Sample solution: 20 mg/mL of Polyethylene Glycol 3350 in *Mobile phase*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: Differential refractive index

Columns

Guard: 6-mm \times 4-cm; 6- μm packing L25

Analytical: 7.8-mm \times 30-cm; 6- μm packing L25

Temperatures

Detector: $35 \pm 1^\circ$

Column: $24 \pm 1^\circ$

■ $35 \pm 1^\circ$ ■ 2S (USP39)

Flow rate: 0.8 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[Note—The retention time for polyethylene glycol 3350 is about 8.5 min.]

Suitability requirements

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of polyethylene glycol 3350 [$\text{H}(\text{OCH}_2\text{CH}_2)_n\text{OH}$] in the portion of Polyethylene Glycol 3350 taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Polyethylene Glycol 3350 RS in the *Standard solution* (mg/mL)

C_U = concentration of Polyethylene Glycol 3350 in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** { 281 }

Sample: 2 g

Analysis: Proceed as directed, moistening the residue with 2 mL of sulfuric acid.

Acceptance criteria: NMT 0.1%

Delete the following:

- **Heavy Metals** { 231 }

Test preparation: 4.0 g in 5.0 mL of 0.1 N hydrochloric acid. Dilute with water to 25 mL.

Acceptance criteria: NMT 5 μ g/g (Official 1-Dec-2015)

- **Limit of Ethylene Oxide and Dioxane**

Analysis: Proceed as directed in *Ethylene Oxide and Dioxane* { 228 }, Method II.

Acceptance criteria

Ethylene oxide: NMT 1 μ g/g

Dioxane: NMT 10 μ g/g

- **Limit of Ethylene Glycol and Diethylene Glycol**

Mobile phase: 50 μ g/mL of sodium azide in water

Eluant: Water

Standard stock solution: 10 mg/g of USP Diethylene Glycol RS and 10 mg/g of USP

Ethylene Glycol RS in *Eluant*

Standard solution: Transfer 0.1 g of *Standard stock solution* to a 100-mL volumetric flask. Dilute with water to volume. The *Standard solution* contains 0.01 mg/mL of USP Diethylene Glycol RS and 0.01 mg/mL of USP Ethylene Glycol RS.

Sample solution: 10 mg/mL of Polyethylene Glycol 3350 in *Eluant*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Differential refractive index

Column: 7.8-mm × 30-cm; 7-μm packing L89, 125-Å pore size

Temperatures

Detector: 35 ± 1°

Column: 35 ± 1°

Flow rate: 0.5 mL/min

Injection volume: 100 μL

Run time: 30 min

System suitability

Sample: *Standard solution*

[Note—The relative retention times for diethylene glycol and ethylene glycol are 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.5 between diethylene glycol and ethylene glycol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of diethylene glycol (or ethylene glycol) in the portion of Polyethylene Glycol 3350 taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diethylene glycol (or ethylene glycol) from the *Sample solution*

r_S = peak response of diethylene glycol (or ethylene glycol) from the *Standard solution*

C_S = concentration of USP Diethylene Glycol RS (or USP Ethylene Glycol RS) in the *Standard solution* (mg/mL)

C_U = concentration of Polyethylene Glycol 3350 in the *Sample solution* (mg/mL)

Acceptance criteria

Ethylene glycol: NMT 0.062%

Sum of diethylene glycol and ethylene glycol: NMT 0.2%

Change to read:

- **Limit of Formaldehyde and Acetaldehyde**

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time ^a (min)	Solution A (%)	Solution B (%)
0	50	50
11	0	100
a The equilibration time is 5 min.		

[Note—Use amber containers and amber autosampler vials.]

2,4-DNPH solution: Transfer 250 mg of 2,4-dinitrophenylhydrazine (2,4-DNPH) to a 50-mL volumetric flask, add 20.0 mL of acetonitrile, and swirl to mix. Add 3.0 mL of hydrochloric acid to the flask, and swirl to mix. Sonicate until all solids are dissolved, and dilute with acetonitrile to volume.

Formaldehyde-2,4-DNPH solution: 100 µg/mL of aldehyde equivalent in acetonitrile¹

Acetaldehyde-2,4-DNPH solution: 1000 µg/mL of aldehyde equivalent in acetonitrile²

Formaldehyde stock solution: Transfer 500 µL of *Formaldehyde-2,4-DNPH solution* to a 10-mL volumetric flask. Dilute with acetonitrile to volume. The formaldehyde concentration is 5.0 µg/mL.

Acetaldehyde stock solution: Transfer 500 µL of *Acetaldehyde-2,4-DNPH solution* to a 10-mL volumetric flask. Dilute with acetonitrile to volume. The acetaldehyde concentration is 50.0 µg/mL.

Standard solution: Transfer 1.5 mL of *Formaldehyde stock solution* and 1.2 mL of *Acetaldehyde stock solution* to a 10-mL volumetric flask. Dilute with acetonitrile to volume, and mix well. The concentrations of formaldehyde and acetaldehyde are 0.75 and 6.0 µg/mL, respectively.

Sample solution: Transfer 0.5 g of Polyethylene Glycol 3350 to a 10-mL volumetric flask. Add 1.0 mL of acetonitrile to the flask, and swirl to dissolve the sample. Add 2.0 mL of *2,4-DNPH solution* to the flask, and swirl to mix. Allow the solution to react for 15 min, then dilute with acetonitrile to volume.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 360 nm

Column: 3.0-mm × 15-cm; 3.5-µm packing L7, 80-Å pore size

Column temperature: 30 ± 1°

Flow rate: 0.65 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for formaldehyde and acetaldehyde are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 2.0 between formaldehyde and acetaldehyde

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the content of formaldehyde (or acetaldehyde), in µg/g, in the portion of Polyethylene Glycol 3350 taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U)$$

r_U peak response of formaldehyde (or acetaldehyde) from the *Sample solution*

r_S peak response of formaldehyde (or acetaldehyde) from the *Standard solution*

C_S concentration of formaldehyde (or acetaldehyde) in the *Standard solution* ($\mu\text{g/mL}$)

C_U concentration of Polyethylene Glycol 3350 in the *Sample solution* (g/mL)

Acceptance criteria

Formaldehyde: NMT 30 $\mu\text{g/g}$

■ NMT 15 $\mu\text{g/g}$ ■ 2S (USP39)

Sum of formaldehyde and acetaldehyde: NMT 200 $\mu\text{g/g}$

SPECIFIC TESTS

Change to read:

- **Apparent Weight-Average Molecular Weight and Polydispersity**

Mobile phase: ~~Water~~

Standard solution: ~~5 mg/mL each of five polyethylene glycol standards with molecular weights of 960, 1960, 3020, 6430, and 12,300 Daltons (g/mol) or equivalent standards ranging from 900 to 15,000 Daltons (g/mol) in *Mobile phase*~~

Dextran solution: ~~5 mg/mL of dextran standard of weight-average molecular weight of 3,500,000 Daltons in *Mobile phase*~~

Sample solution: ~~5 mg/mL of Polyethylene Glycol 3350 in *Mobile phase*~~

Chromatographic system

(See *Chromatography* ~~621~~, *System Suitability*.)

Mode: ~~LC~~

Detector: ~~Differential refractive index~~

Columns: ~~Two 7.5 mm \times 30 cm; 8 μm packing L##~~

Temperatures

Detector: ~~$35 \pm 1^\circ$~~

Column: ~~$35 \pm 1^\circ$~~

Flow rate: ~~1.0 mL/min~~

Injection volume: ~~20 μL~~

Run time: ~~40 min~~

Analysis

Samples: ~~*Standard solution*, *Dextran solution*, and *Sample solution*~~

~~Determine the void volume using *Dextran solution*.~~

~~Separately inject equal volumes of the *Standard solution* and the *Sample solution* into the chromatograph, record the chromatograms, and determine the elution peak maxima and the corresponding retention times for the five polyethylene glycol standards.~~

Calibration curve: ~~Plot the retention times on the x-axis against the $\log M_p$ (M_p : peak molecular weights) on the y-axis for the peaks from the polyethylene glycol standard to generate a calibration curve using suitable gel permeation chromatography or size~~

exclusion chromatography (GPC/SEC) software.

Calculations: Compute the data using the same GPC/SEC software, and determine the number- and weight-average molecular weights, M_N and M_W , in g/mol, respectively, for the chromatogram of the *Sample solution*.

Calculate the polydispersity for Polyethylene Glycol 3350:

$$\text{Result} = M_W/M_N$$

M_W weight-average molecular weight from the *Sample solution* (g/mol)

M_N number-average molecular weight from the *Sample solution* (g/mol)

■ Mobile phase:

Water

Standard solution: 1.0 mg/mL each of five polyethylene glycol standards with molecular weights of 1000, 2000, 3000, 4000, and 6000 g/mol (Da) in *Mobile phase*. Pass a portion of the solution through a 0.45- μ m PTFE (polytetrafluoroethylene) syringe filter.³ Discard the first 2 mL, and transfer the solution for analysis.

Sample solution: 1.0 mg/mL of Polyethylene Glycol 3350 in *Mobile phase*. Pass a portion of the solution through a 0.45- μ m PTFE (polytetrafluoroethylene) syringe filter.³ Discard the first 2 mL, and transfer the solution for analysis.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: Differential refractive index

Column: 7.8-mm \times 30-cm; 6- μ m packing L37

Temperatures

Detector: 35 \pm 1 $^\circ$

Column: 35 \pm 1 $^\circ$

Flow rate: 0.8 mL/min

Injection volume: 10 μ L

Run time: 18 min

Analysis

Samples: *Standard solution* and *Sample solution*

Separately inject equal volumes of the *Standard solution* and the *Sample solution* into the chromatograph, record the chromatograms, and determine the elution peak maxima and the corresponding retention times for the five polyethylene glycol standards.

Calibration curve: Plot the retention times on the x-axis against the log M_p (M_p : peak molecular weights) on the y-axis for the peaks from the polyethylene glycol standard to generate a calibration curve using suitable gel permeation chromatography or size exclusion chromatography (GPC/SEC) software.

Calculations: Compute the data using the same GPC/SEC software, and determine the number- and weight-average molecular weights, M_N and M_W , in g/mol (Da), respectively, for the chromatogram of the *Sample solution*.

Calculate the polydispersity for Polyethylene Glycol 3350:

$$\text{Result} = M_W/M_N$$

$M_{\overline{w}}$ weight-average molecular weight from the *Sample solution* (g/mol)

$M_{\overline{n}}$ number-average molecular weight from the *Sample solution* (g/mol)

■ 2S (USP39)

Acceptance criteria: The value of apparent weight-average molecular weight is 3015–3685 g/mol. Polydispersity is 90%–110% of the value stated on the label or within the range indicated on the label.

Add the following:

● **Hydroxyl Value**

Phthalic anhydride solution: Place 49.0 g of phthalic anhydride into an amber bottle, and dissolve in 300 mL of pyridine from a freshly opened bottle or pyridine that has been freshly distilled over phthalic anhydride. Shake vigorously until completely dissolved. Add 7 g of imidazole, swirl carefully to dissolve, and allow to stand for 16 h before using.

Sample solution: Carefully introduce 25.0 mL of *Phthalic anhydride solution* into a dry, heat-resistant pressure bottle. Add 12.0 g of Polyethylene Glycol 3350. Add 25 mL of pyridine, from a freshly opened bottle or pyridine that has been freshly distilled over phthalic anhydride. Swirl to dissolve, insert the stopper in the bottle, and wrap it securely in a cloth bag.

Blank: 25.0 mL of *Phthalic anhydride solution* plus any additional pyridine added to the bottle

Analysis: Immerse the bottle in a water bath maintained at a temperature between 96° and 100°, to the same depth as that of the mixture in the bottle. Remove the bottles from the bath after 5 min and, without unwrapping, swirl for 30 s to homogenize. Heat in the water bath for 60 min, then remove from the bath, and allow it to cool to room temperature. Uncap the bottle carefully to release any pressure, remove from the bag, add 10 mL of water, and swirl thoroughly. Wait 2 min, add 0.5 mL of a solution of phenolphthalein in pyridine (1 in 100), and titrate with 0.5 N sodium hydroxide VS to the first pink color that persists for 15 s. Perform a blank determination. Calculate the hydroxyl value:

$$\text{Result} = [M_r \times (V_B - V_S) \times N]/W$$

M_r molecular weight of potassium hydroxide, 56.11

V_B volume of 0.5 N sodium hydroxide consumed in the blank test (mL)

V_S volume of 0.5 N sodium hydroxide consumed in the actual test (mL)

N exact normality of the sodium hydroxide solution

W weight of Polyethylene Glycol 3350 taken for the test (g)

Acceptance criteria: 30–38 ■ 2S (USP39)

● **Acidity and Alkalinity**

Sample solution: Dissolve 5.0 g of Polyethylene Glycol 3350 in 100 mL of carbon dioxide-free water.

Analysis: Add 0.3 mL of a saturated solution of potassium chloride into the *Sample*

solution. The test solution should be maintained at $25 \pm 2^\circ$ during the measurement.

Measure the pH following *pH* 〈 791 〉.

Acceptance criteria: 4.5–7.5

● **Water Determination** 〈 921 〉, *Method I*

Sample: 2.0 g

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, protected from direct sunlight. Store at room temperature.
- **Labeling:** Label it to indicate its polydispersity (M_w/M_n) or its polydispersity range. Label it to indicate the name and amount of any added antioxidant.
- **USP Reference Standards** 〈 11 〉
 - USP Diethylene Glycol RS
 - USP Ethylene Glycol RS
 - USP Polyethylene Glycol 3350 RS

■ 2S (USP39)

■ 1

Available from Sigma-Aldrich Corporation, or equivalent. ■ 2S (USP39)

■ 2

Available from Sigma-Aldrich Corporation, or equivalent. ■ 2S (USP39)

■ 3

Millipore® Millex® LCR HPLC syringe filters with hydrophilic PTFE membrane is suitable, or any other equivalent filter. ■ 2S (USP39)

BRIEFING

Potassium Chloride in Dextrose Injection, *USP 38* page 4935. It is proposed to make the following revisions to the monograph.

1. In preparation for the omission of the flame tests from *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to delete the reference to 〈 191 〉 under *Identification* test *B* and include a complete description of the flame test in the monograph. Manufacturers are encouraged to submit a replacement wet chemistry or an instrumental procedure for consideration by the Expert Committee.
2. An equation is added to calculate the percentage of the labeled amount of potassium chloride in the *Assay* for *Potassium Chloride*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R.S. Prasad, M. Koleck.)
Correspondence Number—C158124

Comment deadline: September 30, 2015

Potassium Chloride in Dextrose Injection

DEFINITION

Potassium Chloride in Dextrose Injection is a sterile solution of Potassium Chloride and Dextrose in Water for Injection. It contains NLT 95.0% and NMT 110.0% of the labeled amount of potassium chloride (KCl) and NLT 95.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It contains no antimicrobial agents.

IDENTIFICATION

• A.

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection in water

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

Delete the following:

- ~~• B. Identification Tests—General, Potassium (191): Meets the requirements of the Flame test ■ 2S (USP39)~~

Add the following:

- **B.** The sample imparts a violet color to a nonluminous flame. Since the presence of small quantities of sodium masks the color, screen out the yellow color produced by the sodium by viewing through a blue filter that blocks emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.] ■ 2S (USP39)

- **C. Identification Tests—General (191), Chloride:** Meets the requirements

ASSAY

• Dextrose

Sample solution: Nominally 20–50 mg/mL of dextrose from Injection prepared as follows.

Transfer a volume of Injection, containing 2–5 g of dextrose, to a 100-mL volumetric flask.

Add 0.2 mL of 6 N ammonium hydroxide and dilute with water to volume.

Analysis

Sample: *Sample solution*

Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation (781)*).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* ($^{\circ}$)

l = length of the polarimeter tube (dm)

α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)

$M_{\overline{r}}$ = molecular weight of dextrose monohydrate, 198.17

$M_{\overline{z}}$ = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 95.0%–105.0%

Change to read:

● **Potassium Chloride**

Sample solution: Transfer a volume of Injection, equivalent to 75–150 mg of potassium chloride, to a conical flask. Add water, if necessary, to bring the volume to 10 mL, and add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Indicator: Eosin Y TS

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

Each mL of *Titrant* is equivalent to 7.455 mg of potassium chloride (KCl).

■ Calculate the percentage of the labeled amount of potassium chloride (KCl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V

= *Titrant* volume consumed by the *Sample solution* (mL)

N

= actual normality of the *Titrant* (mEq/mL)

F

= equivalency factor, 74.55 mg/mEq

W

= nominal amount of potassium chloride in the *Sample solution* (mg)

■ 2S (USP39)

Acceptance criteria: 95.0%–110.0%

IMPURITIES

● **Limit of 5-Hydroxymethylfurfural and Related Substances**

Sample solution: Nominally 2.0 mg/mL of dextrose from Injection in water

Instrumental conditions

Mode: UV

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution and Blank*

Determine the absorbance of the *Sample solution*.

Acceptance criteria: NMT 0.25

SPECIFIC TESTS

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 0.5 USP Endotoxin Units/mL
- **pH** 〈 791 〉
Sample solution: Nominally 5% of dextrose from a portion of Injection in water
Acceptance criteria: 3.5–6.5
- **Other Requirements:** It meets the requirements under *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers are preferably Type I or Type II.
- **Labeling:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol/mL. The content of potassium, in mEq, is prominently displayed on the label.
- **USP Reference Standards** 〈 11 〉
USP Endotoxin RS

BRIEFING

Potassium Chloride in Dextrose and Sodium Chloride Injection, *USP 38* page 4936. It is proposed to make the following revisions to the monograph.

1. In preparation for the omission of the flame tests from *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to delete the reference to 〈 191 〉 under *Identification* test *A* and include a complete description of the flame test in the monograph. Manufacturers are encouraged to submit a replacement wet chemistry or an instrumental procedure for consideration by the Expert Committee.
2. An equation is added to calculate the percentage of the labeled amount of chloride in the *Assay* for *Chloride*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R.S. Prasad, M. Koleck.)

Correspondence Number—C158125

Comment deadline: September 30, 2015

Potassium Chloride in Dextrose and Sodium Chloride Injection

DEFINITION

Potassium Chloride in Dextrose and Sodium Chloride Injection is a sterile solution of Potassium Chloride, Dextrose, and Sodium Chloride in Water for Injection. It contains NLT 95.0% and NMT 110.0% of the labeled amounts of potassium (K) and chloride (Cl) and NLT 95.0% and NMT 105.0% of the labeled amounts of dextrose ($C_6H_{12}O_6 \cdot H_2O$) and sodium (Na). It contains no antimicrobial agents.

IDENTIFICATION

Delete the following:

- ~~A. Identification Tests—General, Sodium (191): Meets the requirements of the flame test. ■ 2S (USP39)~~

Add the following:

- A. The sample imparts an intense yellow color to a nonluminous flame. ■ 2S (USP39)
- B.
Analysis: To 2 mL of Injection, add 5 mL of sodium cobaltinitrite TS.
Acceptance criteria: A yellow precipitate is formed immediately. If necessary, centrifuge the solution and examine the precipitate (presence of potassium).
- C. Identification Tests—General (191), Chloride: Meets the requirements
- D.
Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection in water
Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.
Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

ASSAY

Change to read:

- Chloride
Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride, to a suitable conical flask. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Endpoint detection: Visual
Analysis
Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint. Each mL of *Titrant* is equivalent to

3.545 mg of chloride (Cl). Each mg of chloride is equivalent to 0.0282 mEq of chloride (Cl).

- Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V

= *Titrant* volume consumed by the *Sample solution* (mL)

N

= actual normality of the *Titrant* (mEq/mL)

F

= equivalency factor, 35.45 mg/mEq

W

= nominal amount of chloride in the *Sample solution* (mg)

- 2S (USP39)

Acceptance criteria: 95.0%–110.0%

- **Dextrose**

Sample solution: Nominally 20–50 mg/mL of dextrose from Injection prepared as follows.

Transfer a volume of Injection, containing 2–5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide and dilute with water to volume.

Analysis

Sample: *Sample solution*

Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* 〈 781 〉).

Calculate the percentage of the labeled amount of dextrose (C₆H₁₂O₆·H₂O) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* (°)

l = length of the polarimeter tube (dm)

α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 95.0%–105.0%

Change to read:

- **Potassium and Sodium**

Internal standard solution: 1.04 mg/mL of lithium nitrate in water prepared as follows.

Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic

surfactant, and dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (equivalent to 1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride

■(equivalent to 1 mEq/mL of sodium) ■2S (USP39)

prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with water to volume.

Standard stock solution: 0.0391J mg/mL of potassium (K) from the *Potassium stock solution* and 0.02299J' mg/mL of sodium (Na) from the *Sodium stock solution* prepared as follows. Transfer 0.1J mL of the *Potassium stock solution* and 0.1J' mL of the *Sodium stock solution* to a 100-mL volumetric flask, where J and J' are the labeled amounts in (mEq/L) of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 500-mL volumetric flask, and dilute with the *Internal standard solution* to volume.

Sample solution: Transfer 5.0 mL of Injection to a 500-mL volumetric flask, and dilute with the *Internal standard solution* to volume.

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: 766 nm

Sodium: 589 nm

Lithium: 671 nm

Blank: *Internal standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and the *Sample solution*.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{U}}$ emission reading ratio of potassium to lithium from the *Sample solution*

$R_{\bar{S}}$ emission reading ratio of potassium to lithium from the *Standard solution*

$C_{\bar{S}}$ concentration of potassium (K) in the *Standard stock solution* (mg/mL)

$C_{\bar{U}}$ nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[Note—Each mg of potassium is equivalent to 0.02558 mEq of potassium.]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\bar{U}}$ emission reading ratio of sodium to lithium from the *Sample solution*

$R_{\bar{S}}$ emission reading ratio of sodium to lithium from the *Standard solution*

$C_{\bar{S}}$ concentration of sodium (Na) in the *Standard stock solution* (mg/mL)

$C_{\bar{c}}$ nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[Note—Each mg of sodium is equivalent to 0.04350 mEq of sodium.]

Acceptance criteria

Potassium: 95.0%–110.0%

Sodium: 95.0%–105.0%

IMPURITIES

Delete the following:

•• Heavy Metals 〈 231 〉

Sample solution: Transfer a volume of Injection as determined by the calculation below to an appropriate vessel.

Calculate the volume of Injection to use to two significant figures:

$$\text{Result} = 0.2 / [(G_K L_K) + (G_D L_D) + (G_S L_S)]$$

G_K labeled amount of potassium chloride in each 100 mL of Injection (g)

L_K limit of heavy metals for potassium chloride, 0.001%

G_D labeled amount of dextrose in each 100 mL of Injection (g)

L_D limit of heavy metals for dextrose, 0.0005%

G_S labeled amount of sodium chloride in each 100 mL of Injection (g)

L_S limit of heavy metals for sodium chloride, 0.0005%

Adjust the volume by evaporation or addition of water to 25 mL, as necessary: it passes the test.

Acceptance criteria: Meets the requirements • (Official 1-Dec-2015)

• 5-Hydroxymethylfurfural and Related Substances

Sample solution: Nominally 2.0 mg/mL of dextrose from Injection in water

Instrumental conditions

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution* and *Blank*

Acceptance criteria: NMT 0.25

SPECIFIC TESTS

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 0.5 USP Endotoxin Units/mL
- **pH** 〈 791 〉: 3.5–6.5
- **Other Requirements:** It meets the requirements under *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass

containers are preferably of Type I or Type II glass.

- **Labeling:** The label states the potassium, sodium, and chloride contents in terms of mEq in a given volume. The label also states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.
- **USP Reference Standards** 〈 11 〉
USP Endotoxin RS

BRIEFING

Potassium Chloride in Lactated Ringer's and Dextrose Injection, USP 38 page 4937. It is proposed to make the following revisions to the monograph:

1. In preparation for the omission of the flame tests from *Identification Tests—General* 〈 191 〉, proposed in PF 41(2) [Mar.–Apr. 2015], it is proposed to delete the references to *Sodium* and *Potassium* in 〈 191 〉 under *Identification* test *B*, and include a complete description of each flame test in the monograph. Manufacturers are encouraged to submit replacement wet chemistry or instrumental procedures for consideration by the Expert Committee.
2. Update the *Assay* procedure for *Calcium* by removing an unneeded reference to the equivalent amount of calcium in the *Sample solution*.
3. Add or revise equations in the *Assay* procedures for *Calcium*, for *Chloride*, for *Lactate*, for *Potassium Chloride*, and for *Sodium* to be consistent with the *Definition* section.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM4: H. Joyce, M. Koleck.)

Correspondence Number—C158118

Comment deadline: September 30, 2015

Potassium Chloride in Lactated Ringer's and Dextrose Injection

DEFINITION

Potassium Chloride in Lactated Ringer's and Dextrose Injection is a sterile solution of Calcium Chloride, Potassium Chloride, Sodium Chloride, and Sodium Lactate in Water for Injection. It contains, in each 100 mL, NLT 285.0 mg and NMT 315.0 mg of sodium [as sodium chloride (NaCl) and anhydrous sodium lactate (C₃H₅NaO₃)], NLT 4.90 mg and NMT 6.00 mg of calcium (Ca) [equivalent to NLT 18.0 mg and NMT 22.0 mg of calcium chloride (CaCl₂·2H₂O)], and NLT 231.0 mg and NMT 261.0 mg of lactate (C₃H₅O₃) [equivalent to NLT 290.0 mg and NMT 330.0 mg of anhydrous sodium lactate (C₃H₅NaO₃)]. It contains NLT 95.0% and NMT 105.0% of the labeled amount of potassium chloride (KCl), NLT 90.0% and NMT 105.0% of the labeled amount of dextrose (C₆H₁₂O₆·H₂O), and NLT 90.0% and NMT 110.0% of the labeled amount of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride (CaCl₂·2H₂O)]. It contains no antimicrobial agents.

IDENTIFICATION

- **A.**

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection in water

Analysis: Add a few drops of *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

Change to read:

- **B. Identification Tests—General** (191), ~~Sodium, (191) Potassium,~~

- **2S (USP39)**

Calcium and Chloride: Meets the requirements of the flame tests for ~~Sodium and Potassium,~~

- **2S (USP39)**

of the ammonium oxalate test for *Calcium* and the test for *Chloride*.

Add the following:

- **C. Sodium:** The sample imparts an intense yellow color to a nonluminous flame. ■ **2S (USP39)**

Add the following:

- **D. Potassium:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

- **2S (USP39)**

Change to read:

- ~~C.~~

- **E.** ■ **2S (USP39)**

The retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Lactate*.

ASSAY

Change to read:

- **Calcium**

[Note—Concentrations of the *Standard solution* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Lanthanum chloride solution: 88.45 g/L of *lanthanum chloride* prepared as follows.

Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Calcium stock solution: 1 mg/mL of calcium (Ca) prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of *diluted hydrochloric acid*, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Lanthanum chloride solution*, add 1.0, 1.5, and 2.0 mL of *Calcium stock solution*, respectively. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 30.0 mL of Injection (~~equivalent to about 1.6 mg of calcium~~)

■ ■ 2S (USP39)

to a 100-mL volumetric flask containing 5.0 mL of *Lanthanum chloride solution*. Dilute with water to volume.

Blank: Transfer 5.0 mL of *Lanthanum chloride solution* to a 100-mL volumetric flask and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* { 852 } .)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in mg/mL, of calcium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (*C*), in mg/mL, of calcium in the *Sample solution*.

Calculate the quantity (mg) of calcium (Ca) in each 100 mL of Injection taken:

$$\text{Result} = C/3$$

~~C = concentration of calcium in the *Sample solution*, as determined from the graph (mg/mL)~~

■

$$\text{Result} = C \times D \times F$$

C

= concentration of calcium in the *Sample solution*, as determined from the graph (mg/mL)

D

= dilution factor of the *Sample solution*, 3.3

F

= conversion factor for each 100 mL of Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 4.90–6.00 mg of calcium (Ca) in each 100 mL

Change to read:

● **Chloride**

Sample solution: Transfer 10 mL of Injection into a conical flask. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint. ~~Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl).~~

- Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V

= volume of *Titrant* consumed by the *Sample solution* (mL)

N

= actual normality of the *Titrant* (mEq/mL)

F

= equivalency factor, 35.45 mg/mEq

W

= nominal amount of chloride in the *Sample solution* (mg)

- 2S (USP39)

Acceptance criteria: 90.0%–110.0%

- **Dextrose**

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis

Sample: *Sample solution*

Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* 〈 781 〉).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution*, in degrees

l = length of the polarimeter tube, in decimeters

α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution*, g/100 mL

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–105.0%

Change to read:

- **Lactate**

Mobile phase: Add 1 mL of formic acid and 1 mL of dicyclohexylamine per L of water.

System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium

Lactate RS in water

Standard solution: 3 mg/mL of USP Sodium Lactate RS in water

Sample solution: Use the undiluted Injection

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between acetate and lactate, *System suitability solution*

Tailing factor: NMT 2.0 for lactate, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (mg/mL) of lactate ($C_3H_5O_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sodium Lactate in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of lactate, 89.07

M_{r2} = molecular weight of anhydrous sodium lactate, 112.06

■ Calculate the quantity (mg) of lactate ($C_3H_5O_3$) in each 100 mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V$$

r_U

= peak response of lactate from the *Sample solution*

r_S

= peak response of lactate from the *Standard solution*

C_S

= concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)

M_{r1}

= molecular weight of lactate, 89.07

M_{r2}

= molecular weight of anhydrous sodium lactate, 112.06

V

= volume of the Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 231.0–261.0 mg of lactate ($C_3H_5O_3$) in each 100 mL

Change to read:

● **Potassium**

Solution A: Suitable nonionic wetting agent (1 in 500)

Standard stock solution A: 100 µg/mL of potassium in water prepared as follows. Dissolve 190.7 mg of potassium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer the resulting solution to a 1-L volumetric flask, and dilute with water to volume.

Standard stock solution B: 10.93 mg/mL of sodium chloride in water

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of potassium prepared as follows. Transfer 10 mL of *Standard stock solution B* to each of four 100-mL volumetric flasks containing 10.0 mL of *Solution A*. To each flask add, respectively, 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution A*, and dilute with water to volume.

Sample solution: ~~Transfer 10 mL of Injection to a 100-mL volumetric flask. Add 10.0 mL of *Solution A*. Dilute with water to volume.~~

■ Nominally 0.015 mg/mL of potassium prepared as follows. Transfer a suitable portion of Injection into an appropriate volumetric flask. Add 10% of the final flask volume of *Solution A*. Dilute with water to volume. ■ 2S (USP39)

Blank: Transfer 10 mL of *Standard stock solution B* to a 100-mL volumetric flask containing 10.0 mL of *Solution A*. Dilute with water to volume.

Instrumental conditions

Mode: Atomic emission spectrophotometry

Emission wavelength: 766 nm

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set a suitable flame photometer for maximum transmittance at a wavelength of 766 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of potassium. From the graph so obtained, read the percentage transmittance of the *Sample solution*. ~~and calculate the potassium content, in mg per 100 mL, of Injection.~~

■ Calculate the percentage of the labeled amount of potassium chloride (KCl) in the portion of Injection taken:

$$\text{Result} = (C/C_U) \times (M_r/A_r) \times 100$$

C

= concentration of potassium in the *Sample solution*, as determined from the graph (mg/mL)

C_U

= nominal concentration of potassium in the *Sample solution* (mg/mL)

M_r

= molecular weight of potassium chloride, 74.55

A_r

= atomic weight of potassium, 39.10

■ 2S (USP39)

Acceptance criteria: 95.0%–105.0%

Change to read:

• **Sodium**

Solution A: Suitable nonionic wetting agent (1 in 500)

Standard stock solution: 100 µg/mL of sodium in water prepared as follows. Dissolve

254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer the resulting solution to a 1-L volumetric flask, and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium prepared as follows.

Transfer 10 mL of *Solution A* to each of four 100-mL volumetric flasks. To each flask add, respectively, 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*, and dilute with water to volume.

Sample solution: Transfer 5 mL of Injection to a 1-L volumetric flask containing 100 mL of *Solution A*. Dilute with water to volume.

Blank: Transfer 10 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Atomic emission spectrophotometry

Emission wavelength: 589 nm

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set a suitable flame photometer for maximum transmittance at a wavelength of 589 nm.

Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of sodium. From the graph so obtained, read the percentage transmittance of the *Sample solution*. ~~and calculate the sodium content, in mg per 100 mL, of Injection.~~

■ Calculate the quantity (mg) of sodium (Na) in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C

= concentration of sodium in the *Sample solution*, as determined from the graph (mg/mL)

D

= dilution factor of the *Sample solution*, 200

F

= conversion factor for each 100 mL of Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 285.0–315.0 mg of sodium (Na) in each 100 mL

IMPURITIES

Delete the following:

●● Heavy Metals (231)

Sample solution: Transfer a volume of Injection as determined by the calculation below to an appropriate vessel. Adjust the volume by evaporation or addition of water to 25 mL, as necessary.

Calculate the volume of Injection to use to two significant figures:

$$\text{Result} = 0.2 / [(G_{S\bar{L}S}) + (G_{K\bar{L}K}) + (G_{C\bar{L}C}) + (G_{L\bar{L}L}) + (G_{D\bar{L}D})]$$

$G_{S\bar{L}S}$ labeled amount of sodium chloride in each 100 mL of Injection (g)

$L_{S\bar{L}S}$ limit of heavy metals under *Sodium Chloride*

$G_{K\bar{L}K}$ labeled amount of potassium chloride in each 100 mL of Injection (g)

$L_{K\bar{L}K}$ limit of heavy metals under *Potassium Chloride*

$G_{C\bar{L}C}$ labeled amount of calcium chloride in each 100 mL of Injection (g)

$L_{C\bar{L}C}$ limit of heavy metals under *Calcium Chloride*

$G_{L\bar{L}L}$ labeled amount of sodium lactate in each 100 mL of Injection (g)

$L_{L\bar{L}L}$ limit of heavy metals under *Sodium Lactate*

$G_{D\bar{L}D}$ labeled amount of dextrose in each 100 mL of Injection (g)

$L_{D\bar{L}D}$ limit of heavy metals under *Dextrose*

Acceptance criteria: Meets the requirements (Official 1-Dec-2015)

● Limit of 5-Hydroxymethylfurfural and Related Substances

Sample solution: Nominally 2.0 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) from Injection in water

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution* and *Blank*

Determine the absorbance of the *Sample solution* with a suitable spectrophotometer.

Acceptance criteria: The absorbance is NMT 0.25.

SPECIFIC TESTS

- **Bacterial Endotoxins Test (85):** It contains NMT 0.5 USP Endotoxin Units/mL.
- **pH (791):** 3.5–6.5
- **Other Requirements:** It meets the requirements in *Injections (1)*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **Labeling:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL. The label includes also the warning: "Not for use in the treatment of lactic acidosis."
- **USP Reference Standards** { 11 }
USP Endotoxin RS
USP Sodium Lactate RS

BRIEFING

Potassium Chloride in Sodium Chloride Injection, *USP 38* page 4938. It is proposed to make the following revisions to the monograph:

1. In preparation for the omission of the flame tests from *Identification Tests—General* { 191 }, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to delete the reference to *Sodium* in { 191 } under *Identification* test A and include a complete description of the flame test in the monograph. Manufacturers are encouraged to submit a replacement wet chemistry or an instrumental procedure for consideration by the Expert Committee.
2. An equation is added to calculate the percentage of the labeled amount of chloride in the *Assay* for *Chloride*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: R.S. Prasad, M. Koleck.)

Correspondence Number—C158126

Comment deadline: September 30, 2015

Potassium Chloride in Sodium Chloride Injection**DEFINITION**

Potassium Chloride in Sodium Chloride Injection is a sterile solution of Potassium Chloride and Sodium Chloride in Water for Injection. It contains NLT 95.0% and NMT 110.0% of the labeled amounts of potassium (K) and chloride (Cl) and NLT 95.0% and NMT 105.0% of the labeled amount of sodium (Na). It contains no antimicrobial agents.

IDENTIFICATION**Delete the following:**

- ~~**A. Identification Tests—General, Sodium** { 191 }:~~ Meets the requirements of the flame test ■ 2S (*USP39*)

Add the following:

- **A. Sodium:** The sample imparts an intense yellow color to a nonluminous flame. ■ 2S (*USP39*)

- **B.**

Analysis: To 2 mL of Injection add 5 mL of sodium cobaltinitrite TS.

Acceptance criteria: A yellow precipitate is formed immediately. If necessary, centrifuge the solution and examine the precipitate (presence of potassium).

- **C. Identification Tests—General** (191), *Chloride*: Meets the requirements

ASSAY

Change to read:

- **Potassium and Sodium**

Internal standard solution: 1.04 mg/mL of lithium nitrate in water prepared as follows.

Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, and dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (equivalent to 1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride

■ (equivalent to 1 mEq/mL of sodium) ■ 2S (USP39)

prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with water to volume.

Standard stock solution: 0.0391J mg/mL of potassium (K) from the *Potassium stock solution* and 0.02299J' mg/mL of sodium (Na) from the *Sodium stock solution* prepared as follows. Transfer 0.1J mL of *Potassium stock solution* and 0.1J' mL of *Sodium stock solution* to a 100-mL volumetric flask, where J and J' are the labeled amounts (in mEq/L) of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 500-mL volumetric flask, and dilute with the *Internal standard solution* to volume.

Sample solution: Transfer 5.0 mL of Injection to a 500-mL volumetric flask, and dilute with the *Internal standard solution* to volume.

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: 766 nm

Sodium: 589 nm

Lithium: 671 nm

Blank: *Internal standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and the *Sample solution*.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\overline{K}}$ emission reading ratio of potassium to lithium from the *Sample solution*

$R_{\overline{S}}$ emission reading ratio of potassium to lithium from the *Standard solution*

$C_{\overline{S}}$ concentration of potassium (K) in the *Standard solution* (mg/mL)

$C_{\overline{U}}$ nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[Note—Each mg of potassium is equivalent to 0.02558 mEq of potassium.]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_{\overline{Na}}$ emission reading ratio of sodium to lithium from the *Sample solution*

$R_{\overline{S}}$ emission reading ratio of sodium to lithium from the *Standard solution*

$C_{\overline{S}}$ concentration of sodium (Na) in the *Standard solution* (mg/mL)

$C_{\overline{U}}$ nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[Note—Each mg of sodium is equivalent to 0.04350 mEq of sodium.]

Acceptance criteria

Potassium: 95.0%–110.0%

Sodium: 95.0%–105.0%

Change to read:

• Chloride

Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride, to a suitable conical flask. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

- Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V

= *Titrant* volume consumed by the *Sample solution* (mL)

N

= actual normality of the *Titrant* (mEq/mL)

F

= equivalency factor, 35.45 mg/mEq

W

= nominal amount of chloride in the *Sample solution* (mg)

■ 2S (USP39)

[Note—Each mL of *Titrant* is equivalent to 3.545 mg of chloride (Cl).]

■ 2S (USP39)

Each mg of chloride is equivalent to 0.0282 mEq of chloride (Cl).]

Acceptance criteria: 95.0%–110.0%

IMPURITIES

Delete the following:

●● Heavy Metals 〈 231 〉

Sample solution: Transfer a volume of Injection as determined by the calculation below to an appropriate vessel. Adjust the volume by evaporation or addition of water to 25 mL, as necessary.

Calculate the volume of Injection to use to two significant figures.

$$\text{Result} = 0.2 / [(G_K L_K) + (G_S L_S)]$$

G_K labeled amount of potassium chloride in each 100 mL of Injection (g)

L_K limit of heavy metals for potassium chloride, 0.001%

G_S labeled amount of sodium chloride in each 100 mL of Injection (g)

L_S limit of heavy metals for sodium chloride, 0.0005%

Acceptance criteria: Meets the requirements ● (Official 1-Dec-2015)

SPECIFIC TESTS

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 0.5 USP Endotoxin Units/mL

SPECIFIC TESTS

- **pH** 〈 791 〉: 3.5–6.5
- **Other Requirements:** It meets the requirements under *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I or Type II glass, or of a suitable plastic.
- **Labeling:** The label states the potassium, sodium, and chloride contents in terms of mEq in a given volume. The label also states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.
- **USP Reference Standards** 〈 11 〉
USP Endotoxin RS

BRIEFING

Promethazine Hydrochloride, *USP* 38 page 5027. As part of the *USP* monograph modernization effort, the following changes are proposed.

1. The nonspecific titrimetric procedure for the *Assay* is replaced with a specific liquid chromatographic procedure that is based on validated methods of analysis performed with the Waters μ Bondapak C18 brand of L1 column. The typical retention time for promethazine is about 4 min.
2. The TLC test for *Organic Impurities* is replaced with a stability-indicating liquid chromatographic procedure. This procedure was validated using the Phenomenex Luna C18 (2) brand of L1 column. The typical retention time for promethazine is about 8 min.
3. *Identification* test C, based on a retention time match in the *Assay*, is added as an orthogonal identification procedure to strengthen the monograph.
4. The test for *Completeness and Clarity of Solution* is deleted because the monograph contains sufficient tests and specifications to ensure the quality of the article. The test for *Completeness and Clarity of Solution* is a nonspecific test that does not provide any added value to the monograph.
5. *USP* Promethazine Related Compound B RS is added to the *USP Reference Standards* section to support the proposed procedures in the *Assay* and in the test for *Organic Impurities*.

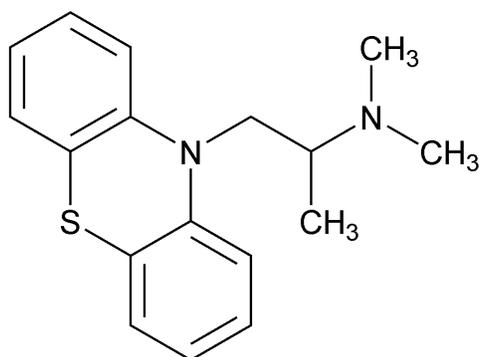
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: M. Koleček.)

Correspondence Number—C114808

Comment deadline: September 30, 2015

Promethazine Hydrochloride



$C_{17}H_{20}N_2S \cdot HCl$ 320.88

10*H*-Phenothiazine-10-ethanamine, *N,N,\alpha*-trimethyl-, monohydrochloride, (\pm)-; (\pm)-10-[2-(Dimethylamino)propyl]phenothiazine monohydrochloride [58-33-3].

DEFINITION

Change to read:

Promethazine Hydrochloride contains NLT 97.0% and NMT 101.5% of promethazine

hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$), calculated on the dried basis.

■ [

Note—Throughout the following procedures, protect the samples, the Reference Standards, and the solutions containing them by conducting the procedures without delay under subdued light or using low actinic glassware.] ■ 2S (USP39)

IDENTIFICATION

Change to read:

- **A. Infrared Absorption** 〈 197K 〉 :

■ [Note—Methods described in 〈 197K 〉 or 〈 197A 〉 may be used.] ■ 2S (USP39)

- **B. Identification Tests—General** 〈 191 〉 , Chloride

Add the following:

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

ASSAY

Change to read:

- **Procedure**

~~**Solution A:** Glacial acetic acid and mercuric acid TS (75:10)~~

~~**Sample solution:** Dissolve 700 mg of Promethazine Hydrochloride in *Solution A*.~~

~~**Blank:** *Solution A*~~

~~**Titrimetric system**~~

~~**Mode:** Direct titration~~

~~**Titrant:** 0.1 N perchloric acid VS~~

~~**Endpoint detection:** Visual~~

~~**Analysis**~~

~~**Samples:** *Sample solution* and *Blank*~~

~~Add 1 drop of crystal violet TS to the *Sample solution* and titrate with *Titrant* to a blue endpoint. Perform a *Blank* determination and make any necessary correction. Each ml of 0.1N perchloric acid is equivalent to 32.09 mg of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$).~~

~~**Acceptance criteria:** 97.0%–101.5% on the dried basis~~

■ **Diluent:** Dissolve 8.2 mL of *hydrochloric acid* in 1000 mL of water.

Mobile phase: Acetonitrile, water, and *triethylamine* (850:270:1)

System suitability stock solution: 1.2 mg/mL of USP Promethazine Hydrochloride Related Compound B RS in *Diluent*. Sonicate to dissolve.

Standard solution: 0.1 mg/mL of USP Promethazine Hydrochloride RS in *Diluent*. Sonicate to dissolve.

System suitability solution: 0.09 mg/mL of USP Promethazine Hydrochloride RS and 0.12 mg/mL of USP Promethazine Hydrochloride Related Compound B RS in *Diluent* from the *Standard solution* and *System suitability stock solution*, respectively.

Sample solution: 0.1 mg/mL of Promethazine Hydrochloride in *Diluent*. Sonicate to dissolve.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-μm packing L1

Flow rate: 2.5 mL/min

Injection volume: 20 μL

Run time: NLT 2.5 times the retention time of promethazine

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for promethazine related compound B and promethazine are 0.82 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between promethazine and promethazine related compound B, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) in the portion of Promethazine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= concentration of Promethazine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–101.5% on the dried basis ■_{2S} (USP39)

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Change to read:

- **Organic Impurities**

Protect all solutions containing Promethazine Hydrochloride from light.

Standard stock solution: 10.0 mg/mL of USP Promethazine Hydrochloride RS in methylene chloride

Standard solution A: 0.2 mg/mL of USP Promethazine Hydrochloride RS from the *Standard stock solution* in methylene chloride

Standard solution B: 0.1 mg/mL of USP Promethazine Hydrochloride RS from the *Standard stock solution* in methylene chloride

Standard solution C: 0.05 mg/mL of USP Promethazine Hydrochloride RS from the *Standard stock solution* in methylene chloride

Standard solution D: 0.025 mg/mL of USP Promethazine Hydrochloride RS from the *Standard stock solution* in methylene chloride

[Note—These solutions correspond to 2.0% (*Standard solution A*), 1.0% (*Standard solution B*), 0.5% (*Standard solution C*), and 0.25% (*Standard solution D*), respectively, of impurities.]

Sample solution: 10 mg/mL of Promethazine Hydrochloride in methylene chloride

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25 mm layer of silica gel mixture, 20 cm × 20 cm plate

Application volume: 10 µL

Developing solvent system: Ethyl acetate, acetone, alcohol, and ammonium hydroxide (90:45:2:1)

Analysis

Samples: *Standard stock solution*, each of the *Standard solutions*, and *Sample solution*

Apply each solution 2.5 cm from the lower edge of the plate. Develop the plate in an unsaturated tank containing *Developing solvent system*. After the solvent has moved NLT 10 cm, air-dry the plate, and view under short-wavelength UV light. Estimate the concentration of any other spots other than the principal spot observed in the lane for the *Sample solution* by comparison with the *Standard solutions*.

Acceptance criteria: The R_f value of the principal spot from the *Sample solution* corresponds to that from the *Standard stock solution*. The sum of the impurities is NMT 2.0%, and no single impurity is NMT 1.0%.

■ **Diluent:** Methanol and triethylamine (999:1)

Buffer: 3.7 g/L of ammonium acetate in water

Solution A: Acetonitrile and Buffer (300:700)

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	60	40
18	60	40

Time (min)	Solution A (%)	Solution B (%)
18.1	100	0
25	100	0

System suitability stock solution: 0.5 mg/mL of USP Promethazine Related Compound B RS in *Diluent*

Standard stock solution: 0.5 mg/mL of USP Promethazine Hydrochloride RS in *Diluent*

System suitability solution: 5 µg/mL each of USP Promethazine Hydrochloride RS and USP Promethazine Related Compound B RS from the *Standard stock solution* and *System suitability stock solution*, respectively

Standard solution: 5 µg/mL of USP Promethazine Hydrochloride RS from the *Standard stock solution*

Sensitivity solution: 0.25 µg/mL of USP Promethazine Hydrochloride RS from the *Standard solution*

Sample solution: 0.5 mg/mL of Promethazine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 234 and 249 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.4 mL/min

Injection volume: 15 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between promethazine and promethazine related compound B, *System suitability solution*

Relative standard deviation: NMT 3.0% at 234 and 249 nm, *Standard solution*

Signal-to-noise ratio: NLT 10 at 234 and 249 nm, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of promethazine sulfoxide in the portion of Promethazine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U

= peak response of promethazine sulfoxide at 234 nm from the *Sample solution*

r_S

= peak response of promethazine hydrochloride at 234 nm from the *Standard solution*

C_S

= concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= concentration of Promethazine Hydrochloride in the *Sample solution* (mg/mL)

F

= relative response factor (see *Table 2*)

Calculate the percentage of all other impurities in the portion of Promethazine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U

= peak response of each impurity at 249 nm from the *Sample solution*

r_S

= peak response of promethazine hydrochloride at 249 nm from the *Standard solution*

C_S

= concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= concentration of Promethazine Hydrochloride in the *Sample solution* (mg/mL)

F

= relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks that are less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Promethazine sulfoxide ^a	0.28	2.1	0.1
Desmethyl promethazine ^b	0.71	1.0	0.2
Promethazine	1.0	—	—
Promethazine related compound B	1.3	1.0	0.8
Phenothiazine	1.7	2.0	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.2

^a *N,N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.

^b *N*-Methyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine.

■ 2S (USP39)

SPECIFIC TESTS

Delete the following:

■ • ~~Completeness and Clarity of Solution~~

~~Sample solution 1:~~ 100 mg/mL of Promethazine Hydrochloride in water

~~Sample solution 2:~~ 100 mg/mL of Promethazine Hydrochloride in chloroform

~~Acceptance criteria:~~ *Sample solution 1* and *Sample solution 2* are practically clear and show NMT a light yellow color. ■ 2S (USP39)

• pH 〈 791 〉

Sample solution: 50 mg/mL of Promethazine Hydrochloride

Acceptance criteria: 4.0–5.0

• Loss on Drying 〈 731 〉

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

• **USP Reference Standards** 〈 11 〉

USP Promethazine Hydrochloride RS

■ USP Promethazine Related Compound B RS

Isopromethazine;

N,N-Dimethyl-2-(10*H*-phenothiazin-10-yl)propan-1-amine.

C₁₇H₂₀N₂S 284.42

■ 2S (USP39)

BRIEFING

Promethazine Hydrochloride Injection, *USP 38* page 5027. As part of the USP monograph modernization effort, the following changes are proposed:

1. The current *Identification* test *A* is replaced with the HPLC retention time agreement from the proposed *Assay* procedure. The current test uses toxic solvents in determining the IR absorption spectrum.
2. *Identification* test *B* is added to strengthen the monograph.
3. The current HPLC procedure in the *Assay* is replaced with the HPLC procedure that is the same as the one proposed for the *Organic Impurities* test. The typical retention time for promethazine is about 8 min.
4. An HPLC procedure is added to the *Organic Impurities* test to monitor the purity of the product. This procedure uses the Phenomenex Luna C18 (2) brand of L1 column.
5. USP Promethazine Related Compound B RS is added to the *USP Reference Standards* section to support the proposed procedures in the *Assay* and the test for *Organic*

Impurities.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: W. Yang.)

Correspondence Number—C139184

Comment deadline: September 30, 2015

Promethazine Hydrochloride Injection

DEFINITION

Promethazine Hydrochloride Injection is a sterile solution of Promethazine Hydrochloride in Water for Injection. It contains NLT 95.0% and NMT 110.0% of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$).

[Note—Throughout the following procedures, protect the samples, the Reference Standards, and the solutions containing them, by conducting the procedures without delay under subdued light or using low-actinic glassware.]

IDENTIFICATION

Change to read:

- **A.**

~~**Sample solution:** Nominally 2.5 mg/mL of promethazine hydrochloride from a volume of Injection prepared as follows. Add 50 mg of promethazine hydrochloride from a volume of Injection to 20 mL of dilute hydrochloric acid (1 in 1000) in a separator.~~

~~**Analysis:** Wash the solution with a 20 mL portion of methylene chloride, discarding the washing. Add 2 mL of 1 N sodium hydroxide and 20 mL of methylene chloride, and shake for 2 min. Evaporate the methylene chloride extract on a steam bath with the aid of a stream of nitrogen to dryness. Dissolve the residue in 4 mL of carbon disulfide, filter through paper, if necessary, and determine the IR absorption spectrum as directed under *Identification—Organic Nitrogenous Bases* (181), obtaining the spectrum of USP Promethazine Hydrochloride RS as directed.~~

~~**Acceptance criteria:** The Injection meets the requirements of the test.~~

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

Add the following:

- • **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

ASSAY

Change to read:

- **Procedure**

~~**Mobile phase:** Dissolve sodium 1-pentanesulfonate in water, then add acetonitrile and~~

glacial acetic acid to result in a concentration of 0.995 mg/mL in a solvent mixture of acetonitrile, glacial acetic acid, and water (100:1:100).

Standard solution: 0.1 mg/mL of USP Promethazine Hydrochloride RS in *Mobile phase*

Sample solution: Nominally 0.1 mg/mL of Promethazine Hydrochloride from a volume of Injection diluted with *Mobile phase*

System suitability solution: 10 µg/mL of phenothiazine in *Standard solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6 mm × 30 cm; packing L11

Flow rate: 1.5 mL/min

Injection size: 30 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for promethazine and phenothiazine are 1.0 and 1.6, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the promethazine and phenothiazine peaks

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{17}H_{20}N_2S$ ·HCl in each mL of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–110.0%

■ **Buffer:** 3.7 g/L of ammonium acetate in water

Solution A: Acetonitrile and *Buffer* (30:70)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Acetonitrile (%)
0	100	0
10	60	40
18	60	40
18.1	100	0
25	100	0

Diluent: 0.1% Triethylamine in methanol

System suitability solution: 1.0 µg/mL each of USP Promethazine Hydrochloride RS and

USP Promethazine Related Compound B RS in *Diluent*

Standard solution: 0.05 mg/mL of USP Promethazine Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.05 mg/mL of promethazine hydrochloride from a volume of Injection in *Diluent*

[Note—Sonication may be used in the preparation of *System suitability solution*, *Standard solution*, and *Sample solution*.]

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 254 nm. For *Identification test B*, use a diode-array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 5-μm packing L1

Temperatures

Column: 30°

Autosampler: 4°

Flow rate: 1.4 mL/min

Injection volume: 15 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for promethazine and promethazine related compound B are 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 5.0 between the promethazine and promethazine related compound B peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of promethazine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–110.0% ■ 2S (USP39)

IMPURITIES

Add the following:

■ ● Organic Impurities

Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 1.0 µg/mL each of USP Promethazine Hydrochloride RS and USP Promethazine Related Compound B RS in *Diluent*

Sample solution: Nominally 500 µg/mL of promethazine hydrochloride from a volume of Injection in *Diluent*

[Note—Sonication may be used in the preparation of *Standard solution* and *Sample solution*.]

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between the promethazine and promethazine related compound B peaks

Relative standard deviation: NMT 2.0% for promethazine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of promethazine from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Promethazine sulfoxide ^a	0.3	0.29	1.5
Desmethyl promethazine ^b	0.7	1.1	0.2
Promethazine	1.0	—	—
Promethazine related compound B ^c	1.3	—	—
Phenothiazine ^d	1.7	2.3	0.2
Any individual unspecified degradation product	—	1.0	0.1
Total degradation product	—	—	2.5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
a <i>N,N</i> -Dimethyl-1-(10 <i>H</i> -phenothiazin-10-yl)propan-2-amine sulfoxide.			

b *N*-Methyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine.

c This is a process impurity that is controlled in the drug substance and is included for identification only.

d 10*H*-Phenothiazine.

■ 2S (USP39)

SPECIFIC TESTS

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 5.0 USP Endotoxin Units/mg of promethazine hydrochloride
- **pH** 〈 791 〉: 4.0–5.5
- **Other Requirements:** It meets the requirements in *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

Change to read:

- **USP Reference Standards** 〈 11 〉

USP Endotoxin RS

USP Promethazine Hydrochloride RS

- USP Promethazine Related Compound B RS

Isopromethazine;

N,N-Dimethyl-2-(10*H*-phenothiazin-10-yl)propan-1-amine.

C₁₇H₂₀N₂S 284.42 ■ 2S (USP39)

BRIEFING

Promethazine Hydrochloride Tablets, *USP 38* page 5029. As part of the USP monograph modernization effort, the following changes are proposed:

1. The nonspecific UV-Vis procedure for the *Assay* is replaced with a specific liquid chromatographic procedure that is based on validated methods of analysis performed with the Waters μ Bondapak C18 brand of L1 column. The typical retention time for promethazine is about 4 min.
2. *Identification* test *B*, based on a retention time match in the *Assay*, is added as an orthogonal procedure to strengthen the monograph.
3. A stability-indicating liquid chromatographic procedure in the test for *Organic*

Impurities is added. This procedure was validated using the Phenomenex Luna C18 (2) brand of L1 column. The typical retention time for promethazine is about 8 min.

4. The reference to *Spectrophotometry and Light-Scattering* 〈 851 〉 is deleted from *Dissolution* tests 1 and 2, and an equation to calculate the amount of promethazine hydrochloride dissolved is added to *Dissolution Test 1*.
5. The *Procedure for content uniformity* in the test for *Uniformity of Dosage Units* is deleted to allow flexibility in performing the test.
6. The *Packaging and Storage* section is updated to be consistent with approved manufacturers' package inserts.
7. USP Promethazine Related Compound B RS is added to the *USP Reference Standards* section to support the proposed procedures for the *Assay* and the test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: M. Koleck.)

Correspondence Number—C114810

Comment deadline: September 30, 2015

Promethazine Hydrochloride Tablets

DEFINITION

Promethazine Hydrochloride Tablets contain NLT 95.0% and NMT 110.0% of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$).

[Note—Throughout the following procedures, protect the samples, the Reference Standards, and the solutions containing them, by conducting the procedures without delay under subdued light or using low-actinic glassware.]

IDENTIFICATION

• **A.**

Standard solution: In a separator, dissolve 50 mg of USP Promethazine Hydrochloride RS in 40 mL of dilute hydrochloric acid (1 in 1000).

Sample solution: Shake a quantity of powdered Tablets, equivalent to 50 mg of promethazine hydrochloride, with 30 mL of chloroform, and filter into a beaker. Evaporate the chloroform, dissolve the residue in 40 mL of dilute hydrochloric acid (1 in 1000), and transfer the liquid to a separator.

Analysis: Separately treat the *Sample solution* and the *Standard solution* as follows. Add 2 mL of 1 N sodium hydroxide and 15 mL of carbon disulfide to the separators, and shake for 2 min. Centrifuge if necessary to clarify the lower phase, and pass through a dry filter, collecting the filtrate from each separator in a small flask provided with a glass stopper. Reduce the volume of the carbon disulfide extracts to 4–5 mL, and proceed as directed in *Identification—Organic Nitrogenous Bases* 〈 181 〉, beginning with "Determine the absorption spectra".

Acceptance criteria: Meet the requirements

Add the following:

Mobile phase: Acetonitrile, water, and *triethylamine* (850:270:1)

System suitability stock solution: 1.2 mg/mL of USP Promethazine Hydrochloride Related Compound B RS in *Diluent*. Sonicate to dissolve.

Standard solution: 0.1 mg/mL of USP Promethazine Hydrochloride RS in *Diluent*. Sonicate to dissolve.

System suitability solution: 0.09 mg/mL of USP Promethazine Hydrochloride RS and 0.12 mg/mL of USP Promethazine Hydrochloride Related Compound B RS in *Diluent* from the *Standard solution* and *System suitability stock solution*, respectively.

Sample stock solution: Nominally 2.5–5.0 mg/mL of promethazine hydrochloride prepared as follows. Transfer 20 Tablets to a volumetric flask of an appropriate size and add 50% of the flask volume of *Diluent*. Sonicate with swirling for NLT 20 min, or until the Tablets have fully disintegrated. Shake the flask for NLT 15 min and dilute with *Diluent* to volume.

Sample solution: Nominally 0.1 mg/mL of promethazine hydrochloride in *Diluent* from the *Sample stock solution*. Pass a portion through a filter of 0.45- μ m pore size and use the clear filtrate.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; 10- μ m packing L1

Flow rate: 2.5 mL/min

Injection volume: 20 μ L

Run time: NLT 2.5 times the retention time of promethazine

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for promethazine related compound B and promethazine are 0.82 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between promethazine and promethazine related compound B, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria:** 95.0%–110.0% ■ 2S (USP39)**PERFORMANCE TESTS****Change to read:**● **Dissolution** 〈 711 〉**Test 1****Medium:** 0.01 N hydrochloric acid; 900 mL**Apparatus 1:** 100 rpm**Time:** 45 min**Standard solution:** Prepare a solution with a known concentration of USP Promethazine Hydrochloride RS in *Medium*.**Sample solution:** A filtered portion of the solution under test, suitably diluted with *Medium***Instrumental conditions**~~(See *Spectrophotometry and Light Scattering* 〈 851 〉.)~~

■ 2S (USP39)

Mode: UV**Analytical wavelength:** Absorption maximum at about 249 nm**Analysis****Samples:** *Standard solution* and *Sample solution*~~Determine the amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) dissolved.~~■ Calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times D \times 100$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (mg/mL) D = dilution factor for the *Sample solution*■ **2S (USP39)****Tolerances:** NLT 75% (Q) of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.**Medium:** 0.01 N hydrochloric acid; 900 mL**Apparatus 1:** 100 rpm**Time:** 15 min**Standard solution:** ($L/900$) mg/mL of USP Promethazine Hydrochloride RS in *Medium*, where L is the label claim (mg/Tablet)**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.**Instrumental conditions**~~(See *Spectrophotometry and Light Scattering* **851**.)~~■ **2S (USP39)****Mode:** UV**Analytical wavelength:** Absorption maximum at about 249 nm**Cell:** 0.2 cm**Blank:** *Medium***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

 A_U absorbance of the *Sample solution* A_S absorbance of the *Standard solution* C_S concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL) V = volume of *Medium*, 900 mL L = label claim (mg/Tablet)**Tolerances:** NLT 80% (Q) of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) is dissolved.**Change to read:**

- **Uniformity of Dosage Units** **905**: Meet the requirements

Procedure for content uniformity**Diluent:** 10 g/L of citric acid in water**Standard solution:** 50 µg/mL of USP Promethazine Hydrochloride RS in *Diluent***Sample solution:** Transfer 1 finely powdered Tablet to a 100-mL volumetric flask, add 50 mL of *Diluent*, and shake by mechanical means for 15 min. Dilute with *Diluent* to volume, and centrifuge about 50 mL of the mixture. Dilute a portion of the clear solution, equivalent to 5 mg of promethazine hydrochloride, with *Diluent* to 100 mL.**Instrumental conditions**~~(See Spectrophotometry and Light Scattering (851).)~~**Mode:** UV**Analytical wavelength:** Absorption maximum at about 298 nm**Cell:** 1 cm**Analysis****Samples:** *Standard solution*, *Sample solution*, and *Blank*Concomitantly determine the absorbance of the *Standard solution* and *Sample solution*, using *Diluent* as the *Blank*.Calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

 ~~A_U~~ absorbance of the *Sample solution* ~~A_S~~ absorbance of the *Standard solution* ~~C_S~~ concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL) ~~C_U~~ nominal concentration of promethazine hydrochloride in the *Sample solution* (µg/mL)

■ ■ 2S (USP39)

IMPURITIES**Add the following:**■ ● **Organic Impurities****Diluent:** Methanol and triethylamine (999:1)**Buffer:** 3.7 g/L of ammonium acetate in water**Solution A:** Buffer and acetonitrile (700:300)**Solution B:** Acetonitrile**Mobile phase:** See Table 1.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	60	40
18	60	40
18.1	100	0

Time (min)	Solution A (%)	Solution B (%)
25	100	0

System suitability stock solution: 0.5 mg/mL of USP Promethazine Related Compound B RS in *Diluent*

Standard stock solution: 0.5 mg/mL of USP Promethazine Hydrochloride RS in *Diluent*

System suitability solution: 5 µg/mL each of USP Promethazine Hydrochloride RS and USP Promethazine Related Compound B RS from the *Standard stock solution* and *System suitability stock solution*, respectively

Standard solution: 5 µg/mL of USP Promethazine Hydrochloride RS from the *Standard stock solution*

Sensitivity solution: 0.25 µg/mL of USP Promethazine Hydrochloride RS from the *Standard solution*

Sample solution: Nominally 0.5 mg/mL of promethazine hydrochloride from powdered Tablets (NLT 20) prepared as follows. Transfer a quantity of powdered Tablets, equivalent to 50 mg of promethazine hydrochloride, to a volumetric flask of appropriate size and add 75% of the flask volume of *Diluent*. Shake the flask for NLT 5 min and dilute with *Diluent* to volume. Pass a portion through a suitable filter.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 234 and 249 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.4 mL/min

Injection volume: 15 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between promethazine and promethazine related compound B, *System suitability solution*

Relative standard deviation: NMT 3.0% at 234 and 249 nm, *Standard solution*

Signal-to-noise ratio: NLT 10 at 234 and 249 nm, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of promethazine sulfoxide in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U peak response of promethazine sulfoxide at 234 nm from the *Sample solution*

r_S peak response of promethazine hydrochloride at 234 nm from the *Standard solution*

C_S concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U nominal concentration of promethazine hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Calculate the percentage of all other degradation products in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product at 249 nm from the *Sample solution*

r_S = peak response of promethazine hydrochloride at 249 nm from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks that are less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Promethazine sulfoxide ^a	0.28	2.1	0.5
Desmethyl promethazine ^b	0.71	1.0	0.5
Promethazine	1.0	—	—
Promethazine related compound B ^c	1.3	—	—
Phenothiazine	1.7	2.0	0.5
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.0

^a *N,N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.

^b *N*-Methyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine.

^c This is a process impurity and is included for identification only. It is not to be reported and not to be included in the total degradation products.

■ 2S (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

■ Store at controlled room temperature. ■ 2S (USP39)

- **Labeling:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

Change to read:

- **USP Reference Standards** < 11 >

USP Promethazine Hydrochloride RS

■ USP Promethazine Related Compound B RS

Isopromethazine;
N,N-Dimethyl-2-(10*H*-phenothiazin-10-yl)propan-1-amine.
 $C_{17}H_{20}N_2S$ 284.42

■ 2S (USP39)

BRIEFING

Oral Rehydration Salts, *USP 38* page 5145. In preparation for the omission of the flame tests from *Identification Tests—General* (191), proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to delete the references to *Sodium* and *Potassium* under *Identification* test A and include a complete description of each flame test in the monograph. Manufacturers are encouraged to submit replacement wet chemistry or instrumental procedures for consideration by the Expert Committee.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: H. Joyce, M. Koleck.)
 Correspondence Number—C158119

Comment deadline: September 30, 2015

Oral Rehydration Salts

DEFINITION

Oral Rehydration Salts is a dry mixture of Sodium Chloride, Potassium Chloride, Sodium Bicarbonate, and Dextrose (anhydrous). Alternatively, it may contain Sodium Citrate (anhydrous or dihydrate) instead of Sodium Bicarbonate. It may contain Dextrose (monohydrate) instead of Dextrose (anhydrous), provided that the Sodium Bicarbonate or Sodium Citrate is packaged in a separate, accompanying container. It contains the equivalent of NLT 90.0% and NMT 110.0% of the amounts of sodium (Na), potassium (K), chloride (Cl), and bicarbonate (HCO_3) or citrate ($C_6H_5O_7$) calculated from the labeled amounts of Sodium Chloride, Potassium Chloride, and Sodium Bicarbonate [or Sodium Citrate (anhydrous or dihydrate)]. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of anhydrous dextrose ($C_6H_{12}O_6$) or dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$). It may contain suitable flavors.

IDENTIFICATION

Change to read:

- **A.** ~~*Identification Tests—General, Sodium* (191) and *Potassium* (191)~~

■ **Sodium:** ■ 2S (USP39)

~~**Acceptance criteria:** Meets the requirements of the flame tests~~

■ The sample imparts an intense yellow color to a nonluminous flame. ■ 2S (USP39)

Add the following:

- **B. Potassium:** The sample imparts a violet color to a nonluminous flame. Since the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm

(sodium), but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

■ 2S (USP39)

Change to read:

• ~~B.~~

■ C. ■ 2S (USP39)

Identification Tests—General 〈 191 〉, *Chloride*: Meets the requirements

Change to read:

• ~~C.~~

■ D. ■ 2S (USP39)

Identification Tests—General 〈 191 〉, *Bicarbonate*

Analysis: Collect the gas that evolves upon dissolution.

Acceptance criteria: Where it contains Sodium Bicarbonate, it dissolves with effervescence, and the collected gas so obtained meets the requirements.

Change to read:

• ~~D.~~

■ E. ■ 2S (USP39)

Identification Tests—General 〈 191 〉, *Citrate*

Sample solution: Constitute the Oral Rehydration Salts as directed in the labeling.

Analysis: Add 3–5 drops of the *Sample solution* to 20 mL of the mixture of pyridine and acetic anhydride.

Acceptance criteria: Where it contains Sodium Citrate, it meets the requirements.

Change to read:

• ~~E.~~

■ F. ■ 2S (USP39)

Sample solution: Constitute the Oral Rehydration Salts as directed in the labeling.

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: Where it contains Dextrose, a copious red precipitate of cuprous oxide is formed (presence of dextrose).

ASSAY

Change to read:

• **Dextrose**

Sample stock solution: Nominally 200 mg/mL of dextrose from Oral Rehydration Salts prepared as follows. Transfer the contents of 1 or more unit-dose containers of Oral Rehydration Salts, or a portion of the contents of 1 multiple-unit container equivalent to 20 g of dextrose, to a 100-mL volumetric flask. Dilute with water to volume.

Sample solution: Nominally 100 mg/mL of dextrose from the *Sample stock solution* prepared as follows. Transfer 50.0 mL of the *Sample stock solution* to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

[Note—Reserve the remaining *Sample stock solution* for the *Assay* procedures for *Sodium and Potassium, Chloride, Bicarbonate, and Citrate*.]

Analysis: Determine the angular rotation in a suitable polarimeter tube (see *Optical*

Rotation (781A), Angular Rotation).

Sample: Sample solution

Where Oral Rehydration Salts is labeled to contain anhydrous dextrose, calculate the observed concentration (g/100 mL) of anhydrous dextrose ($C_6H_{12}O_6$) in the portion of Oral Rehydration Salts taken:

$$\text{Result} = A \times R \times (1/F)$$

~~A = 100 mm divided by the length of the polarimeter tube (mm)~~

~~R = observed rotation (degrees)~~

~~F = midpoint of the specific rotation range for anhydrous dextrose, 52.9°~~

$$\text{Result} = (100 \times a)/(l \times \alpha)$$

a

= observed angular rotation of the *Sample solution* ($^\circ$)

l

= length of the polarimeter tube (dm)

α

= midpoint of the specific rotation range for anhydrous dextrose, 52.9°

■ 2S (USP39)

Where Oral Rehydration Salts is labeled to contain dextrose monohydrate, calculate the observed concentration (g/100 mL) of dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Oral Rehydration Salts taken:

$$\text{Result} = A \times R \times (M_{r1}/M_{r2}) \times (1/F)$$

~~A = 100 mm divided by the length of the polarimeter tube (mm)~~

~~R = observed rotation (degrees)~~

~~M_{r1} = molecular weight of dextrose monohydrate, 198.17~~

~~M_{r2} = molecular weight of anhydrous dextrose, 180.16~~

~~F = midpoint of the specific rotation range for anhydrous dextrose, 52.9°~~

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (M_{r1}/M_{r2})$$

a

= observed angular rotation of the *Sample solution*, in degrees

l

= length of the polarimeter tube, in decimeters

α

= midpoint of the specific rotation range for anhydrous dextrose, 52.9°

M_{r1}

= molecular weight of dextrose monohydrate, 198.17

 M_{r2}

= molecular weight of anhydrous dextrose, 180.16

■ 2S (USP39)

Calculate the percentage of the labeled amount of anhydrous dextrose ($C_6H_{12}O_6$) or dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Oral Rehydration Salts taken:

$$\text{Result} = (C_{U1}/C_{U2})$$

■ $\times 100$ ■ 2S (USP39) C_{U1} = observed concentration of the *Sample solution* (g/100 mL) C_{U2} = nominal concentration of the *Sample solution* (g/100 mL)**Acceptance criteria:** 90.0%–110.0%**Change to read:**● **Sodium and Potassium****Sodium stock solution:** 58.44 mg/mL of sodium chloride prepared as follows. Transfer

14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride prepared as follows.

Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Diluent: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Add a suitable nonionic surfactant, then add water to volume.**Standard stock solution:** 0.5844 mg/mL of sodium chloride and 0.7456 mg/mL of potassium chloride in water from the *Sodium stock solution* and the *Potassium stock solution*, respectively**Standard solution:** 0.01150 mg/mL of sodium and 0.01955 mg/mL of potassium from the *Standard stock solution* in *Diluent***Sample stock solution A:** Nominally 0.23 mg/mL of sodium from the *Sample stock solution* remaining from the *Assay for Dextrose* in water**Sample solution A:** Nominally 0.0115 mg/mL of sodium from *Sample stock solution A* in *Diluent***Sample stock solution B:** Nominally 0.39 mg/mL of potassium from the *Sample stock solution* remaining from the *Assay for Dextrose* in water**Sample solution B:** Nominally 0.01955 mg/mL of potassium from *Sample stock solution B* in *Diluent***Instrumental conditions****Mode:** Flame photometer**Analytical wavelengths****Potassium:** Maximum absorbance at about

■ ■ 2S (USP39)

766 nm

Sodium: Maximum absorbance at about

■ ■ 2S (USP39)

589 nm

Blank: Diluent

Analysis

Samples: Standard solution, Sample solution A, Sample solution B, and Blank

Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution*, *Sample solution A*, and *Sample solution B*.

Calculate the percentage of the labeled amount of sodium (Na) in the unit-dose container or containers taken or in the portion of Oral Rehydration Salts taken from the multiple-unit container:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U photometer reading of sodium from *Sample solution A*

r_S photometer reading of sodium from the *Standard solution*

C_S concentration of sodium in the *Standard solution* (mg/mL)

C_U nominal concentration of sodium in *Sample solution A* (mg/mL)

Calculate the percentage of the labeled amount of potassium (K) in the unit-dose container or containers taken or in the portion of Oral Rehydration Salts taken from the multiple-unit container:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U photometer reading of potassium from *Sample solution B*

r_S photometer reading of potassium from the *Standard solution*

C_S concentration of potassium in the *Standard solution* (mg/mL)

C_U nominal concentration of potassium in *Sample solution B* (mg/mL)

Acceptance criteria

Potassium: 90.0%–110.0%

Sodium: 90.0%–110.0%

● **Chloride**

Sample solution: Transfer a volume of the *Sample stock solution* remaining from the *Assay for Dextrose*, containing nominally 55 mg of chloride (Cl), to a suitable container.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate with *Titrant* until the silver chloride flocculates and the mixture acquires a faint pink color, using potassium chromate TS as the indicator.

Calculate the percentage of the labeled amount of chloride (Cl) in the unit-dose container or containers taken or in the portion of Oral Rehydration Salts taken from the multiple-unit container:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = volume of *Titrant* consumed (mL)

N = normality of *Titrant* (mEq/mL)

F = equivalent weight of chloride, 35.45 mg/mEq

W = nominal amount of chloride in the *Sample solution* (mg)

Acceptance criteria: 90.0%–110.0%

- **Bicarbonate** (if present)

Sample solution: Transfer a volume of the *Sample stock solution* remaining from the *Assay for Dextrose*, containing nominally 100 mg of bicarbonate, to a suitable beaker.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N hydrochloric acid VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Add 25 mL of water and 3 drops of methyl orange TS to the *Sample solution*. Titrate the resulting solution with *Titrant*.

Calculate the percentage of the labeled amount of bicarbonate (HCO_3) in the unit-dose container or containers taken or in the portion of Oral Rehydration Salts taken from the multiple-unit container:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = volume of *Titrant* consumed (mL)

N = normality of *Titrant* (mEq/mL)

F = equivalent weight of bicarbonate, 61.02 mg/mEq

W = nominal amount of bicarbonate in the *Sample solution* (mg)

Acceptance criteria: 90.0%–110.0%

Change to read:

- **Citrate** (if present)

Mobile phase, Standard preparation 1, and Chromatographic system: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* 〈 345 〉.

Sample solution: Transfer a volume of the *Sample stock solution* remaining from the *Assay for Dextrose*, containing nominally 180 mg of citrate, to a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* 〈 345 〉, ~~*Assay Preparation for Citric Acid/Citrate Assay*~~

■ *Procedure.* ■ 2S (USP39)

Analysis

Samples: *Standard preparation 1* and *Sample solution*

Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* 〈 345 〉, *Procedure*. Calculate the percentage of the labeled amount of citrate ($C_6H_5O_7$) in the portion of Oral Rehydration Salts taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of citrate from the *Sample solution*

r_S = peak response of citrate from *Standard preparation 1*

C_S = concentration of citrate in the *Standard preparation 1* ($\mu\text{g/mL}$)

C_U = nominal concentration of citrate in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Minimum Fill** 〈 755 〉: Proceed as directed, except the average net weight of the contents of the 10 containers is NLT the labeled amount, and the net weight of the contents of any single container is NLT 95% and NMT 105% of the labeled amount. If the contents of NMT 1 container are less than 95% but NLT 90% of the labeled amount, or more than 105% but NMT 110% of the labeled amount, determine the net weight of the contents of 20 additional containers. The average net weight of the contents of 30 containers is NLT the labeled amount, and the net weight of the contents of NMT 1 of the 30 containers is less than 95% but NLT 90% of the labeled amount, or more than 105% but NMT 110% of the labeled amount.

[Note—In performing the *Assay* procedures for *Sodium and Potassium, Chloride*, and *Bicarbonate* or *Citrate*, calculate from the labeled amounts of sodium chloride, potassium chloride, and sodium bicarbonate or sodium citrate the total equivalent amounts of sodium (Na), potassium (K), chloride (Cl), and bicarbonate (HCO_3) or citrate ($C_6H_5O_7$) contained therein (see *Table 1*).]

Table 1. mg Equivalent of Each g of Component

Component	Na	K	Cl	HCO_3	$C_6H_5O_7$
Sodium chloride	393.4	—	606.6	—	—
Potassium chloride	—	524.4	475.6	—	—
Sodium bicarbonate	273.6	—	—	726.4	—
Anhydrous sodium citrate	267.2	—	—	—	732.8
Sodium citrate dihydrate	234.5	—	—	—	643.0

SPECIFIC TESTS

- **pH** 〈 791 〉
Sample solution: Constitute the Oral Rehydration Salts as directed in the labeling.
Acceptance criteria: 7.0–8.8
- **Loss on Drying** 〈 731 〉
Analysis: Dry at 50° to constant weight.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and avoid exposure to temperatures in excess of 30°. The Sodium Bicarbonate or Sodium Citrate component may be omitted from the mixture and packaged in a separate, accompanying container.
- **Labeling:** The label indicates prominently whether Sodium Bicarbonate or Sodium Citrate is a component by the placement of the word "Bicarbonate" or "Citrate", as appropriate, in juxtaposition to the official title. The label states the name and quantity, in g, of each component in each unit-dose container, or in a stated quantity, in g, of Oral Rehydration Salts in a multiple-unit container. The label states the net weight in each container and provides directions for constitution. Where packaged in individual unit-dose pouches, the label instructs the user not to open the pouch until the time of use. The label also states that any solution that remains unused 24 h after constitution is to be discarded.
- **USP Reference Standards** 〈 11 〉
USP Citric Acid RS

BRIEFING

Ringer's and Dextrose Injection, *USP 38* page 5183. It is proposed to make the following revisions to the monograph:

1. In preparation for the omission of the flame tests from *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to delete the reference to *Sodium* and *Potassium* under *Identification* test *B*, and include a complete description of each flame test in the monograph. Manufacturers are encouraged to submit replacement wet chemistry or instrumental procedures for consideration by the Expert Committee.
2. Update the *Assay* procedure for *Calcium* by removing an unneeded reference to the equivalent amount of calcium in the *Sample solution*.
3. Add or revise the equations in the *Assay* procedures for *Calcium*, for *Chloride*, for *Potassium Chloride*, and for *Sodium* to be consistent with the *Definition* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: H. Joyce, M. Koleck.)

Correspondence Number—C158120

Comment deadline: September 30, 2015

Ringer's and Dextrose Injection

DEFINITION

Ringer's and Dextrose Injection is a sterile solution of Sodium Chloride, Potassium Chloride, Calcium Chloride, and Dextrose in Water for Injection. It contains, in each 100 mL, NLT 323.0 mg and NMT 354.0 mg of sodium (Na) [equivalent to NLT 820.0 mg and NMT 900.0 mg of sodium chloride (NaCl)], NLT 14.9 mg and NMT 16.5 mg of potassium (K) [equivalent to NLT 28.5 mg and NMT 31.5 mg of potassium chloride (KCl)], NLT 8.20 mg and NMT 9.80 mg of

calcium (Ca) [equivalent to NLT 30.0 mg and NMT 36.0 mg of calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)], and NLT 523.0 mg and NMT 608.5 mg of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)]. It contains NLT 95.0% and NMT 105.0% of the labeled amount of dextrose ($\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$). It contains no antimicrobial agents.

[Note—The potassium, calcium, sodium, and chloride ion contents of Ringer's and Dextrose Injection are approximately 4, 4.5, 147.5, and 156 mEq/L, respectively.]

IDENTIFICATION

Change to read:

- **A.**

Sample solution: ~~1 in 20~~

■ Nominally 50 mg/mL of dextrose from a suitable volume of Injection in water ■ 2S (USP39)

Analysis: Add a few drops of *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

Change to read:

- **B. Identification Tests—General**, ~~Sodium~~ ~~(191)~~, ~~Potassium~~ ~~(191)~~,

■ 2S (USP39)

~~(191)~~, *Calcium* and *Chloride*: Meets the requirements of the flame tests for ~~Sodium~~ and ~~Potassium~~,

■ 2S (USP39)

of the ammonium oxalate test for *Calcium*, and the test for *Chloride*.

Add the following:

■ **C. Sodium:** The sample imparts an intense yellow color to a nonluminous flame. ■ 2S (USP39)

Add the following:

■ **D. Potassium:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

■ 2S (USP39)

ASSAY

Change to read:

- **Calcium**

[Note—Concentrations of the *Standard solution* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Lanthanum chloride solution: 88.45 g/L of lanthanum chloride prepared as follows.

Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Calcium stock solution: 1 mg/mL of calcium (Ca) prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of

water. Carefully add 5 mL of diluted hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium (Ca) prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Lanthanum chloride solution*, add 1.0, 1.5, and 2.0 mL, respectively, of *Calcium stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 20.0 mL of Injection (~~equivalent to about 1.8 mg of calcium~~)

■ ■ 2S (USP39)

to a 100-mL volumetric flask containing 5.0 mL of *Lanthanum chloride solution*. Dilute with water to volume.

Blank: Transfer 5.0 mL of *Lanthanum chloride solution* to a 100-ml volumetric flask and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* 〈 852 〉 .)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in mg/mL, of calcium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (*C*), in mg/mL, of calcium in the *Sample solution*.

Calculate the quantity (mg) of calcium (Ca) in each 100 mL of Injection taken:

$$\text{Result} = C \times 0.5$$

~~C = concentration of calcium in the *Sample solution*, as determined from the graph ($\mu\text{g/mL}$)~~

■

$$\text{Result} = C \times D \times F$$

C

= concentration of calcium in the *Sample solution*, as determined from the graph (mg/mL)

D

= dilution factor of the *Sample solution*, 5

F

= conversion factor for each 100 mL of Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 8.20–9.80 mg of calcium (Ca) in each 100 mL

Change to read:

• Chloride

Sample solution: Transfer 10 mL of Injection into a conical flask. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.

Titrimetric system**Mode:** Direct titration**Titrant:** 0.1 N silver nitrate VS**Endpoint detection:** Visual**Analysis****Sample:** *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint. ~~Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl).~~

- Calculate the amount, in mg, of chloride (Cl) in each 100 mL of Injection taken:

$$\text{Result} = V \times N \times D \times F$$

$$V$$

= volume of *Titrant* consumed by the *Sample solution* (mL)

$$N$$

= actual normality of the *Titrant* (mEq/mL)

$$D$$

= dilution factor of the *Sample solution*, 10

$$F$$

= equivalency factor, 35.45 mg/mEq

- 2S (USP39)

Acceptance criteria: 523.0–608.5 mg of chloride (Cl) in each 100 mL

- **Dextrose**

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis**Sample:** *Sample solution*

Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* 〈 781 〉).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* ($^\circ$)

l = length of the polarimeter tube (dm)

α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution*, g/100 mL

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 95.0%–105.0%

Change to read:

• **Potassium**

Solution A: Suitable nonionic wetting agent (1 in 500)

Solution B: 10.93 mg/mL of sodium chloride in water

Standard stock solution: 100 µg/mL of potassium in water prepared as follows. Dissolve 190.7 mg of potassium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer the resulting solution to a 1-L volumetric flask, and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of potassium prepared as follows. Transfer 10 mL of *Solution B* to each of four 100-mL volumetric flasks containing 10.0 mL of *Solution A*. To each flask add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*, respectively, and dilute with water to volume.

Sample solution: Transfer 10 mL of Injection to a 100-mL volumetric flask. Add 10.0 mL of *Solution A*. Dilute with water to volume.

Blank: Transfer 10 mL of *Solution B* to a 100-mL volumetric flask containing 10.0 mL of *Solution A*. Dilute with water to volume.

Instrumental conditions

Mode: Atomic emission spectrophotometry

Emission wavelength: 766 nm

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set a suitable flame photometer for maximum transmittance at a wavelength of 766 nm.

Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of potassium. From the graph so obtained, read the percentage transmittance of the *Sample solution*. ~~and calculate the potassium content, in mg per 100 mL, of Injection~~

■ Calculate the quantity (mg) of potassium (K) in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C

= concentration of potassium in the *Sample solution*, as determined from the graph (mg/mL)

D

= dilution factor of the *Sample solution*, 10

F

= conversion factor for each 100 mL of Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 14.9–16.5 mg of potassium (K) in each 100 mL

Change to read:

• **Sodium**

Solution A: Suitable nonionic wetting agent (1 in 500)

Standard stock solution: 100 µg/mL of sodium in water prepared as follows. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer the resulting solution to a 1-L volumetric flask, and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium prepared as follows. Transfer 10 mL of *Solution A* to each of four 100-mL volumetric flasks. To each flask add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*, respectively, and dilute with water to volume.

Sample solution: Transfer 5 mL of Injection to a 1-L volumetric flask containing 100 mL of *Solution A*. Dilute with water to volume.

Blank: Transfer 10 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Atomic emission spectrophotometry

Emission wavelength: 589 nm

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set a suitable flame photometer for maximum transmittance at a wavelength of 589 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of sodium. From the graph so obtained, read the percentage transmittance of the *Sample solution*. ~~and calculate the sodium content, in mg per 100 mL, of Injection.~~

■ Calculate the quantity (mg) of sodium (Na) in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C

= concentration of sodium in the *Sample solution*, as determined from the graph (mg/mL)

D

= dilution factor of the *Sample solution*, 200

F

= conversion factor for each 100 mL of Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 323.0–354.0 mg of sodium (Na) in each 100 mL

IMPURITIES

Delete the following:

●● **Heavy Metals** (231)

Sample solution: Transfer a volume of Injection as determined by the calculation below to an appropriate vessel. Adjust the volume by evaporation or addition of water to 25 mL, as necessary.

Calculate the volume of Injection to use to two significant figures:

$$\text{Result} = 0.2 / [(G_S L_S) + (G_K L_K) + (G_C L_C) + (G_D L_D)]$$

G_S labeled amount of sodium chloride in each 100 mL of Injection (g)

L_S limit of heavy metals under *Sodium Chloride*

G_K labeled amount of potassium chloride in each 100 mL of Injection (g)

L_K limit of heavy metals under *Potassium Chloride*

G_C labeled amount of calcium chloride in each 100 mL of Injection (g)

L_C limit of heavy metals under *Calcium Chloride*

G_D labeled amount of dextrose in each 100 mL of Injection (g)

L_D limit of heavy metals under *Dextrose*

Acceptance criteria: Meets the requirements (Official 1-Dec-2015)

- **Limit of 5-Hydroxymethylfurfural and Related Substances**

Sample solution: Nominally 2.0 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) from Injection in water

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution* and *Blank*

Determine the absorbance of the *Sample solution* with a suitable spectrophotometer.

Acceptance criteria: The absorbance is NMT 0.25

SPECIFIC TESTS

- **Bacterial Endotoxins Test** (85): NMT 0.5 USP Endotoxin Units/mL
- **pH** (791): 3.5–6.5
- **Other Requirements:** It meets the requirements in *Injections* (1).

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **Labeling:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.
- **USP Reference Standards** (11)
USP Endotoxin RS

BRIEFING

Lactated Ringer's Injection, *USP 38* page 5184. It is proposed to revise the monograph as follows:

1. In preparation for the omission of the flame tests from *Identification Tests—General* (191), proposed in *PF 41(2)* [Mar.–Apr. 2015], delete the reference to (191)

flame tests for sodium and potassium in *Identification* test *A* and include a complete description of the flame test for the two elements in the monograph. Manufacturers are encouraged to submit a replacement wet-chemistry or an instrumental procedure for the Expert Committee's consideration.

2. Revise the units for the concentration of calcium in the *Analysis* in the *Assay* for *Calcium* from $\mu\text{g/mL}$ to mg/mL .
3. Add the equation for calculating the concentration of potassium in the *Assay* for *Potassium*.
4. Add the equation for calculating the concentration of sodium in the *Assay* for *Sodium*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: S. Ramakrishna, D. Vicchio.)
Correspondence Number—C158121

Comment deadline: September 30, 2015

Lactated Ringer's Injection

DEFINITION

Lactated Ringer's Injection is a sterile solution of Calcium Chloride, Potassium Chloride, Sodium Chloride, and Sodium Lactate in Water for Injection. It contains, in each 100 mL, NLT 285.0 and NMT 315.0 mg of sodium [as sodium chloride (NaCl) and sodium lactate ($\text{C}_3\text{H}_5\text{NaO}_3$)], NLT 14.2 and NMT 17.3 mg of potassium (K) [equivalent to NLT 27.0 and NMT 33.0 mg of potassium chloride (KCl)], NLT 4.90 and NMT 6.00 mg of calcium (Ca) [equivalent to NLT 18.0 and NMT 22.0 mg of calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)], NLT 368.0 and NMT 428.0 mg of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)], and NLT 231.0 and NMT 261.0 mg of lactate ($\text{C}_3\text{H}_5\text{O}_3$) [equivalent to NLT 290.0 and NMT 330.0 mg of sodium lactate ($\text{C}_3\text{H}_5\text{NaO}_3$)]. It contains no antimicrobial agents.

[Note—The calcium, potassium, and sodium contents of Lactated Ringer's Injection are approximately 2.7, 4, and 130 mEq/L, respectively.]

IDENTIFICATION

Change to read:

- **A. Identification Tests—General, Sodium** ~~(191)~~ (flame test), **Potassium** ~~(191)~~ (flame test), **Calcium** (ammonium oxalate test) ~~(191)~~, and **Chloride** ~~(191)~~

■ **Identification Tests—General** (191), **Chloride and Calcium:**

Meets the requirements of the test for *Chloride* and the test for ammonium oxalate in *Calcium*

■ 2S (USP39)

Add the following:

- **B. Potassium:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [Note—Traditionally,

cobalt glass has been used, but other suitable filters are commercially available.]

■ 2S (USP39)

Add the following:

■ ● **C. Sodium:** The sample imparts an intense yellow color to a nonluminous flame. ■ 2S (USP39)

Change to read:

● **B**

■ **D.** ■ 2S (USP39)

The retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Lactate*.

ASSAY

Change to read:

● **Calcium**

[Note—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: Transfer 17.69 g of lanthanum chloride to a 200-mL volumetric flask, add 100 mL of water, carefully add 50 mL of hydrochloric acid, and allow to cool. Dilute with water to volume.

Calcium stock solution: 1 mg/mL of calcium prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask and add 10 mL of water. Carefully add 5 mL of dilute hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium (Ca), prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 1.0, 1.5, and 2.0 mL, respectively, of *Calcium stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Dilute with water, 20.0 mL of Injection, equivalent to ~~1.8 mg~~

■ 1 mg ■ 2S (USP39)

of calcium (Ca), in a 100-mL volumetric flask containing 5.0 mL of *Solution A* to volume, and mix.

Blank: 5.0 mL of *Solution A* diluted with water to 100.0 mL

Instrumental conditions

(See *Atomic Absorption Spectroscopy* { 852 } .)

Mode: Atomic absorption spectrophotometer

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Concomitantly determine the absorbance of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus concentration in ~~µg/mL~~

■ in mg/mL, ■ 2S (USP39)

of calcium, and draw the straight line best fitting the three plotted points. From this graph, determine the concentration (C)

■ in mg/mL, ■_{2S} (USP39)

of calcium in the *Sample solution*.

Calculate the quantity of calcium in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of calcium in the *Sample solution* (mg/mL)

D = dilution factor of the *Sample solution*, 5

F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 4.90–6.00 mg of calcium per 100 mL of Injection

Change to read:

- **Potassium**

Solution A: Suitable nonionic wetting solution (1 in 500)

Solution B: 10.93 mg/mL of sodium chloride in water

Standard stock solution: 0.100 mg/mL of potassium, prepared as follows. Transfer 190.7 mg of potassium chloride, previously dried at 105° for 2 h, to a 1-L volumetric flask and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of potassium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A* and 10 mL of *Solution B*, add 5.0, 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Pipet 10 mL of Injection to a 100-mL volumetric flask containing 10 mL of *Solution A*, dilute with water to volume, and mix.

Blank: Transfer 10.0 mL of *Solution A* and 10 mL of *Solution B* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: Maximum transmittance at 766 nm

Analysis

Samples: ~~Standard solutions and Sample solution~~

~~Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the Blank. Adjust the instrument to 100% transmittance with the most concentrated of the Standard solutions. Read the percentage transmittance of the other Standard solutions, and plot transmittances versus concentration of potassium.~~

~~Read the percentage transmittance of the Sample solution.~~

~~Calculate the potassium content, in mg/100 mL, of Injection.~~

■ **Samples:** *Standard solutions, Sample solution, and Blank*

Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus the concentration, in mg/mL, of potassium. Draw the straight line best fitting the four

plotted points. From this graph, calculate the concentration of potassium in the *Sample solution*.

Calculate the quantity, in mg, of potassium in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C

= concentration of potassium in the *Sample solution* (mg/mL)

D

= *Sample solution* dilution factor, 10

F

= conversion factor for each 100 mL of Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 14.2–17.3 mg of potassium per 100 mL of Injection

Change to read:

● **Sodium**

Solution A: Suitable nonionic wetting solution (1 in 500)

Standard stock solution: 0.100 mg/mL of sodium, prepared as follows. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer this solution to a 1-L volumetric flask and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A*, add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 5 mL of Injection in a 1-L volumetric flask containing 100.0 mL of *Solution A* and dilute with water to volume.

Blank: Transfer 10.0 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: Maximum transmittance at 589 nm

Analysis

Samples: ~~Standard solutions and Sample solution~~

~~Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the Blank. Adjust the instrument to 100% transmittance with the most concentrated of the Standard solutions. Read the percentage transmittance of the other Standard solutions, and plot transmittances versus concentration of sodium.~~

~~Read the percentage transmittance of the Sample solution.~~

~~Calculate the sodium content, in mg/100 mL, of Injection.~~

■ **Samples:** *Standard solutions, Sample solution, and Blank*

Set the flame photometer for maximum transmittance. Adjust the instrument to zero

transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus concentration, in mg/mL, of sodium, and draw the straight line best fitting the four plotted points. From this graph, calculate the concentration of sodium in the *Sample solution*.

Calculate the quantity of sodium in each 100 mL of Injection:

$$\text{Result} = C \times D \times F$$

C

= concentration of sodium in the *Sample solution* (mg/mL)

D

= dilution factor of the *Sample solution*, 200

F

= conversion factor for each 100 mL of Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 285.0–315.0 mg of sodium per 100 mL of Injection

● **Chloride**

Sample solution: Transfer 10 mL of Injection into a conical flask, and add 10 mL of glacial acetic acid and 75 mL of methanol.

Titrimetric system

■ (See *Titrimetry* 〈 541 〉). ■ 2S (USP39)

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate the *Sample solution*, with shaking, with 0.1 N silver nitrate VS to a pink endpoint using 3 drops of eosin Y TS, as an indicator. ~~Each mL of *Titrant* is equivalent to 3.545 mg of chloride (Cl).~~

Calculate the labeled amount of chloride (Cl) in 100 mL of Injection:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V

= *Titrant* volume consumed by the *Sample solution* (mL)

N

= actual normality of the *Titrant* (mEq/mL)

F

= equivalency factor, 35.45 mg/mEq

W

= nominal amount of chloride in the *Sample solution* (mg)

■ 2S (USP39)

Analysis: Titrate the *Sample solution*, with shaking, with 0.1 N silver nitrate VS to a pink endpoint using 3 drops of eosin Y TS, as an indicator. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl).

Acceptance criteria: 368.0–428.0 mg of chloride per 100 mL of Injection

● **Lactate**

Mobile phase: Dicyclohexylamine, formic acid, and water (1:1:998)

System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water

Standard solution: 3 mg/mL of USP Sodium Lactate RS in water

Sample solution: Use undiluted Injection.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between the acetate and lactate peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount of lactate (C₃H₅O₃) in each 100 mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of lactate, 89.07

M_{r2} = molecular weight of anhydrous sodium lactate, 112.06

Acceptance criteria: 231.0–261.0 mg of lactate (C₃H₅O₃) per 100 mL of Injection

IMPURITIES

Delete the following:

- **Heavy Metals** 〈 231 〉: Evaporate 67 mL to a volume of 20 mL, add 2 mL of 1 N acetic acid, and dilute with water to 25 mL: the limit is 0.3 ppm. ●(Official 1-Dec-2015)

SPECIFIC TESTS

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 0.5 USP Endotoxin Units/mL
- **pH** 〈 791 〉: 6.0–7.5
- **Other Requirements:** It meets the requirements in *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **Labeling:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL. The label also includes the warning: "Not for use in the treatment of lactic acidosis".
- **USP Reference Standards** 〈 11 〉
USP Endotoxin RS
USP Sodium Lactate RS

BRIEFING

Lactated Ringer's and Dextrose Injection, *USP 38* page 5185. It is proposed to revise the monograph as follows:

1. In preparation for the omission of the flame tests from *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], delete the reference to 〈 191 〉 flame tests for sodium and potassium in *Identification* test *B* and include a complete description of the flame test for the two elements in the monograph. Manufacturers are encouraged to submit a replacement wet-chemistry or an instrumental procedure for the Expert Committee's consideration.
2. Under *Analysis* in the *Assay for Calcium*, revise the equivalent amount of calcium in the *Sample solution* to get the solution within the linearity range evaluated. Add a clarifying statement describing how absorbance data are used to calculate the concentration of calcium.
3. Add the equation for calculating the concentration of potassium in the *Assay for Potassium*.
4. Add the equation for calculating the concentration of sodium in the *Assay for Sodium*.
5. Update the equation in the *Assay for Dextrose* to be consistent with current *USP* style.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: S. Ramakrishna, D. Vicchio.)
Correspondence Number—C158122

Comment deadline: September 30, 2015

Lactated Ringer's and Dextrose Injection

DEFINITION

Lactated Ringer's and Dextrose Injection is a sterile solution of Calcium Chloride, Potassium Chloride, Sodium Chloride, Sodium Lactate, and Dextrose in Water for Injection. It contains, in each 100 mL, NLT 285.0 and NMT 315.0 mg of sodium (Na) [as sodium chloride (NaCl) and sodium lactate ($C_3H_5NaO_3$)], NLT 14.2 and NMT 17.3 mg of potassium (K) [equivalent to NLT 27.0 and NMT 33.0 mg of potassium chloride (KCl)], NLT 4.90 and NMT 6.00 mg of calcium (Ca) [equivalent to NLT 18.0 and NMT 22.0 mg of calcium chloride ($CaCl_2 \cdot 2H_2O$)], NLT 368.0 and NMT 428.0 mg of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride ($CaCl_2 \cdot 2H_2O$)], and NLT 231.0 and NMT 261.0 mg of lactate ($C_3H_5O_3$) [equivalent to NLT 290.0 mg and NMT 330.0 mg of sodium lactate ($C_3H_5NaO_3$)]. It contains NLT 90.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It contains no antimicrobial agents.

[Note—The calcium, potassium, and sodium contents of Lactated Ringer's and Dextrose Injection are approximately 2.7, 4, and 130 mEq/L, respectively.]

IDENTIFICATION

- **A.**

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

Delete the following:

- ~~• **B. Identification Tests—General** (191): Meets the requirements of the flame tests for Sodium (191) and Potassium (191), of the test for Chloride (191), and of the test for ammonium oxalate for Calcium (191) ■2S (USP39)~~

Add the following:

- **B. Identification Tests—General** (191), **Chloride and Calcium:** Meets the requirements of the test for Chloride and the test for ammonium oxalate in Calcium ■2S (USP39)

Add the following:

- **C. Potassium:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.] ■2S (USP39)

Add the following:

- **D. Sodium:** The sample imparts an intense yellow color to a nonluminous flame. ■2S (USP39)

Change to read:

- €

■E. ■2S (USP39)

The retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Lactate*.

ASSAY**Change to read:**

- Calcium

[Note—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: Transfer 17.69 g of lanthanum chloride to a 200-mL volumetric flask, add 100 mL of water, carefully add 50 mL of hydrochloric acid, and allow to cool. Dilute with water to volume.

Calcium stock solution: 1 mg/mL of calcium, prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask and add 10 mL of water. Carefully add 5 mL of dilute hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium (Ca), prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 1.0, 1.5, and 2.0 mL, respectively, of *Calcium stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 50.0

■25.0 ■2S (USP39)

mL of Injection (equivalent to about 1.4 mg of calcium, to a 100-mL volumetric flask containing 5.0 mL of *Solution A*. Dilute with water to volume and mix.

Blank: 5.0 mL of *Solution A* diluted with water to 100.0 mL

Instrumental conditions

(See *Atomic Absorption Spectroscopy* { 852 } .)

Mode: Atomic absorption spectrophotometer

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air-acetylene

Blank: *Blank*

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in mg/mL, of calcium, and draw the straight line best fitting the three plotted points.

■ From this graph, calculate the concentration of calcium in the *Sample solution*. ■2S (USP39)

Calculate the quantity of calcium in each 100 mL of the Injection:

$$\text{Result} = C \times D \times F$$

C = concentration of calcium in the *Sample solution* (mg/mL)

D = dilution factor of the *Sample solution*, 4

F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 4.90–6.00 mg of calcium per 100 mL of Injection

Change to read:

• **Potassium**

Solution A: Suitable nonionic wetting solution (1 in 500)

Solution B: 10.93 mg/mL of sodium chloride in water

Standard stock solution: 0.100 mg/mL of potassium, prepared as follows. Transfer 190.7 mg of potassium chloride, previously dried at 105° for 2 h, to a 1-L volumetric flask and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of potassium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A* and 10 mL of *Solution B*, add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Pipet 10 mL of Injection to a 100-mL volumetric flask containing 10 mL of *Solution A*, dilute with water to volume, and mix.

Blank: Transfer 10.0 mL of *Solution A* and 10 mL of *Solution B* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: Maximum transmittance at 766 nm

Analysis

~~**Samples:** *Standard solutions* and *Sample solution*~~

~~Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the blank. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of potassium.~~

~~Read the percentage transmittance of the *Sample solution*.~~

~~Calculate the potassium content, in mg/100 mL, of Injection.~~

■ **Samples:** *Standard solutions*, *Sample solution*, and *Blank*

Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus the concentration, in mg/mL, of potassium. Draw the straight line best fitting the four plotted points. From this graph, calculate the concentration of potassium in the *Sample solution*.

Calculate the quantity of potassium, in mg, in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C

= concentration of potassium in the *Sample solution* (mg/mL)

D= dilution factor of the *Sample solution*, 10*F*

= conversion factor for each 100 mL of Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 14.2–17.3 mg of potassium per 100 mL of Injection**Change to read:**● **Sodium****Solution A:** Suitable nonionic wetting solution (1 in 500)**Standard stock solution:** 0.100 mg/mL of sodium, prepared as follows. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer this solution to a 1-L volumetric flask and dilute with water to volume.**Standard solutions:** 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A*, add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*. Dilute the contents of each flask with water to volume.**Sample solution:** Pipet 5 mL of Injection into a 1-L volumetric flask containing 100 mL of *Solution A* and dilute with water to volume.**Blank:** Transfer 10.0 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.**Instrumental conditions****Mode:** Flame photometry**Analytical wavelength:** Maximum transmittance at 589 nm**Analysis-****Samples:** ~~Standard solutions and Sample solution~~~~Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the blank. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of sodium.~~~~Read the percentage transmittance of the *Sample solution*.~~~~Calculate the sodium content, in mg/100 mL, of Injection.~~■ **Samples:** *Standard solutions, Sample solution, and Blank*Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus concentration, in mg/mL, of sodium, and draw the straight line best fitting the four plotted points. From this graph, calculate the concentration of sodium in the *Sample solution*.

Calculate the quantity of sodium in each 100 mL of Injection:

$$\text{Result} = C \times D \times F$$

C

= concentration of sodium in the *Sample solution* (mg/mL)

D

= *Sample solution* dilution factor, 200

F

= conversion factor for each 100 mL of Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 285.0–315.0 mg of sodium per 100 mL of Injection**Change to read:**● **Chloride****Sample solution:** Transfer 10 mL of Injection into a conical flask, and add 10 mL of glacial acetic acid and 75 mL of methanol.**Analysis****Sample:** *Sample solution*Titrate the *Sample solution*, with shaking, with 0.1 N silver nitrate VS to a pink endpoint, using 3 drops of eosin Y TS as an indicator. ~~Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl).~~

■

Calculate the labeled amount of chloride (Cl) in 100 mL of Injection:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V

= *Titrant* volume consumed by the *Sample solution* (mL)

N

= actual normality of the *Titrant* (mEq/mL)

F

= equivalency factor, 35.45 mg/mEq

W

= nominal amount of chloride in the *Sample solution* (mg)

■ 2S (USP39)

Acceptance criteria: 368.0–428.0 mg of chloride per 100 mL of Injection● **Lactate****Mobile phase:** Dicyclohexylamine, formic acid, and water (1:1:998)**System suitability solution:** 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS, in water**Standard solution:** 3 mg/mL of USP Sodium Lactate RS in water**Sample solution:** Use undiluted Injection.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between the acetate and lactate peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount of lactate ($C_3H_5O_3$) in each 100 mL of Injection:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of lactate, 89.07

M_{r2} = molecular weight of anhydrous sodium lactate, 112.06

Acceptance criteria: 231.0–261.0 mg of lactate ($C_3H_5O_3$) per 100 mL of Injection

Change to read:

- Dextrose**

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis: Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* { 781 }).

Calculate the percentage, in g/100mL, of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = (100/M) \times (M_{r1}/M_{r2}) \times AR$$

M = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

A = 100 mm divided by the length of the polarimeter tube (mm)

R = observed rotation (°)

■ Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a

= observed angular rotation of the *Sample solution* ($^{\circ}$)

l

= length of the polarimeter tube (dm)

α

= midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U

= nominal concentration of dextrose in the *Sample solution* (g/100 mL)

M_{r1}

= molecular weight of dextrose monohydrate, 198.17

M_{r2}

= molecular weight of anhydrous dextrose, 180.16

■ 2S (USP39)

Acceptance criteria: 90.0%–105.0%

IMPURITIES

Delete the following:

- **Heavy Metals** (231): Transfer to a suitable vessel a volume, in mL, of Injection, calculated to two significant figures:

$$\text{Result} = 0.2/[(G_{S-L_S}) + (G_{K-L_K}) + (G_{C-L_C}) + (G_{L-L_L}) + (G_{D-L_D})]$$

G_S labeled amount of sodium chloride in each 100 mL of Injection (g)

L_S limit for *Heavy Metals* specified under *Sodium Chloride* (percentage)

G_K labeled amount of potassium chloride in each 100 mL of Injection (g)

L_K limit for *Heavy Metals* specified under *Potassium Chloride* (percentage)

G_C labeled amount of calcium chloride in each 100 mL of Injection (g)

L_C limit for *Heavy Metals* specified under *Calcium Chloride* (percentage)

G_L labeled amount of sodium lactate in each 100 mL of Injection (g)

L_L limit for *Heavy Metals* specified under *Sodium Lactate Solution* (percentage)

G_D labeled amount of dextrose in each 100 mL of Injection (g)

L_D limit for *Heavy Metals* specified under *Dextrose* (percentage)

Adjust the volume by evaporation or addition of water to 25 mL, as necessary: it meets the requirements of the test. ●(Official 1-Dec-2015)

- **Limit of 5-Hydroxymethylfurfural and Related Substances**

Sample solution: Nominally equivalent to 2.0 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) from a suitable volume of Injection, in water

Instrumental conditions

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Acceptance criteria: The absorbance is NMT 0.25.

SPECIFIC TESTS

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 0.5 USP Endotoxin Units/mL
- **pH** 〈 791 〉: 4.0–6.5
- **Other Requirements:** It meets the requirements in *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **Labeling:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL. The label also includes the warning: "Not for use in the treatment of lactic acidosis".
- **USP Reference Standards** 〈 11 〉
USP Endotoxin RS
USP Sodium Lactate RS

BRIEFING

Half-Strength Lactated Ringer's and Dextrose Injection, *USP 38* page 5186. It is proposed to revise the monograph as follows:

1. In preparation for the omission of the flame tests from *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], delete the reference to 〈 191 〉 flame tests for sodium and potassium in *Identification* test *B* and include a complete description of the flame test for the two elements in the monograph. Manufacturers are encouraged to submit a replacement wet-chemistry or an instrumental procedure for the Expert Committee's consideration.
2. Add a clarifying statement describing how absorbance data are used to calculate the concentration of calcium in the *Assay for Calcium*.
3. Add the equation for calculating the concentration of potassium in the *Assay for Potassium*.
4. Add the equation for calculating the concentration of sodium in the *Assay for Sodium*.
5. Update the equation in the *Assay for Dextrose* to be consistent with current *USP* style.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: D. Vicchio.)

Correspondence Number—C158316

Comment deadline: September 30, 2015**Half-Strength Lactated Ringer's and Dextrose Injection****DEFINITION**

Half-Strength Lactated Ringer's and Dextrose Injection is a sterile solution of Calcium Chloride, Potassium Chloride, Sodium Chloride, Sodium Lactate, and Dextrose in Water for Injection. It contains, in each 100 mL, NLT 142.5 and NMT 157.5 mg of sodium (Na) [as sodium chloride (NaCl) and sodium lactate (C₃H₅NaO₃)], NLT 7.08 and NMT 8.65 mg of potassium (K) [equivalent to NLT 13.5 and NMT 16.5 mg of potassium chloride (KCl)], NLT 2.45 and NMT 3.00 mg of calcium (Ca) [equivalent to NLT 9.0 and NMT 11.0 mg of calcium chloride (CaCl₂·2H₂O)], NLT 184.0 and NMT 214.0 mg of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride (CaCl₂·2H₂O)], and NLT 115.5 and NMT 130.5 mg of lactate (C₃H₅O₃) [equivalent to NLT 145.0 and NMT 165.0 mg of sodium lactate (C₃H₅NaO₃)]. It contains NLT 90.0% and NMT 105.0% of the labeled amount of dextrose (C₆H₁₂O₆·H₂O). It contains no antimicrobial agents.

[Note—The calcium, potassium, and sodium contents of Half-Strength Lactated Ringer's and Dextrose Injection are approximately 1.4, 2, and 65 mEq/L, respectively.]

IDENTIFICATION● **A.**

Sample solution: Nominally 50 mg/mL of dextrose from Injection in water

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

Delete the following:

- ● **B. Identification Tests—General** ~~(191)~~: Meets the requirements of the flame tests for *Sodium* ~~(191)~~ and *Potassium* ~~(191)~~, of the test for *Chloride* ~~(191)~~, and of the test for ammonium oxalate for *Calcium* ~~(191)~~ ■ 2S (USP39)

Add the following:

- ● **B. Identification Tests—General** (191), **Chloride and Calcium:** Meets the requirements of the test for *Chloride* and the test for ammonium oxalate in *Calcium* ■ 2S (USP39)

Add the following:

- ● **C. Potassium:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.] ■ 2S (USP39)

Add the following:

- • **D. Sodium:** The sample imparts an intense yellow color to a nonluminous flame. ■ 2S (USP39)

Change to read:

- Ca^{2+}

- **E.** ■ 2S (USP39)

The retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay for Lactate.

ASSAY

Change to read:

- **Calcium**

[Note—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Calcium stock solution: 1000 $\mu\text{g/mL}$ of calcium, prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask and add 10 mL of water. Carefully add 5 mL of dilute hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium (Ca), prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 1.0, 1.5, and 2.0 mL, respectively, of *Calcium stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Nominally 0.014 mg/mL of calcium, prepared as follows. Transfer 50.0 mL of Injection, equivalent to 1.4 mg of calcium, to a 100-mL volumetric flask containing 5.0 mL of *Solution A*, and dilute with water to volume.

Blank: 5.0 mL of *Solution A* diluted with water to 100.0 mL

Instrumental conditions

(See *Atomic Absorption Spectroscopy* { 852 } .)

Mode: Atomic absorption spectrophotometer

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Concomitantly determine the absorbance of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus concentration, in mg/mL, of calcium, and draw the straight line best fitting the three plotted points.

- From this graph, calculate the concentration of calcium in each 100 mL of Injection taken: ■ 2S (USP39)

$$\text{Result} = C \times D \times F$$

C = concentration of calcium in the *Sample solution* (mg/mL)

D = dilution factor of the *Sample solution*, 2

F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 2.45–3.00 mg of calcium (Ca) in each 100 mL

Change to read:

● **Potassium**

Solution A: Suitable nonionic wetting solution (1 in 500)

Solution B: 10.93 mg/mL of sodium chloride

Standard stock solution: 0.100 mg/mL of potassium, prepared as follows. Transfer 190.7 mg of potassium chloride, previously dried at 105° for 2 h, to a 1-L volumetric flask and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of potassium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A* and 10 mL of *Solution B*, add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 20.0 mL of Injection to a 100-mL volumetric flask, add 10.0 mL of *Solution A*, and dilute with water to volume.

Blank: Transfer 10.0 mL of *Solution A* and 10 mL of *Solution B* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: Maximum transmittance at 766 nm

Analysis

~~**Samples:** *Standard solutions* and *Sample solution*~~

~~Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of potassium.~~

~~Read the percentage transmittance of the *Sample solution*.~~

~~Calculate the potassium content, in mg/100 mL, of Injection.~~

■ **Samples:** *Standard solutions*, *Sample solution*, and *Blank*

Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus the concentration, in mg/mL, of potassium. Draw the straight line best fitting the four plotted points.

From this graph, calculate the concentration of potassium in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C

= concentration of potassium in the *Sample solution* (mg/mL)

D

= dilution factor of the *Sample solution*, 5

F

= conversion factor for each 100 mL of Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 7.08–8.65 mg of potassium (K) in each 100 mL

Change to read:

● **Sodium**

Solution A: Suitable nonionic wetting solution (1 in 500)

Standard stock solution: 0.100 mg/mL of sodium, prepared as follows. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer this solution to a 100-mL volumetric flask and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A* add 5.0, 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 10 mL of Injection to a 1-L volumetric flask containing 100.0 mL of *Solution A*. Dilute with water to volume.

Blank: Transfer 10.0 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: Maximum transmittance at 589 nm

Analysis

~~**Samples:** *Standard solutions* and *Sample solution*~~

~~Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of sodium.~~

~~Read the percentage transmittance of the *Sample solution*.~~

~~Calculate the sodium content, in mg/100 mL, of Injection.~~

■ **Samples:** *Standard solutions*, *Sample solution*, and *Blank*

Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus the concentration, in mg/mL, of sodium, and draw the straight line best fitting the four plotted points.

From this graph, calculate the concentration of sodium in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C

= concentration of sodium in the *Sample solution* (mg/mL)

D

= dilution factor for the *Sample solution*, 100

F

= conversion factor for each 100 mL of Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 142.5–157.5 mg of sodium (Na) in each 100 mL● **Chloride****Sample solution:** Transfer 10 mL of Injection into a conical flask, and add 10 mL of glacial acetic acid and 75 mL of methanol.**Analysis:** Titrate the *Sample solution*, with shaking, with 0.1 N silver nitrate VS to a pink endpoint using 3 drops of eosin Y TS as an indicator. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl).**Acceptance criteria:** 184.0–214.0 mg of chloride (Cl) in each 100 mL of Injection● **Lactate****Mobile phase:** Dicyclohexylamine, formic acid, and water (1:1:998)**System suitability solution:** 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS, in water**Standard solution:** 1.6 mg/mL of USP Sodium Lactate RS, in water**Sample solution:** Use undiluted Injection.**Chromatographic system**(See *Chromatography* { 621 }, *System Suitability*.)**Mode:** LC**Detector:** UV 210 nm**Column:** 4.6-mm × 10-cm; packing L1**Flow rate:** 1 mL/min**Injection volume:** 20 µL**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 2 between the acetate and lactate peaks, *System suitability solution***Tailing factor:** NMT 2.0, *Standard solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the amount of lactate (C₃H₅O₃) in each 100 mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution*

C_S = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of lactate, 89.07

M_{r2} = molecular weight of anhydrous sodium lactate, 112.06

Acceptance criteria: 115.5–130.5 mg of lactate ($C_3H_5O_3$) in each 100 mL

Change to read:

• **Dextrose**

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis: Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* (781)).

Calculate the percentage, in g/100mL, of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = (100/M) \times (M_{r1}/M_{r2}) \times AR$$

M = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

A = 100 mm divided by the length of the polarimeter tube (mm)

R = observed rotation ($^\circ$)

■ Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a

= observed angular rotation of the *Sample solution* ($^\circ$)

l

= length of the polarimeter tube (dm)

α

= midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U

= nominal concentration of dextrose in the *Sample solution* (g/100 mL)

M_{r1}

= molecular weight of dextrose monohydrate, 198.17

M_{r2}

= molecular weight of anhydrous dextrose, 180.16

■ 2S (USP39)

Acceptance criteria: 90.0%–105.0%

IMPURITIES

Delete the following:

- **Heavy Metals** 〈 231 〉: Transfer to a suitable vessel a volume, in mL, of Injection, calculated to two significant figures:

$$\text{Result} = 0.2 / [(G_S L_S) + (G_K L_K) + (G_C L_C) + (G_L L_L) + (G_D L_D)]$$

G_S labeled amount of sodium chloride in each 100 mL of Injection (g)

L_S limit for *Heavy Metals* specified under *Sodium Chloride* (percentage)

G_K labeled amount of potassium chloride in each 100 mL of Injection (g)

L_K limit for *Heavy Metals* specified under *Potassium Chloride* (percentage)

G_C labeled amount of calcium chloride in each 100 mL of Injection (g)

L_C limit for *Heavy Metals* specified under *Calcium Chloride* (percentage)

G_L labeled amount of sodium lactate in each 100 mL of Injection (g)

L_L limit for *Heavy Metals* specified under *Sodium Lactate* (percentage)

G_D labeled amount of dextrose in each 100 mL of Injection (g)

L_D limit for *Heavy Metals* specified under *Dextrose* (percentage)

Adjust the volume by evaporation or addition of water to 25 mL, as necessary: it meets the requirements of the test. • (Official 1-Dec-2015)

- **Limit of 5-Hydroxymethylfurfural and Related Substances**

Sample solution: Nominally 2.0 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) from Injection, in water

Instrumental conditions

Mode: UV

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution* and *Blank*

Acceptance criteria: The absorbance is NMT 0.25.

SPECIFIC TESTS

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 0.5 USP Endotoxin Units/mL
- **pH** 〈 791 〉: 4.0–6.5
- **Other Requirements:** It meets the requirements in *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **Labeling:** The label states the total osmolar concentration in mOsmol/L. Where the contents

are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL. The label also includes the warning: "Not for use in the treatment of lactic acidosis".

- **USP Reference Standards** 〈 11 〉

USP Endotoxin RS

USP Sodium Lactate RS

BRIEFING

Modified Lactated Ringer's and Dextrose Injection, *USP 38* page 5187. It is proposed to revise the monograph as follows:

1. In preparation for the omission of the flame tests from *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], delete the reference to 〈 191 〉 flame tests for sodium and potassium in *Identification* test *B* and include a complete description of the flame test for the two elements in the monograph. Manufacturers are encouraged to submit a replacement wet-chemistry or an instrumental procedure for the Expert Committee's consideration.
2. In the *Assay Analysis* for *Calcium*, revise the equivalent amount of calcium in the *Sample solution* to get the solution within the linearity range evaluated. A clarifying statement is also added, describing how absorbance data are used to calculate the concentration of calcium.
3. Add the equation for calculating the concentration of potassium in the *Assay* for *Potassium*.
4. Add the equation for calculating the concentration of sodium in the *Assay* for *Sodium*.
5. Update the equation in the *Assay* for *Dextrose* to be consistent with current *USP* style.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: S. Ramakrishna, D. Vicchio.)

Correspondence Number—C158123

Comment deadline: September 30, 2015

Modified Lactated Ringer's and Dextrose Injection

DEFINITION

Modified Lactated Ringer's and Dextrose Injection is a sterile solution of Calcium Chloride, Potassium Chloride, Sodium Chloride, Sodium Lactate, and Dextrose in Water for Injection. It contains, in each 100 mL, NLT 57.0 and NMT 63.0 mg of sodium (Na) [as sodium chloride (NaCl) and sodium lactate, (C₃H₅NaO₃)], NLT 2.82 and NMT 3.46 mg of potassium (K) [equivalent to NLT 5.4 and NMT 6.6 mg of potassium chloride (KCl)], NLT 0.98 and NMT 1.20 mg of calcium (Ca) [equivalent to NLT 3.6 and NMT 4.4 mg of calcium chloride (CaCl₂·2H₂O)], NLT 73.6 and NMT 85.6 mg of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride (CaCl₂·2H₂O)], NLT 46.2 and NMT 52.20 mg of lactate (C₃H₅O₃) [equivalent to NLT 58.0 and NMT 66.0 mg of sodium lactate (C₃H₅NaO₃)]. It contains NLT 90.0% and NMT 105.0% of the labeled amount of dextrose (C₆H₁₂O₆·H₂O). It contains no antimicrobial agents.

[Note—The calcium, potassium, and sodium contents of Modified Lactated Ringer's and Dextrose Injection are approximately 0.5, 0.8, and 26 mEq/L, respectively.]

IDENTIFICATION

• A.

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

Delete the following:

- ~~• B. Identification Tests—General (191): Meets the requirements of the flame tests for Sodium (191) and Potassium (191), of the test for Chloride (191), and of the test for ammonium oxalate for Calcium (191) ■ 2S (USP39)~~

Add the following:

- **B. Identification Tests—General (191), Chloride and Calcium:** Meets the requirements of the test for *Chloride* and the test for ammonium oxalate in *Calcium* ■ 2S (USP39)

Add the following:

- **C. Potassium:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.] ■ 2S (USP39)

Add the following:

- **D. Sodium:** The sample imparts an intense yellow color to a nonluminous flame. ■ 2S (USP39)

Change to read:

• E.

- **E.** ■ 2S (USP39)

The retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Lactate*.

ASSAY

Change to read:

• Calcium

[Note—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Calcium stock solution: 1 mg/mL of calcium, prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask and add 10 mL of water. Carefully add 5 mL of dilute hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium (Ca), prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 1.0, 1.5, and 2.0 mL, respectively, of *Calcium stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Add 5.0 mL of *Solution A* to a 100-mL volumetric flask, and dilute with Injection to volume.

Blank: 5.0 mL of *Solution A* diluted with water to 100.0 mL

Instrumental conditions

(See *Atomic Absorption Spectroscopy* { 852 } .)

Mode: Atomic absorption spectrophotometer

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air-acetylene

Blank: *Blank*

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Concomitantly determine the absorbance of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus the concentration of calcium, in mg/mL, and draw the straight line best fitting the three plotted points.

■ From this graph, calculate the concentration of calcium in the *Sample solution*.

■ 2S (USP39)

Calculate the quantity, in mg, of calcium in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of calcium in the *Sample solution* (mg/mL)

D = dilution factor of the *Sample solution*, 1.055

F = conversion factor for each 100 mL of Injection, 100

Acceptance criteria: 0.98 mg–1.20 mg of calcium per 100 mL of Injection

Change to read:

● Potassium

Solution A: Suitable nonionic wetting solution (1 in 500)

Solution B: 10.93 mg/mL of sodium chloride in water

Standard stock solution: 0.100 mg/mL of potassium, prepared as follows. Transfer 190.7 mg of potassium chloride, previously dried at 105° for 2 h, to a 1-L volumetric flask and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of potassium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A* and 10 mL of *Solution B*, add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 50.0 mL of Injection to a 100-mL volumetric flask containing 10 mL of *Solution A*, dilute with water to volume, and mix.

Blank: Transfer 10.0 mL of *Solution A* and 10 mL of *Solution B* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: Maximum transmittance at 766 nm

Analysis

Samples: ~~Standard solutions and Sample solution~~

~~Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the blank. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of potassium.~~

~~Read the percentage transmittance of the *Sample solution*.~~

~~Calculate the potassium content, in mg/100 mL, of Injection.~~

■ **Samples:** *Standard solutions, Sample solution, and Blank*

Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus the concentration, in mg/mL, of potassium. Draw the straight line best fitting the four plotted points. From this graph, calculate the concentration of potassium in the *Sample solution*.

Calculate the quantity of potassium in each 100 mL of Injection:

$$\text{Result} = C \times D \times F$$

C

= concentration of potassium in the *Sample solution* (mg/mL)

D

= dilution factor of the *Sample solution*, 2

F

= conversion factor for each 100 mL of Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 2.82–3.46 mg of potassium per 100 mL of Injection

Change to read:

● **Sodium**

Solution A: Suitable nonionic wetting solution (1 in 500)

Standard stock solution: 0.100 mg/mL of sodium, prepared as follows. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer this solution to a 1-L volumetric flask and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A*, add

5.0, 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 25.0 mL of Injection in a 1-L volumetric flask containing 100.0 mL of *Solution A* and dilute with water to volume.

Blank: Transfer 10.0 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: Maximum transmittance at 589 nm

Analysis

~~Analysis~~

~~**Samples:** *Standard solutions* and *Sample solution*~~

~~Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the blank. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of sodium.~~

~~Read the percentage transmittance of the *Sample solution*.~~

~~Calculate the sodium content, in mg/100 mL, of Injection.~~

■ **Samples:** *Standard solutions*, *Sample solution*, and *Blank*

Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus concentration, in mg/mL, of sodium, and draw the straight line best fitting the four plotted points. From the graph, calculate the concentration of sodium in the *Sample solution*.

Calculate the quantity of sodium in each 100 mL of Injection:

$$\text{Result} = C \times D \times F$$

C

= concentration of sodium in the *Sample solution* (mg/mL)

D

= dilution factor of the *Sample solution*, 40

F

= conversion factor for each 100 mL of Injection, 100 mL

■ 2S (USP39)

Acceptance criteria: 57.0–63.0 mg of sodium per 100 mL of Injection

Change to read:

- **Chloride**

Sample solution: Transfer a volume of Injection equivalent to 55 mg of chloride (1.55

mEq) to a suitable conical flask, and add water, if necessary, to bring the volume to 10 mL. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS.

Titrimetric system

■ (See *Titrimetry* 〈 541 〉). ■ 2S (USP39)

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate the *Sample solution*, with shaking, with *Titrant* to a pink endpoint, using 3 drops of eosin Y TS. Each mL of *Titrant* is equivalent to 3.545 mg of chloride (Cl).

Calculate the labeled amount of chloride (Cl) in 100 mL of Injection:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V

= *Titrant* volume consumed by the *Sample solution* (mL)

N

= actual normality of the *Titrant* (mEq/mL)

F

= equivalency factor, 35.45 mg/mEq

W

= nominal amount of chloride in the *Sample solution* (mg)

■ 2S (USP39)

Acceptance criteria: 73.6–85.6 mg of chloride per 100 mL

• Lactate

Mobile phase: Dicyclohexylamine, formic acid, and water (1:1:998)

System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water

Standard solution: 0.6 mg/mL of USP Sodium Lactate RS, in water

Sample solution: Use undiluted Injection.

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between the acetate and lactate peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount of lactate ($C_3H_5O_3$) in each 100 mL of Injection:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of lactate, 89.07

M_{r2} = molecular weight of anhydrous sodium lactate, 112.06

Acceptance criteria: 46.2–52.20 mg of lactate ($C_3H_5O_3$) per 100 mL of Injection

Change to read:

• Dextrose

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis: Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* 〈 781 〉).

Calculate the percentage, in g/100mL, of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = (100/M) \times (M_{r1}/M_{r2}) \times AR$$

M = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

A = 100 mm divided by the length of the polarimeter tube (mm)

R = observed rotation ($^\circ$)

■ Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a

= observed angular rotation of the *Sample solution* ($^\circ$)

l

= length of the polarimeter tube (dm)

α

= midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U

= nominal concentration of dextrose in the *Sample solution* (g/100 mL)

M_{r1}

= molecular weight of dextrose monohydrate, 198.17

M_{r2}

= molecular weight of anhydrous dextrose, 180.16

■ 2S (USP39)

Acceptance criteria: 90.0%–105.0%

IMPURITIES

Delete the following:

- **Heavy Metals** $\langle 231 \rangle$: Transfer to a suitable vessel a volume, in mL, of Injection, calculated to two significant figures:

$$\text{Result} = 0.2 / [(G_{S-L_S}) + (G_{K-L_K}) + (G_{C-L_C}) + (G_{L-L_L}) + (G_{D-L_D})]$$

G_S labeled amount of sodium chloride in each 100 mL of Injection (g)

L_S limit for *Heavy Metals* specified under *Sodium Chloride* (percentage)

G_K labeled amount of potassium chloride in each 100 mL of Injection (g)

L_K limit for *Heavy Metals* specified under *Potassium Chloride* (percentage)

G_C labeled amount of calcium chloride in each 100 mL of Injection (g)

L_C limit for *Heavy Metals* specified under *Calcium Chloride* (percentage)

G_L labeled amount of sodium lactate in each 100 mL of Injection (g)

L_L limit for *Heavy Metals* specified under *Sodium Lactate* (percentage)

G_D labeled amount of dextrose in each 100 mL of Injection (g)

L_D limit for *Heavy Metals* specified under *Dextrose* (percentage)

Adjust the volume by evaporation or addition of water to 25 mL, as necessary: it meets the requirements of the test. ● (Official 1-Dec-2015)

- **Limit of 5-Hydroxymethylfurfural and Related Substances**

Sample solution: Nominally 2.0 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) from a suitable volume of Injection, in water

Instrumental conditions

Analytical wavelength: Maximum transmittance at 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution* and *Blank*

Acceptance criteria: The absorbance is NMT 0.25.

SPECIFIC TESTS

- **Bacterial Endotoxins Test** 〈 85 〉: NMT 0.5 USP Endotoxin Units/mL
- **pH** 〈 791 〉: 4.0–6.5
- **Other Requirements:** It meets the requirements in *Injections* 〈 1 〉.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **Labeling:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL. The label also includes the warning: "Not for use in the treatment of lactic acidosis".
- **USP Reference Standards** 〈 11 〉
USP Endotoxin RS
USP Sodium Lactate RS

BRIEFING

Sodium Nitroprusside, *USP 38* page 5327. In preparation for the omission of the flame tests from *Identification Tests—General* 〈 191 〉, proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to replace the flame test for sodium in *Identification* test C with a colorimetric test using zinc uranyl acetate. This test is adopted from the identity tests described in the *Sodium Nitroprusside* monograph in the fourth edition of *The International Pharmacopoeia*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM2: S. Ramakrishna.)
Correspondence Number—C155173

Comment deadline: September 30, 2015

Sodium Nitroprusside

$\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}] \cdot 2\text{H}_2\text{O}$ 297.95

$\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$ 261.92

Ferrate(2-), pentakis(cyano-C)nitrosyl-, disodium, dihydrate, (OC-6-22)-;
Disodium pentacyanonitrosylferrate(2-) dihydrate;
Sodium nitroferricyanide dihydrate [13755-38-9].
Anhydrous [14402-89-2].

DEFINITION

Sodium Nitroprusside contains NLT 99.0% of sodium nitroprusside ($\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}] \cdot 2\text{H}_2\text{O}$).

IDENTIFICATION

- **A. Ultraviolet Absorption** 〈 197U 〉
Use low-actinic glassware.

Sample solution: 0.74 mg/mL in water

Wavelength range: 350–700 nm

Acceptance criteria: Meets the requirements

- **B.** Dissolve 5 mg of Sodium Nitroprusside in 2 mL of water. Add 2 drops of acetone and 0.5 mL of 2 N sodium hydroxide. An orange color is produced that changes to a purple color upon adding 2 mL of acetic acid.

Change to read:

- **C.** ~~Identification Tests—General (191), Sodium~~

~~**Sample solution:** 250 mg/mL~~

~~**Acceptance criteria:** Meets the requirements~~

■ **Sample solution:** 100 mg/mL of Sodium Nitroprusside solution acidified with 5 N acetic acid

Analysis: Treat the *Sample solution* with *zinc uranyl acetate TS*.

Acceptance criteria: A yellow crystalline precipitate is produced. ■2S (USP39)

ASSAY

- **Procedure**

Sample: 500 mg of Sodium Nitroprusside

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 130 mL of chloride-free water. Titrate with *Titrant*, using a silver–silver chloride electrode system, and determine the endpoint. Each mL of 0.1 N silver nitrate is equivalent to 14.90 mg of sodium nitroprusside ($\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}] \cdot 2\text{H}_2\text{O}$).

Acceptance criteria: NLT 99.0%

IMPURITIES

- **Insoluble Substances**

Sample solution: Dissolve 10.0 g of Sodium Nitroprusside in 50 mL of water.

Analysis: Heat the *Sample solution* on a steam bath for 30 min. Filter, wash the residue with water, and dry at 105° to constant weight.

Acceptance criteria: NMT 1 mg (0.01%)

- **Chloride**

Solution A: 83 mg/mL of cupric sulfate

Standard chloride solution: Dissolve 42.4 mg of potassium chloride in water to make a 100.0 mL solution. This solution contains 0.2 mg/mL of chloride.

Sample: 1.0 g of Sodium Nitroprusside

Analysis: Transfer the *Sample* and 1.0 mL of *Standard chloride solution* to two separate 250-mL conical flasks and add 85 mL of water to each flask. To the flask containing the substance under analysis, add 15 mL of *Solution A*, and allow any undissolved particles to settle. Carefully add *Solution A* to the flask containing the *Standard chloride solution* by mixing, so that its color matches that of the flask containing the *Sample*. Filter the contents of each flask, and discard the first 25 mL of the filtrate. To 10 mL of the subsequent filtrate from each flask, add 2 mL of nitric acid, add 1 mL of 1 N silver nitrate

and mix again.

Acceptance criteria: The *Sample* so treated becomes no more turbid than the treated *Standard chloride solution* (0.02%).

- **Sulfate**

Standard sulfate solution: Dissolve 15 mg of anhydrous sodium sulfate in water to make a 100.0 mL solution. This solution contains 0.1 mg/mL of sulfate.

Sample solution: Dissolve 5.0 g of Sodium Nitroprusside in water to make a 250.0 mL solution.

Analysis: Filter the *Sample solution* into a flat-bottom 250-mL graduated flask. Transfer 5.0 mL of *Standard sulfate solution* to a similar flask, and dilute to the same volume as the sample under test. To each flask add 10 drops of glacial acetic acid and 5 mL of 1 N barium chloride, and allow to stand for 10 min. Place both flasks over a fluorescent light source, and observe.

Acceptance criteria: The turbidity in the treated *Sample solution* is not more intense than that of the treated *Standard sulfate solution* (0.01%).

- **Limit of Ferricyanide**

Sample solution: Dissolve 500 mg of Sodium Nitroprusside in 20 mL of ammonium acetate TS, previously adjusted with 1 N acetic acid to a pH of 4.62.

Blank solution: Dissolve 250 mg of Sodium Nitroprusside in 10 mL of ammonium acetate TS, previously adjusted with 1 N acetic acid to a pH of 4.62, and dilute with water to 50 mL.

Analysis: Divide the *Sample solution* into halves, and transfer each half into individual 50-mL volumetric flasks. Identify the flasks as A and B. To flask B add 1.0 mL of a freshly prepared 78 µg/mL potassium ferricyanide solution. To both flasks add 5 mL of 1 mg/mL of ferrous ammonium sulfate solution, and dilute with water to volume. Allow the flasks to stand for 1 h, and concomitantly measure the absorbance of the solutions at the wavelength of maximum absorbance at about 720 nm using the *Blank solution* as a blank.

Acceptance criteria: The absorbance of the solution in flask A is NMT the absorbance of the solution in flask B minus the absorbance of the solution in flask A (0.02% of ferricyanide).

- **Limit of Ferrocyanide**

Sample solution: Dissolve 2.0 g of Sodium Nitroprusside in 40 mL of water.

Blank solution: Dissolve 1.0 g of Sodium Nitroprusside in water to make 50 mL.

Analysis: Divide the *Sample solution* into halves, and transfer each half into individual 50-mL volumetric flasks. Identify the flasks as A and B. To flask B add 2 mL of a freshly prepared 200 µg/mL of potassium ferrocyanide solution. To both flasks add 0.2 mL of ferric chloride TS, dilute with water to volume, and mix. Allow to stand for 20 min, accurately timed, and concomitantly measure the absorbance of the solutions at the wavelength of maximum absorbance at about 695 nm using the *Blank solution* as the blank.

Acceptance criteria: The absorbance of the solution in flask A is NMT the absorbance of the solution in flask B minus the absorbance of the solution in flask A (0.02% of ferrocyanide).

SPECIFIC TESTS

- **Water Determination** { 921 }, *Method I*: 9.0%–15.0%

- **Sterility Tests** { 71 } : Where the label states that Sodium Nitroprusside is sterile, it meets the requirements.

- **Bacterial Endotoxins Test** 〈 85 〉: Where the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.05 USP Endotoxin Units/μg of sodium nitroprusside.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at 25°; excursions permitted between 15° and 30°.
- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP Reference Standards** 〈 11 〉
 - USP Endotoxin RS
 - USP Sodium Nitroprusside RS

BRIEFING

Silver Sulfadiazine, *USP 38* page 5383. As part of USP monograph modernization efforts, the following revisions are proposed:

1. Replace the TLC procedure using hazardous solvent (chloroform) in *Identification* test *B* with the retention time agreement proposed in the HPLC-based *Assay*.
2. A more selective, single HPLC procedure is used for both the *Assay* and *Organic Impurities* test. The liquid chromatographic procedure is based on analyses performed with the YMC Pack Pro brand of L1 column. The typical retention time for silver sulfadiazine is about 13 min. The Purospher star LP RP-18 brand of L1 column is a suitable alternate column for this test.
3. Replace the TLC procedure in the test for *Organic Impurities* with a validated HPLC procedure that uses the same chromatographic procedure as proposed for the *Assay*. The *Acceptance criteria* for specified and individual unspecified impurities are proposed based on International Conference on Harmonization guidelines. Stakeholders with different impurities and/or limits than those in this revision are strongly encouraged to submit their FDA-approved acceptance criteria, along with the list of specified degradation products, for the Expert Committee's consideration.
4. Add USP Sulfanilic Acid RS and USP Sulfanilamide RS to the *USP Reference Standards* section to support the proposed revision in the test for *Organic Impurities*.

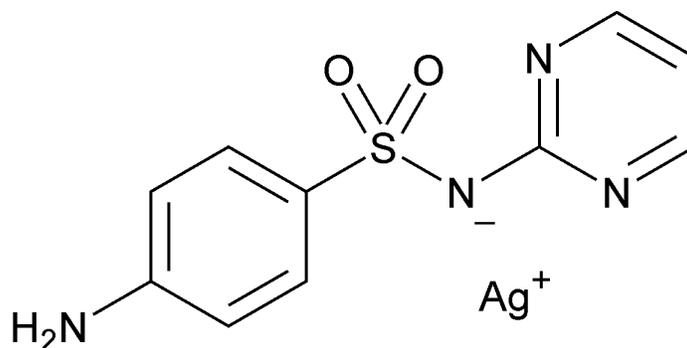
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM1: S. Ramachandran.)

Correspondence Number—C138908

Comment deadline: September 30, 2015

Silver Sulfadiazine



$C_{10}H_9AgN_4O_2S$ 357.14

Benzenesulfonamide, 4-amino-*N*-2-pyrimidinyl-, monosilver(1+) salt;
*N*¹-2-Pyrimidinylsulfanilamide monosilver(1+) salt [22199-08-2].

DEFINITION

Silver Sulfadiazine contains NLT 98.0% and NMT 102.0% of silver sulfadiazine ($C_{10}H_9AgN_4O_2S$), calculated on the dried basis.

IDENTIFICATION

• A. Infrared Absorption (197K)

Change to read:

- B. The R_f value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*, as obtained in the test for *Organic Impurities*.
- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

• C. Identification Tests—General (191), Silver

Sample: 1 g

Analysis: Dissolve the *Sample* in 15 mL of *ammonium hydroxide* and 15 mL of water in a 50-mL volumetric flask, dilute with water to volume, and mix.

Acceptance criteria: Meets the requirements

ASSAY

Change to read:

• Procedure

Mobile phase: Acetonitrile, phosphoric acid, and water (99:1:900)

Diluent: Transfer 100 mL of ammonium hydroxide to a 1-L volumetric flask, and dilute with water to volume.

Internal standard solution: 10 mg/mL of sulfamerazine in *Diluent*

Standard stock solution: 1.25 mg/mL of USP Silver Sulfadiazine prepared as follows.

Dissolve 250 mg of USP Silver Sulfadiazine RS in 100 mL of *Diluent* in a 200-mL volumetric flask, and sonicate for 5 min. Add 25.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.

Standard solution: ~~0.05 mg/mL of USP Silver Sulfadiazine RS prepared as follows. Pipet 2.0 mL of *Standard stock solution* into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.~~

Sample stock solution: ~~Transfer 250 mg of Silver Sulfadiazine to a 50-mL round-bottom centrifuge tube. Add 35 mL of methanol, tightly seal the tube with a cap containing an inert liner, and mix using a vortex mixer for about 15 s. Centrifuge for 15 min to separate the liquid and solid phases. Aspirate, and discard the methanol supernatant layer. Care should be taken to avoid aspirating any of the residue. Pipet 25.0 mL of *Internal standard solution* into a 200-mL volumetric flask. Add 30 mL of *Diluent* to the centrifuge tube, replace the cap, and mix using a vortex mixer, for about 15 s. Quantitatively transfer the contents to the 200-mL volumetric flask, using the *Diluent* to rinse the tube. Repeat the addition of 30 mL of *Diluent*, mixing and quantitatively transferring three more times. Dilute with *Diluent* to volume, and mix. Sonicate if necessary to obtain dissolution of the residue.~~

Sample solution: ~~0.05 mg/mL of Silver Sulfadiazine prepared as follows. Pipet 2.0 mL of *Sample stock solution* into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.~~

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between sulfadiazine and sulfamerazine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of silver sulfadiazine ($C_{10}H_9AgN_4O_2S$) in the portion of Silver Sulfadiazine taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U peak response ratio of sulfadiazine to the internal standard from the *Sample solution*

R_S peak response ratio of sulfadiazine to the internal standard from the *Standard solution*

C_S concentration of USP Silver Sulfadiazine RS in the *Standard solution* (mg/mL)

C_U concentration of Silver Sulfadiazine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

■ **Solution A:** 0.77 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 5.2 ± 0.1.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
4	98	2
16	40	60
20	40	60
22	20	80
32	20	80
34	98	2
40	98	2

Diluent: Acetonitrile, ammonium hydroxide, and water (50:60:900)

Standard solution: 0.05 mg/mL of USP Silver Sulfadiazine RS in *Diluent*. Sonicate, if necessary, to dissolve.

Sample solution: 0.05 mg/mL of Silver Sulfadiazine in *Diluent*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of silver sulfadiazine (C₁₀H₉AgN₄O₂S) in the portion of Silver Sulfadiazine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Silver Sulfadiazine RS in the *Standard solution* (mg/mL)

C_U = concentration of Silver Sulfadiazine in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the dried basis ■2S (USP39)**OTHER COMPONENTS**● **Content of Silver****Sample solution:** Add 500 mg to 150 mL of water and 50 mL of *nitric acid*, and stir for 15 min.**Titrimetric system**(See *Titrimetry* 〈 541 〉.)**Mode:** Direct titration**Titrant:** *Potassium thiocyanate, tenth-normal (0.1 N) VS* or *ammonium thiocyanate, tenth-normal (0.1 N) VS***Endpoint detection:** Potentiometric**Analysis:** Titrate the *Sample solution* with *Titrant* using a silver-based indicator electrode and a double-junction reference electrode. Perform a blank determination, and make any necessary correction. Each mL of *potassium thiocyanate, tenth-normal (0.1 N) VS* or *ammonium thiocyanate, tenth-normal (0.1 N) VS* is equivalent to 10.79 mg of silver.**Acceptance criteria:** 29.3%–30.5% of silver is found.**IMPURITIES**● **Limit of Nitrate****Standard solution:** 200 µg/mL of nitrate from *potassium nitrate***Sample solution:** Add 30.0 mL of water to 2 g of Silver Sulfadiazine, stir for 20 min, and pass through a suitable, nitrate-free filter.**Instrumental conditions****Mode:** UV-Vis**Analytical wavelength:** 408 nm**Blank:** Deionized water**Analysis****Samples:** *Standard solution*, *Sample solution*, and *Blank*

Pipet 3 mL of the *Sample solution* and *Blank* into separate test tubes. Pipet 1 mL of the *Standard solution* and 2 mL of water into a third test tube. Cool the three test tubes in an ice bath. Slowly add 7.0 mL of cold chromotropic acid solution, prepared by dissolving 50 mg of chromotropic acid in 100 mL of cold sulfuric acid, to each test tube, while swirling, and allow the test tubes to remain in the ice bath for 3 min after the addition of the chromotropic acid solution. Remove the test tubes from the ice bath, and allow to stand for 30 min. Concomitantly determine the absorbances of the *Sample solution* and the *Standard solution* with a suitable spectrophotometer, against the *Blank*.

Calculate the percentage of nitrate content in the portion of Silver Sulfadiazine taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

 $A_{\bar{U}}$ absorbance of the *Sample solution* $A_{\bar{S}}$ absorbance of the *Standard solution*

$C_{\bar{s}}$ concentration of nitrate in the *Standard solution* ($\mu\text{g/mL}$)

$C_{\bar{f}}$ concentration of Silver Sulfadiazine in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: NMT 0.1%

Change to read:

• **Organic Impurities**

Standard solution A: 5 mg/mL of USP Silver Sulfadiazine RS prepared as follows. Transfer 50 mg of USP Silver Sulfadiazine RS to a 10-mL volumetric flask, and dissolve in 3.0 mL of ammonium hydroxide. Dilute with methanol to volume, and mix.

Standard solution B: 0.05 mg/mL of USP Silver Sulfadiazine RS in a mixture of methanol and ammonium hydroxide (4:1) from *Standard solution A*

Sample solution: 5 mg/mL of Silver Sulfadiazine prepared as follows. Transfer 50 mg of Silver Sulfadiazine to a 10-mL volumetric flask, and dissolve in 3.0 mL of ammonium hydroxide. Dilute with methanol to volume, and mix.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μL

Developing solvent system: Chloroform, methanol, and ammonium hydroxide (7:4:1). Mix the chloroform and methanol, then add the ammonium hydroxide.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Separately apply *Samples* to the chromatographic plate. Allow the spots to dry, and place the plate in the chromatographic chamber. When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light.

Acceptance criteria: 1.0%; no secondary spot of the *Sample solution* is larger or more intense than the principal spot of *Standard solution B*. The sum of all secondary spots observed is NMT 2.0%.

■ **Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system:**

Proceed as directed in the *Assay*.

Standard solution: 0.001 mg/mL each of USP Silver Sulfadiazine RS, USP Sulfanilic Acid RS, USP Sulfanilamide RS, and *sulfaguanidine* in *Diluent*

Sample solution: 1.0 mg/mL of Silver Sulfadiazine in *Diluent*

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between *sulfaguanidine* and *sulfanilamide*

Relative standard deviation: NMT 5.0% for the silver sulfadiazine peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Silver Sulfadiazine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U

= peak response of each impurity from the *Sample solution*

r_S

= peak response of silver sulfadiazine from the *Standard solution*

C_S

= concentration of USP Silver Sulfadiazine RS in the *Standard solution* (mg/mL)

C_U

= concentration of Silver Sulfadiazine in the *Sample solution* (mg/mL)

F

= relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Sulfanilic acid	0.27	0.27	0.1
Sulfaguanidine ^a	0.65	0.85	0.1
Sulfanilamide	0.71	1.0	0.1
2-Aminopyrimidine ^b	0.82	0.48	0.1
Sulfadiazine ^c	1.00	—	—
Individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	2.0

^a 4-Amino-*N*-carbamimidoylbenzenesulfonamide.

^b Pyrimidin-2-amine.

^c 4-Amino-*N*-2-pyrimidinyl-benzenesulfonamide.

■ 2S (USP39)

SPECIFIC TESTS

• Loss on Drying (731)

Analysis: Dry at 105° for 1 h.

Acceptance criteria: NMT 0.5%

• Particle Size

Perform in subdued light.

Sample: 0.5 g of Silver Sulfadiazine

Analysis: Wrap a 1-L flask in aluminum foil, add the *Sample* and 1000 mL of a suitable isotonic solution, and mix for 2 h. Add 5 or 6 drops of a suitable dispersant. Place the container in an ultrasonic bath, sonicate for 15 s, and immediately analyze, using a suitable electronic particle counter equipped with a population counter and 140- and 30- μm apertures.

Acceptance criteria: The average particle size is NMT 10 μm , and the size of NMT 10% of the particles is greater than 40 μm .

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers.

Change to read:

- **USP Reference Standards** $\langle 11 \rangle$

USP Silver Sulfadiazine RS

- USP Sulfanilamide RS

4-Aminobenzenesulfonamide.

$\text{C}_6\text{H}_8\text{N}_2\text{O}_2\text{S}$ 172.20

USP Sulfanilic Acid RS

4-Aminobenzenesulfonic acid.

$\text{C}_6\text{H}_7\text{NO}_3\text{S}$ 173.19 ■_{2S} (USP39)

BRIEFING

Teriparatide Injection. Because there is no existing *USP* monograph for this drug product, a new monograph based on validated methods of analysis is being proposed. The liquid chromatographic procedure in the *Assay* and the test for *Product-Related Impurities* is based on analyses performed with the Zorbax 300SB-C18 brand of L1 column. In the *Assay*, the retention time for the teriparatide peak is approximately 8 min.

(BIO1: E. Chang.)

Correspondence Number—C151705

Comment deadline: September 30, 2015

Add the following:

- **Teriparatide Injection**

DEFINITION

Teriparatide Injection is a sterile solution of Teriparatide in Water for Injection. It contains NLT 90.0% and NMT 105.0% of the labeled amount of teriparatide ($\text{C}_{181}\text{H}_{291}\text{N}_{55}\text{O}_{51}\text{S}_2$).

IDENTIFICATION

- **A.** The ratio of the retention time of the main peak of the *Sample solution* to that of the *Standard solution* is 1.00 ± 0.03 , as obtained in the *Assay*.

ASSAY

- **Procedure**

0.2 M sulfate buffer: 28.4 g/L of anhydrous sodium sulfate in water. Adjust with 85% phosphoric acid to a pH of 2.3.

Solution A: Acetonitrile and 0.2 M sulfate buffer (10:90)

Solution B: Acetonitrile and 0.2 M sulfate buffer (50:50)

Mobile phase: *Solution A* and *Solution B* (61:39). [Note—The *Mobile phase* composition may be adjusted to obtain the retention time of approximately 8 min for the teriparatide main peak.]

Diluent for Standard solution: Acetonitrile and 0.2 M sulfate buffer (25:75)

0.27 M sulfate buffer: 38.8 g/L of anhydrous sodium sulfate in water. Adjust with 85% phosphoric acid to a pH of 2.3.

Diluent for Sample solution: Acetonitrile and 0.27 M sulfate buffer (31:69)

Standard solutions: Prepare three solutions, each containing approximately 100 µg/mL of USP Teriparatide RS in *Diluent for Standard solution*. *Standard solutions* are stable for 48 h when stored at 2°–8° in a sealed container.

Sample solutions: Prepare two dilutions of approximately 50–100 µg/mL of teriparatide in *Diluent for Sample solution*. *Sample solutions* are stable for 48 h when stored at 2°–8° in a sealed container.

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Temperatures

Column: 40°

Autosampler: 5°

Flow rate: 0.8 mL/min

Injection volume: 25 µL

Run time: 20 min

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for the teriparatide peak

Relative standard deviation: NMT 1.25% calculated from three injections of one *Standard solution*

Analysis

Samples: *Standard solutions* and *Sample solutions*

Measure the peak responses corresponding to teriparatide.

Calculate the concentration of teriparatide (C_S), in µg/mL, in each of the *Standard solutions*:

$$C_S = (L_S/V_S)$$

L_S content of teriparatide in USP Teriparatide RS (µg)

V_S volume of *Diluent for Standard solution* used for each *Standard solution* (mL)

Determine the average concentration of teriparatide (C_M) for all three *Standard*

solutions.

Calculate the mean response factor (F_M) for all three *Standard solutions*:

$$F_M = (r_M/C_M)$$

$r_{\overline{M}}$ average peak response of teriparatide from the *Standard solutions*

$C_{\overline{M}}$ average concentration of teriparatide in the *Standard solutions* ($\mu\text{g/mL}$)

Calculate the concentration of teriparatide (C_U), in $\mu\text{g/mL}$, in the portion of Injection taken:

$$C_U = (r_U \times F)/F_M$$

r_U peak response of teriparatide from the *Sample solution*

F = dilution factor used to prepare the *Sample solution*

$F_{\overline{M}}$ mean response factor for all three *Standard solutions*

Calculate the percentage of the label claim of teriparatide in the portion of Injection taken:

$$\text{Result} = (C_U/C_L) \times 100$$

C_U concentration of teriparatide in the portion of Injection taken ($\mu\text{g/mL}$)

C_L labeled concentration of teriparatide in the portion of Injection taken ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–105.0%

PRODUCT RELATED SUBSTANCES IMPURITIES

• Product-Related Impurities

0.2 M sulfate buffer: 28.4 g/L of anhydrous sodium sulfate in water. Adjust with 85% phosphoric acid to a pH of 2.3.

Solution A: Acetonitrile and 0.2 M sulfate buffer (10:90)

Solution B: Acetonitrile and 0.2 M sulfate buffer (50:50)

[Note—If the sodium sulfate precipitates, gentle heating and continuous stirring may be required. The sodium sulfate should not re-precipitate if this procedure is followed.]

Mobile phase: See Table 1. [Note—The *Mobile phase* composition may be adjusted to obtain the desired retention time of the teriparatide peak. *Solution B* (%) at 8 and 68 min may also be changed, if necessary, to obtain the desired retention time, but the same gradient slopes should be maintained. A change of 0.5% of *Solution B* will alter the retention time of the main peak approximately 100 s.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
8	76	24
68	60	40
75	0	100
80	0	100

System suitability solution: Use an appropriate solution containing approximately 0.8% of the first post-main peak in *Solution A*. [Note—Teriparatide containing the first post-main peak may be prepared by dissolving teriparatide in water to obtain a concentration of 2 mg/mL. Adjust with hydrochloric acid to a pH of 3.0. Incubate this solution at 50° for 9 days. The solution may be aliquotted and stored frozen. Dilute 1:1 with *Solution A* prior to injection. The first post-main peak is a degradation product resulting from this process and elutes immediately after the teriparatide peak.]

Sample solution: Use the solution from an undiluted Injection container.

Blank: *Solution A*

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

Temperatures

Column: 40°

Autosampler: 5°

Flow rate: 1.0 mL/min

Injection volume: 50 μL

System suitability

Sample: *System suitability solution*

[Note—The retention time for teriparatide is 60.83–66.67 min.]

Suitability requirements

Peak-to-valley ratio: The ratio of the height of the first post-main peak to the valley between the teriparatide peak and the first post-main peak is NLT 1.5.

Tailing factor: NMT 2.0 for the teriparatide peak

Analysis

Sample: *Sample solution*

Measure the peak responses for all integrated peaks.

Calculate the percentage of a related impurity, rhPTH(1–30) (a cleavage product of teriparatide at Asn 30), in the portion of Injection taken:

$$\text{Result} = (r_{rhPTH(1-30)} / r_T) \times 100$$

$r_{rhPTH(1-30)}$ = peak response of rhPTH(1–30)

r_T = sum of all the peak responses

Calculate the percentage of a related impurity, teriparatide succinimide(30) (formation of succinimide on Asn 30), in the portion of Injection taken:

$$\text{Result} = (r_{Suc} / r_T) \times 100$$

r_{Suc} = peak response of teriparatide succinimide(30)

r_T = sum of all the peak responses

Calculate the percentage of the largest other related impurity of teriparatide in the portion of Injection taken:

$$\text{Result} = (r_i/r_T) \times 100$$

$r_{\overline{T}}$ peak response of the largest other related impurity of teriparatide

$r_{\overline{T}}$ sum of all the peak responses

Calculate the percentage of total related impurities in the portion of Injection taken:

$$\text{Result} = [(r_T - r_S)/r_T] \times 100$$

$r_{\overline{T}}$ sum of all the peak responses

$r_{\overline{S}}$ peak response of teriparatide

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
rhPTH(1-30)	0.77-0.78	1.2
Teriparatide succinimide(30)	0.98-0.99	1.2
Teriparatide	1.0	—
Largest other individual related impurity	—	1.0
Total impurities	—	7.0

SPECIFIC TESTS

- **pH** 〈 791 〉: 3.8–4.5
- **Bacterial Endotoxins Test** 〈 85 〉: NMT 100 USP Endotoxin Units/mg of teriparatide
- **Sterility Tests** 〈 71 〉, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **Particulate Matter in Injections** 〈 788 〉: Meets the requirements for small-volume injections

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Unless otherwise prescribed, store in a sterile, airtight, tamper-proof container, protected from light, at a temperature of 2°–8°. The Injection is not to be frozen.
- **Labeling:** Label it to indicate that the material has been produced by methods based on recombinant DNA technology.
- **USP Reference Standards** 〈 11 〉
 - USP Endotoxin RS
 - USP Teriparatide RS

■ 2S (USP39)

BRIEFING

Testosterone, USP 38 page 5500. As part of USP monograph modernization efforts, the following changes are proposed.

1. The nonspecific UV absorption procedure in the *Assay* is replaced with a specific liquid chromatographic procedure that is based on validated methods of analysis performed with the Waters Symmetry C18 brand of L1 column. The typical retention time for testosterone is about 4 min.
2. *Identification* test *A* is revised to reference *Infrared Absorption* 〈197〉 to allow for more flexibility in performing the analysis.
3. *Identification* test *B*, which uses UV absorption, is replaced with a retention time match of the testosterone peak from the *Assay*.
4. A stability-indicating liquid chromatographic procedure for the *Organic Impurities* test is added. This procedure was validated using the Phenomenex Gemini NX-C18 brand of L1 column. The typical retention time for testosterone is about 16 min.
5. The nonspecific test for *Melting Range or Temperature* is deleted. It is no longer needed because the proposed test for *Organic Impurities* is sufficient.
6. USP Testosterone Related Compound A RS, USP Testosterone Related Compound C RS, USP Exemestane Related Compound C RS, and USP Testosterone Related Compound I RS are added to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

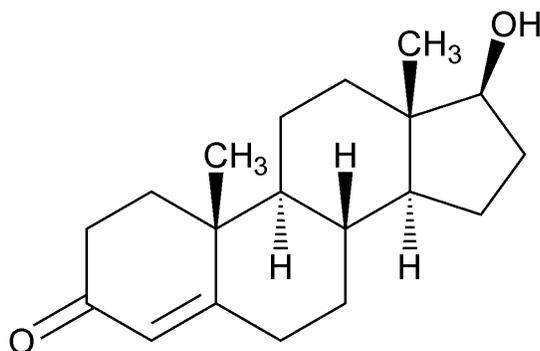
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM4: M. Koleček.)

Correspondence Number—C122363

Comment deadline: September 30, 2015

Testosterone



$C_{19}H_{28}O_2$ 288.42

Androst-4-en-3-one, 17-hydroxy-, (17 β)-;
17 β -Hydroxyandrost-4-en-3-one [58-22-0].

DEFINITION

Testosterone contains NLT 97.0% and NMT 103.0% of testosterone ($C_{19}H_{28}O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- ~~A. Infrared Absorption~~ ~~(197K)~~

- **Infrared Absorption** (197):

[Note—Methods described in (197K) or (197A) may be used.] ■ 2S (USP39)

Delete the following:

- ~~B. Ultraviolet Absorption~~ ~~(197U)~~

~~**Standard solution:** 10 µg/mL of USP Testosterone RS in methanol~~

~~**Sample solution:** 10 µg/mL of testosterone in methanol~~

~~**Acceptance criteria:** Meets the requirements ■ 2S (USP39)~~

Add the following:

- ~~B.~~ The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

ASSAY**Change to read:**

- **Procedure**

~~**Standard preparation:** Prepare as directed under *Single Steroid Assay* (511), using USP Testosterone RS.~~

~~**Assay preparation:** 2 mg/mL of Testosterone, testosterone previously dried, in alcohol and chloroform (1:1). Proceed as directed in *Single Steroid Assay* (511), *Procedure*, using a solvent system consisting of a mixture of benzene and ethyl acetate (1:1), through the fourth sentence of the second paragraph. Then centrifuge the tubes for 5 min and use the supernatant.~~

~~**Instrumental conditions**~~

~~**Mode:** UV-Vis~~

~~**Analytical wavelength:** Maximum absorbance at about 241 nm~~

~~**Cell:** 1 cm~~

~~**Blank:** Benzene and ethyl acetate (1:1)~~

~~**Analysis**~~

~~**Samples:** Supernatant from the *Standard preparation*, supernatant from the *Assay preparation*, and *Blank*~~

~~Calculate the percentage of testosterone (C₁₉H₂₈O₂) in the portion of Testosterone taken:~~

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

~~A_U~~ absorbance of the supernatant from the *Assay preparation*

~~A_S~~ absorbance of the supernatant from the *Standard preparation*

~~C_S~~ concentration of USP Testosterone RS in the *Standard preparation* (mg/mL)

~~C_U~~ concentration of Testosterone in the *Assay preparation* (mg/mL)

~~**Acceptance criteria:** 97.0%–103.0% on the dried basis~~

■ **Mobile phase:** Acetonitrile and water (55:45)

Standard solution: 0.12 mg/mL of USP Testosterone RS in methanol

Sample solution: 0.12 mg/mL of Testosterone in methanol

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 241 nm

Columns

Guard: 3.9-mm × 2-cm; 5-µm packing L1

Analytical: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of testosterone (C₁₉H₂₈O₂) in the portion of Testosterone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Testosterone RS in the *Standard solution* (mg/mL)

C_U = concentration of Testosterone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis ■ 2S (USP39)

IMPURITIES

Add the following:

■ • **Organic Impurities**

Mobile phase: See *Table 1*.

Table 1

Time (min)	Acetonitrile (%)	Water (%)
0	32	68
19.2	32	68

Time (min)	Acetonitrile (%)	Water (%)
29.2	80	20
39.2	80	20
40.2	32	68
50.0	32	68

System suitability solution: 1 mg/mL of USP Testosterone RS, 1 µg/mL of USP Testosterone Related Compound A RS, 5 µg/mL of USP Testosterone Related Compound C RS, 1 µg/mL of USP Exemestane Related Compound C RS, and 2 µg/mL of USP Testosterone Related Compound I RS in methanol

Standard solution: 5 µg/mL of USP Testosterone RS in methanol

Sample solution: 1 mg/mL of Testosterone in methanol

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between testosterone related compound I and exemestane related compound C; NLT 2.0 between exemestane related compound C and testosterone; NLT 1.5 between testosterone related compound A and testosterone related compound C, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Testosterone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of testosterone from the *Standard solution*

C_S = concentration of USP Testosterone RS in the *Standard solution* (mg/mL)

C_U = concentration of Testosterone in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks that are less than 0.05% of the testosterone peak.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Boldenone ^a	0.64	1.0	0.1
Testosterone related compound I	0.79	0.39	0.2
Exemestane related compound C	0.91	1.0	0.1
Testosterone	1.00	—	—
Testosterone related compound A	1.39	1.0	0.1
Testosterone related compound C	1.44	1.0	0.5
Testosterone acetate ^b	1.79	0.67	0.1
Methoxyandrostadiene ^c	1.92	0.73	0.1
Ethoxyandrostadiene ^d	2.03	0.66	0.1
Any other unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.6

^a 17 β -Hydroxyandrosta-1,4-dien-3-one.

^b 3-Oxoandrost-4-en-17 β -yl acetate.

^c 3-Methoxyandrosta-3,5-dien-17-one.

^d 3-Ethoxyandrosta-3,5-dien-17-one.

■ 2S (USP39)

SPECIFIC TESTS

Delete the following:

■ • **Melting Range or Temperature** ~~(741)~~: 153^o – 157^o ■ 2S (USP39)

• **Optical Rotation** ~~(781S)~~, *Specific Rotation*

Sample solution: 10 mg/mL of Testosterone in dioxane

Acceptance criteria: +101^o to +105^o

• **Loss on Drying** ~~(731)~~

Analysis: Dry under vacuum over *phosphorus pentoxide* for 4 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers. Store at 25^o, excursions permitted between 15^o and 30^o.

Change to read:

• **USP Reference Standards** ~~(11)~~

■ USP Exemestane Related Compound C RS
Androstadienedione;

Androsta-1,4-diene-3,17-dione.

$C_{19}H_{24}O_2$ 284.39 ■ 2S (USP39)

USP Testosterone RS

■ USP Testosterone Related Compound A RS

Androstenedione;

Androst-4-ene-3,17-dione.

$C_{19}H_{26}O_2$ 286.41

USP Testosterone Related Compound C RS

Epitestosterone;

17 α -Hydroxyandrost-4-en-3-one.

$C_{19}H_{28}O_2$ 288.42

USP Testosterone Related Compound I RS

Testosterone-6-ene;

17 β -Hydroxyandrosta-4,6-dien-3-one.

$C_{19}H_{26}O_2$ 286.41 ■ 2S (USP39)

BRIEFING

Thiotepa for Injection, USP 38 page 5556. On the basis of comments received, the test for *Completeness of Solution* is replaced with the *Injections and Implanted Drug Products* $\langle 1 \rangle$, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and clarity of solutions* test.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM3: F. Mao.)

Correspondence Number—C139061

Comment deadline: September 30, 2015

Thiotepa for Injection

DEFINITION

Thiotepa for Injection contains NLT 95.0% and NMT 110.0% of the labeled amount of thiotepa ($C_6H_{12}N_3PS$).

IDENTIFICATION

- **A. Infrared Absorption** $\langle 197K \rangle$
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• Procedure

Buffer: 13.6 g/L of monobasic potassium phosphate in water. Adjust with 35 g/L of dibasic sodium phosphate in water to a pH of 7.0.

Mobile phase: Acetonitrile and *Buffer* (13:87)

System suitability solution: Transfer 10 mg of USP Thiotepa RS to a 4-mL vial, add 2 mL of methanol, and mix. Add 50 μ L of 0.1% phosphoric acid solution. Place a cap on the vial, and heat at 65 $^{\circ}$ for 50 s. Cool the solution, add 1 mL of methanol, and mix. [Note—The preparation generates methoxythiotepa.]

Standard solution: 1.5 mg/mL of USP Thiotepa RS in water

Sample solution: Nominally 1.5 mg/mL of thiotepa in water from Thiotepa for Injection

Chromatographic system

(See *Chromatography* { 621 }, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for thiotepa and methoxythiotepa are about 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 3 between methoxythiotepa and thiotepa, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of thiotepa ($C_6H_{12}N_3PS$) in the portion of Thiotepa for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of thiotepa from the *Sample solution*

r_S = peak response of thiotepa from the *Standard solution*

C_S = concentration of USP Thiotepa RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of thiotepa in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–110.0%

IMPURITIES

• **Organic Impurities**

Buffer, Mobile phase, and System suitability solution: Proceed as directed in the *Assay*.

Peak identification solution: Dissolve 15 mg of USP Thiotepa RS in 10 mL of water, add 1 g of sodium chloride, boil in a water bath for 10 min, and cool. [Note—The preparation generates thiotepa chloroethyl analog.]

Standard solution: 3.75 μ g/mL of USP Thiotepa RS in water

Sample solution: Nominally 3.75 mg/mL of thiotepa in water from Thiotepa for Injection. Pass through a suitable filter, and use the filtrate.

Chromatographic system: Proceed as directed in the *Assay*. In addition, the run time is NLT 4 times the retention time of the thiotepa peak.

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 3 between methoxythiotepa and thiotepa

Analysis

Samples: *Peak identification solution, Standard solution, and Sample solution*

Calculate the percentage of each impurity in the portion of thiotepa taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each impurity from the *Sample solution*

r_S peak response of thiotepa from the *Standard solution*

C_S concentration of USP Thiotepa RS in the *Standard solution* (mg/mL)

C_U nominal concentration of thiotepa in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Thiotepa	1.0	—
Thiotepa chloroethyl analog ^a	3.75	0.15
Any individual unspecified impurity	—	0.2
Total impurities ^b	—	0.4

^a *P,P*-Bis(aziridin-1-yl)-*N*-(2-chloroethyl)phosphinothioic amide.
^b The impurity thiotepa chloroethyl analog is excluded.

PERFORMANCE TESTS

- **Uniformity of Dosage Units** (905): Meets the requirements

SPECIFIC TESTS

- **Bacterial Endotoxins Test** (85): NMT 6.25 USP Endotoxin Units/mg of thiotepa
- **Sterility Tests** (71): Meets the requirements
- **pH** (791)

Sample solution: 10 mg/mL of thiotepa constituted as directed in the labeling

Acceptance criteria: 5.5–7.5

Delete the following:

- **Completeness of Solution** (641): The contents of one container dissolved in Sterile Water for Injection, or other diluent as directed in the labeling, to obtain a solution containing 3.75 mg/mL of thiotepa yields a clear solution. ■ 2S (USP39)

Add the following:

- **Injections and Implanted Drug Products** (1), *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and clarity of solutions:* At the

time of use, it meets the requirements. ■2S (USP39)

- **Other Requirements:** It meets the requirements in *Injections* 〈 1 〉, *Labels and Labeling*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described in *Injections* 〈 1 〉, *Packaging, Containers for Sterile Solids*, and store in a refrigerator, protected from light.
- **USP Reference Standards** 〈 11 〉
 - USP Endotoxin RS
 - USP Thiotepa RS

BRIEFING

Tinidazole, *USP 38* page 5587. On the basis of comments received, the following changes are proposed to modernize the monograph:

1. The nonspecific *Identification* test *B* is replaced with a HPLC retention time agreement from the *Assay*.
2. The nonspecific *Identification* test *C* is deleted as the other two tests are sufficient.
3. The titration procedure in the *Assay* is replaced with an HPLC procedure based on the validated methods of analysis.
4. The acceptance criteria in the *Definition* and the *Assay* are revised from NLT 98.0% and NMT 101.0% to NLT 98.0% and NMT 102.0%, which is typical for chromatographic procedures.
5. The TLC procedure in the test for *Organic Impurities* is replaced with an HPLC procedure similar to the *Assay*.
6. The HPLC procedures in the *Assay* and test for *Organic Impurities* are based on analyses performed using the Zorbax SB C8 brand of L7 column. The typical retention time for tinidazole is about 6 min.
7. The *Melting Range or Temperature* test is deleted as the HPLC procedure for *Organic Impurities* is sufficient.

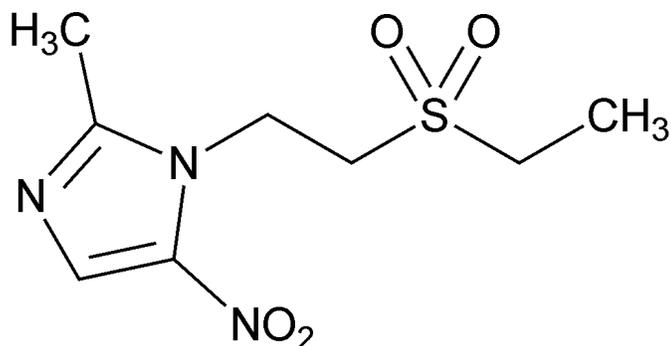
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM1: S. Shivaprasad.)

Correspondence Number—C152566

Comment deadline: September 30, 2015

Tinidazole



$C_8H_{13}N_3O_4S$ 247.27

1*H*-Imidazole, 1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitro- ;
1-[2-(Ethylsulfonyl)ethyl]-2-methyl-5-nitroimidazole [19387-91-8].

DEFINITION

Change to read:

Tinidazole contains NLT 98.0% and NMT $\pm 1.0\%$

■ 102.0% ■ 2S (USP39)

of tinidazole ($C_8H_{13}N_3O_4S$), calculated on the dried basis.

IDENTIFICATION

• A. Infrared Absorption (197K)

Delete the following:

■ • B. Ultraviolet Absorption (197U)

Sample solution: 10 µg/mL in methanol

Acceptance criteria: Meets the requirements ■ 2S (USP39)

Add the following:

- • B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

Delete the following:

- • C. The R_f value and intensity of the principal spot of *Sample solution 2* correspond to those of *Standard solution 1*, as obtained in *Organic Impurities*. ■ 2S (USP39)

ASSAY

Change to read:

• Procedure

Sample solution: 6 mg/mL of Tinidazole in glacial acetic acid

Titrimetric system

(See *Titrimetry* (541).)

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Titrate with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 24.73 mg of tinidazole ($C_8H_{13}N_3O_4S$).

Acceptance criteria: 98.0%–101.0% on the dried basis

■ **Mobile phase:** Acetonitrile, methanol, and water (10:20:70)

Standard solution: 0.1 mg/mL of USP Tinidazole RS prepared as follows. Transfer a suitable amount of USP Tinidazole RS to a suitable volumetric flask and add *methanol* to 10% of the final volume of the flask. Dilute with *Mobile phase* to volume.

Sample solution: 0.1 mg/mL of Tinidazole prepared as follows. Transfer a suitable amount of Tinidazole to a suitable volumetric flask and add *methanol* to 10% of the final volume of the flask. Dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 320 nm

Column: 3.0-mm × 25-cm; 5- μ m packing L7

Flow rate: 0.5 mL/min

Injection volume: 20 μ L

Run time: 1.5 times the retention time of tinidazole

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tinidazole ($C_8H_{13}N_3O_4S$) in the portion of Tinidazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of tinidazole from the *Sample solution*

r_S

= peak response of tinidazole from the *Standard solution*

C_S

= concentration of USP Tinidazole RS in the *Standard solution* (mg/mL)

C_U

= concentration of Tinidazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ■2S (USP39)

IMPURITIES

- **Residue on Ignition** 〈 281 〉: NMT 0.1%

Delete the following:

- **Heavy Metals, Method II** 〈 231 〉: NMT 20 ppm ● (Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

Standard solution 1: 2.0 mg/mL of USP Tinidazole RS in methanol

Standard solution 2: 0.1 mg/mL of USP Tinidazole RS in methanol from *Standard solution 1*

Standard solution 3: 0.04 mg/mL of USP Tinidazole RS in methanol from *Standard solution 2*

Standard solution 4: 0.1 mg/mL of USP Tinidazole Related Compound A RS in methanol

Standard solution 5: 0.1 mg/mL of USP Tinidazole Related Compound B RS in methanol

Sample solution 1: 20 mg/mL of Tinidazole in methanol

Sample solution 2: 2 mg/mL of Tinidazole in methanol from *Sample solution 1*

Chromatographic system

(See *Chromatography* 〈 621 〉, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Developing solvent system: Ethyl acetate and butyl alcohol (30:10)

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, *Standard solution 4*, *Standard solution 5*, *Sample solution 1*, and *Sample solution 2*

Proceed as directed. Activate the plate for at least 1 h at 110°. Examine the plate under short-wavelength UV light.

Acceptance criteria: Any spots due to tinidazole related compound A and tinidazole related compound B from *Sample solution 1* are no more intense than those of *Standard solution 4* and *Standard solution 5*, respectively. Any spot, other than the principal spot, of *Sample solution 1* is not more intense than that of *Standard solution 2*, and NMT one such spot is more intense than that of *Standard solution 3*.

■ **Mobile phase, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

Standard stock solution 1: Use the *Standard solution* from the Assay.

Standard stock solution 2: 0.05 mg/mL each of USP Tinidazole Related Compound A RS and USP Tinidazole Related Compound B RS prepared as follows. Transfer suitable amounts of USP Tinidazole Related Compound A RS and USP Tinidazole Related Compound B RS to a suitable volumetric flask and add *methanol* to 10% of the final volume of the flask. Dilute with *Mobile phase* to volume.

Standard solution: 0.1 μ g/mL of USP Tinidazole RS and 0.2 μ g/mL each of USP

Tinidazole Related Compound A RS and USP Tinidazole Related Compound B RS in *Mobile phase* from *Standard stock solution 1* and *Standard stock solution 2*

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between tinidazole related compound A and tinidazole related compound B

Relative standard deviation: NMT 5.0% for each peak

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of tinidazole related compound A and tinidazole related compound B in the portion of Tinidazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of tinidazole related compound A or tinidazole related compound B from the *Sample solution*

r_S

= peak response of tinidazole related compound A or tinidazole related compound B from the *Standard solution*

C_S

= concentration of USP Tinidazole Related Compound A RS or Tinidazole Related Compound B RS in the *Standard solution* (mg/mL)

C_U

= concentration of Tinidazole in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Tinidazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response of each unspecified impurity from the *Sample solution*

r_S

= peak response of tinidazole from the *Standard solution*

C_S

= concentration of USP Tinidazole RS in the *Standard solution* (mg/mL)

C_U

= concentration of Tinidazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.6	0.2
Tinidazole related compound B	0.7	0.2
Tinidazole	1.0	—
Any unspecified impurity	—	0.10
Total impurities	—	0.4

■ 2S (USP39)

SPECIFIC TESTS

Delete the following:

● **Melting Range or Temperature** ~~(741)~~: 125°–128° ■ 2S (USP39)

● **Loss on Drying** (731)

Analysis: Dry at 100°–105° to constant weight.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

● **Packaging and Storage:** Preserve in tight containers, protected from light, at controlled room temperature.

● **USP Reference Standards** (11)

USP Tinidazole RS

USP Tinidazole Related Compound A RS

2-Methyl-5-nitroimidazole.

C₄H₅N₃O₂ 127.10

USP Tinidazole Related Compound B RS

1-(2-Ethyl-sulfonyl-ethyl)-2-methyl-4-nitroimidazole.

C₈H₁₃N₃O₄S 247.28

BRIEFING

Tretinoin, *USP 38* page 5654. As part of USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Replace the current titration-based *Assay* with a stability-indicating HPLC procedure. The procedure was validated using the Cosmosil Cholester brand of L## column manufactured by Nacalai Tesque in which tretinoin elutes at about 8 min.
2. Replace the UV-based *Identification* test *B* with the retention time agreement proposed in the *Assay*.
3. Replace the current normal-phase HPLC procedure in the test for *Limit of Isotretinoin* with a more specific and reversed-phase HPLC procedure. The proposed test for

Organic Impurities uses the same chromatographic parameters as those proposed in the *Assay*.

- The *Acceptance criteria* for specified, unspecified, and total impurities is proposed based on the limits of the *Tretinoin* monograph in the current edition of the *European Pharmacopoeia*. Manufacturers are strongly encouraged to submit their approved specifications to USP if they are different from those proposed in this revision.

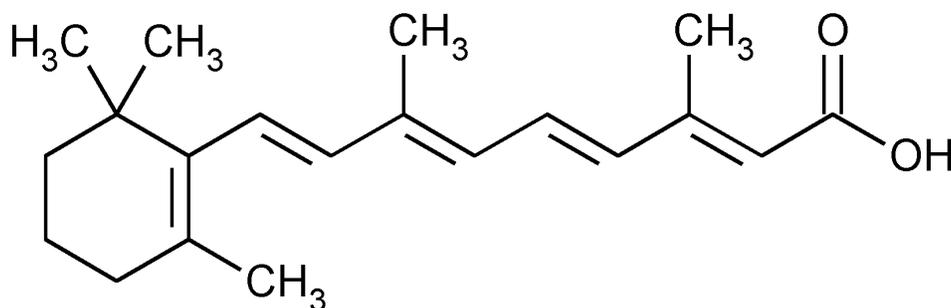
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: D. Min.)

Correspondence Number—C143819

Comment deadline: September 30, 2015

Tretinoin



$C_{20}H_{28}O_2$ 300.44

Retinoic acid;
all-*trans*-Retinoic acid [302-79-4].

DEFINITION

Change to read:

Tretinoin contains NLT 97.0% and NMT 103.0% of tretinoin ($C_{20}H_{28}O_2$), calculated on the dried basis.

~~Avoid exposure to strong light, and use low actinic glassware in the performance of the following procedures.~~

■ ~~2S (USP39)~~

IDENTIFICATION

- **A. Infrared Absorption** (197M)

Delete the following:

- ~~**B. Ultraviolet Absorption** (197U)~~
~~**Analytical wavelength:** 352 nm~~

~~**Medium:** Dilute 1 mL of 0.01 N hydrochloric acid with isopropyl alcohol to 1000 mL.~~

~~**Sample solution:** 4 µg/mL in *Medium*~~

~~**Acceptance criteria:** Absorptivities do not differ by more than 3.0%, calculated on the dried basis. ■2S (USP39)~~

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■2S (USP39)

ASSAY

Change to read:

● **Procedure**

- Avoid exposure to strong light, and use low-actinic glassware in the performance of the following procedures.

■2S (USP39)

~~**Sample:** 240 mg of Tretinoin~~

~~**Titrimetric system**~~

~~**Mode:** Direct titration~~

~~**Titrant:** 0.1 N sodium methoxide VS~~

~~**Endpoint detection:** Visual~~

~~**Analysis:** Dissolve the *Sample* in 50 mL of dimethylformamide, and add 3 drops of a 1-in-100 solution of thymol blue in dimethylformamide. Titrate with *Titrant* to a greenish endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 30.04 mg of tretinoin ($C_{20}H_{28}O_2$).~~

- **Solution A:** 0.1% Trifluoroacetic acid in water

Mobile phase: Methanol and *Solution A* (90:10)

Standard solution: 0.1 mg/mL of USP Tretinoin RS in methanol. [Note—Sonication may be needed to aid dissolution.]

Sample solution: 0.1 mg/mL of Tretinoin in methanol. [Note—Sonication may be needed to aid dissolution.]

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 355 nm

Column: 4.6-mm × 15-cm; 5-µm packing L##

Temperatures

Autosampler: 4°

Column: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 1.5 times the retention time of tretinoin

System suitability

Sample: *Standard solution*

Suitability requirements**Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 1.10%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of tretinoin (C₂₀H₂₈O₂) in the portion of Tretinoin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U= peak response of tretinoin from the *Sample solution**r_S*= peak response of tretinoin from the *Standard solution**C_S*= concentration of USP Tretinoin RS in the *Standard solution* (mg/mL)*C_U*= concentration of Tretinoin in the *Sample solution* (mg/mL)■ **2S (USP39)****Acceptance criteria:** 97.0%–103.0% on the dried basis**IMPURITIES**

- **Residue on Ignition** (281): NMT 0.1%

Delete the following:

- **Heavy Metals, Method II** (231): 20 ppm (Official 1-Dec-2015)

Delete the following:

- **Limit of Isotretinoin**

Mobile phase: Isooctane, isopropyl alcohol, and glacial acetic acid (99.65: 0.25: 0.1)**System suitability stock solution:** 250 µg/mL of USP Tretinoin RS in isooctane prepared as follows. Dissolve a quantity of USP Tretinoin RS in a minimum amount of methylene chloride, and add a suitable amount of isooctane to the known concentration.**Standard stock solution:** 250 µg/mL of USP Isotretinoin RS in isooctane prepared as follows. Dissolve a quantity of USP Isotretinoin RS in a minimum amount of methylene chloride, and add a suitable amount of isooctane to the known concentration.**System suitability solution:** Transfer 5 mL of *Standard stock solution* into a 100 mL volumetric flask, and add *System suitability stock solution* to volume.**Standard solution:** 12.5 µg/mL of USP Isotretinoin RS in isooctane from *Standard stock solution***Sample solution:** Transfer 25 mg of Tretinoin into a 100 mL volumetric flask. Dissolve in a minimum amount of methylene chloride, and add isooctane to volume.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 352 nm

Column: 4.0-mm × 25-cm; packing L3

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for isotretinoin and tretinoin are about 0.84 and 1.00, respectively.]

Suitability requirements

Resolution: NLT 2.0 between isotretinoin and tretinoin

Relative standard deviation: NMT 2.0% for the isotretinoin peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of isotretinoin in the portion of Tretinoin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of isotretinoin from the *Sample solution*

r_S peak response of isotretinoin from the *Standard solution*

C_S concentration of USP Isotretinoin RS in the *Standard solution* (µg/mL)

C_U concentration of Tretinoin in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 5.0% $\pm 2S$ (USP39)

Add the following:

■ ● **Organic Impurities**

Mobile phase and Chromatographic system: Proceed as directed in the Assay.

System suitability solution: 0.1 mg/mL each of USP Isotretinoin RS, 6Z-retinoic acid, and USP Tretinoin RS in methanol. [Note—6Z-Retinoic acid is also named 9-cis retinoic acid.]

Standard solution: 5 µg/mL each of USP Isotretinoin RS and USP Tretinoin RS in methanol

Sample solution: 100 µg/mL of Tretinoin in methanol

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times of isotretinoin, 6Z-retinoic acid, and tretinoin are 0.72, 0.82, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between isotretinoin and 6Z-retinoic acid; NLT 3.0 between 6Z-retinoic acid and tretinoin, *System suitability solution*

Relative standard deviation: NMT 2.0% for isotretinoin and tretinoin, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of isotretinoin in the portion of Tretinoin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of isotretinoin from the *Sample solution*

$r_{\bar{S}}$ peak response of isotretinoin from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Isotretinoin RS in the *Standard solution* ($\mu\text{g/mL}$)

$C_{\bar{U}}$ concentration of Tretinoin in the *Sample solution* ($\mu\text{g/mL}$)

Calculate the percentage of any individual unspecified impurity in the portion of Tretinoin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_{\bar{U}}$ peak response of each unspecified impurity from the *Sample solution*

$r_{\bar{S}}$ peak response of tretinoin from the *Standard solution*

$C_{\bar{S}}$ concentration of USP Tretinoin RS in the *Standard solution* ($\mu\text{g/mL}$)

$C_{\bar{U}}$ concentration of Tretinoin in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 1*. Disregard peaks below 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Isotretinoin	0.72	0.5
Tretinoin	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities	—	1.0

■ 2S (USP39)

SPECIFIC TESTS

- **Loss on Drying** 〈 731 〉

Analysis: Dry under vacuum at room temperature for 16 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, preferably under an atmosphere of an inert gas, protected from light.
- **USP Reference Standards** 〈 11 〉

USP Isotretinoin RS

USP Tretinoin RS

BRIEFING

Tretinoin Cream, *USP 38* page 5655. As part of USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Add a test for *Organic Impurities* with a stability-indicating HPLC procedure based on the one in the *Tretinoin* monograph proposal. The procedure was validated using the Cosmosil Cholester brand of L## column manufactured by Nacalai Tesque in which tretinoin elutes at about 8 min.

2. Replace the current HPLC procedure in the *Assay* that uses an undesirable solvent (tetrahydrofuran) with a more specific HPLC method. The proposed *Assay* uses the same chromatographic parameters as those proposed in the test for *Organic Impurities*.
3. Add *Identification* test *B* based on the UV spectrum in the proposed *Assay*.
4. Add an additional storage condition to the *Packaging and Storage* section based on information from the drug product packaging insert.
5. Add USP Isotretinoin RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SM3: D. Min.)

Correspondence Number—C143857

Comment deadline: September 30, 2015

Tretinoin Cream

DEFINITION

Tretinoin Cream contains NLT 90.0% and NMT 120.0% of the labeled amount of tretinoin (C₂₀H₂₈O₂).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■2S (USP39)

ASSAY

Change to read:

- **Procedure**

Avoid exposure to strong light, and use low-actinic glassware in the performance of the following procedure.

~~Use stabilized tetrahydrofuran in the preparation of the *Standard solution* and the *Sample solution*.~~

~~**Diluted phosphoric acid:** Dilute 10 mL of phosphoric acid with water to 100 mL.~~

~~**Buffer:** 1.38 g/L of monobasic sodium phosphate in water. Adjust with *Diluted phosphoric acid* to a pH of 3.0.~~

~~**Diluent A:** Water and *Diluted phosphoric acid* (9:1)~~

~~**Diluent B:** Tetrahydrofuran and *Diluent A* (3:2)~~

~~**Mobile phase:** Tetrahydrofuran and *Buffer* (42:58)~~

~~**Standard stock solution:** 0.4 mg/mL of USP Tretinoin RS in tetrahydrofuran~~

~~**Standard solution:** 4 µg/mL of USP Tretinoin RS in *Diluent B* from *Standard stock solution*~~

Sample stock solution: ~~Nominally 20 µg/mL of tretinoin in tetrahydrofuran prepared as follows. Transfer a quantity of Cream, equivalent to 1.0 mg of tretinoin, into a 50-mL volumetric flask, and add 20.0 mL of tetrahydrofuran. Shake the flask to disperse the cream, dilute with tetrahydrofuran to volume, mix, and filter if necessary.~~

Sample solution: 4 µg/mL of tretinoin in *Diluent B* from *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 3.9 mm × 15 cm; 4 µm packing L1

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

■ **Solution A:** 0.1% Trifluoroacetic acid in water

Mobile phase: Methanol and *Solution A* (90:10)

Standard solution: 0.02 mg/mL of USP Tretinoin RS in methanol. [Note—Sonication may be needed to aid dissolution.]

Sample solution: Nominally 0.02 mg/mL of tretinoin in methanol prepared as follows. Transfer a portion of Cream, equivalent to 0.5 mg of tretinoin, to a suitable volumetric flask. Add methanol to about 80% of the flask volume and sonicate for 10 min. Dilute with methanol to volume. Centrifuge a portion of the solution at 4°. Use the supernatant. [Note—May centrifuge at 3500 rpm for 10 min.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 355 nm. For *Identification test B*, use a diode array detector in the range of 210–400 nm.

Column: 4.6-mm × 15-cm; 5-µm packing L##

Temperatures

Autosampler: 4°

Column: 30°

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time: NLT 1.5 times the retention time of tretinoin

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tretinoin ($C_{20}H_{28}O_2$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U

= peak response from the *Sample solution*

r_S

= peak response from the *Standard solution*

C_S

= concentration of USP Tretinoin RS in the *Standard solution* (mg/mL)

C_U

= nominal concentration of tretinoin in the *Sample solution* (mg/mL)

■ 2S (USP39)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

- **Minimum Fill** 〈 755 〉: Meets the requirements

IMPURITIES

Add the following:

■ Organic Impurities

Mobile phase and Chromatographic system: Proceed as directed in the *Assay*.

Cold methanol: Methanol stored at 4°

System suitability solution: 20 µg/mL each of USP Isotretinoin RS, 6Z-retinoic acid, and USP Tretinoin RS in *Cold methanol*. [Note—6Z-Retinoic acid is also named 9-cis retinoic acid.]

Standard solution: 0.2 µg/mL of USP Tretinoin RS in *Cold methanol*

Sample solution: Nominally 20 µg/mL of tretinoin in *Cold methanol* prepared as follows. Transfer a portion of Cream, equivalent to 0.5 mg of tretinoin, to a suitable volumetric flask. Add *Cold methanol* to about 80% of the flask volume and sonicate for 10 min. Dilute with *Cold methanol* to volume. Centrifuge a portion of the solution at 4°. Use the supernatant. [Note—May centrifuge at 3500 rpm for 10 min.]

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times of isotretinoin, 6Z-retinoic acid, and tretinoin are 0.72, 0.82, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between isotretinoin and 6Z-retinoic acid; NLT 2.0 between 6Z-retinoic acid and tretinoin, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of any individual unspecified impurity in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response of each unspecified impurity from the *Sample solution*

r_S peak response of tretinoin from the *Standard solution*

C_S concentration of USP Tretinoin RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U nominal concentration of tretinoin in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 1*. Disregard peaks below 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Isotretinoin ^a	0.72	—
Tretinoin	1.0	—
Any individual unspecified impurity	—	1.0
Total impurities	—	3

^a It is a specified impurity and controlled in the drug substance. It is used for resolution measurement only and should not be included in the total impurities.

■ 2S (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in collapsible tubes or in tight, light-resistant containers.
- Store at controlled room temperature. ■ 2S (USP39)

Change to read:

- **USP Reference Standards** 〈 11 〉

- USP Isotretinoin RS

- 2S (USP39)

USP Tretinoin RS

BRIEFING

Vinblastine Sulfate for Injection, USP 38 page 5781. On the basis of comments received, the following revisions to the monograph are proposed:

1. *Identification* test *B* by retention time agreement, based on the *Assay*, is added.
2. The test for *Completeness of Solution* 〈 641 〉 is replaced with the *Injections and Implanted Drug Products* 〈 1 〉, *Product Quality Tests Common to Parenteral Dosage*

Forms, Specific Tests, Completeness and clarity of solutions test.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(SM3: F. Mao.)

Correspondence Number—C139062

Comment deadline: September 30, 2015

Vinblastine Sulfate for Injection

DEFINITION

Vinblastine Sulfate for Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$).

[**Caution**—Handle Vinblastine Sulfate for Injection with great care because it is a potent cytotoxic agent.]

IDENTIFICATION

- **A. Infrared Absorption** 〈 197K 〉

Sample: Use material previously dried in a vacuum at 60° for 16 h.

Acceptance criteria: Meets the requirements

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■2S (USP39)

Change to read:

- **B**

- C.** ■2S (USP39)

Identification Tests—General 〈 191 〉, *Sulfate*

Sample solution: 100 mg/mL in water

Acceptance criteria: Meets the requirements

ASSAY

- **Procedure**

Solution A: Diethylamine and water (14:986). Adjust with phosphoric acid to a pH of 7.5.

Solution B: Acetonitrile and methanol (20:80)

Mobile phase: *Solution A* and *Solution B* (38:62)

Standard solution: 0.4 mg/mL of USP Vinblastine Sulfate RS in water

System suitability solution: 0.4 mg/mL each of vincristine sulfate and vinblastine sulfate in water prepared as follows. Transfer USP Vincristine Sulfate RS or USP Vincristine Sulfate (Assay) RS to a suitable volumetric flask, and dissolve in *Standard solution*.

Sample stock solution: Pipet a suitable volume of water into each of five containers of Vinblastine Sulfate for Injection to obtain a solution in each having a nominal concentration of 1 mg/mL of vinblastine sulfate. Insert the stopper, shake to mix, and combine the solutions from the five containers.

Sample solution: Nominally equivalent to 0.4 mg/mL of vinblastine sulfate in water, from

the *Sample stock solution*

Chromatographic system

(See *Chromatography* 〈 621 〉, *System Suitability*.)

Mode: LC

Detector: UV 262 nm

Columns

Precolumn: Packed with porous silica gel; installed between the pump and the injector

Analytical: 4.6-mm × 15-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 μL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 4.0 between vincristine and vinblastine, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$) in the portion of Vinblastine Sulfate for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U peak response from the *Sample solution*

r_S peak response from the *Standard solution*

C_S concentration of USP Vinblastine Sulfate RS in the *Standard solution* (mg/mL)

C_U nominal concentration of vinblastine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Uniformity of Dosage Units 〈 905 〉

Procedure for content uniformity

Buffer: Dissolve 13.61 g of sodium acetate in 900 mL of water in a 1000-mL volumetric flask. Adjust with glacial acetic acid to a pH of 5.0 while stirring, and dilute with water to volume.

Standard solution: 40 μg/mL of USP Vinblastine Sulfate RS in *Buffer*

Sample solution: Nominally equivalent to 40–50 μg/mL of vinblastine sulfate by dissolving the contents of one container of Vinblastine Sulfate for Injection in *Buffer*

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* 〈 851 〉.)

Mode: UV

Analytical wavelength: 269 nm

Cell: 1 cm

Blank: *Buffer*

Analysis

Samples: *Standard solution* and *Sample solution*

Concomitantly determine the absorbances of the *Sample solution* and the *Standard solution*.

Calculate the percentage of the labeled amount of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$) in the portion of Vinblastine Sulfate for Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_{\bar{U}}$ absorbance of the *Sample solution*

$A_{\bar{S}}$ absorbance of the *Standard solution*

$C_{\bar{S}}$ concentration of USP Vinblastine Sulfate RS in the *Standard solution* (mg/mL)

$C_{\bar{U}}$ nominal concentration of vinblastine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: Meets the requirements

IMPURITIES

• Organic Impurities

Mobile phase, System suitability solution, and System suitability: Prepare as directed in the *Assay*.

Sample solution A: Use the *Sample solution*, prepared as directed in the *Assay*.

Sample solution B: 16 µg/mL of vinblastine sulfate in water, from *Sample solution A*

Chromatographic system: Proceed as directed in the *Assay*, except use an injection volume of 200 µL.

Analysis

Samples: *Sample solution A* and *Sample solution B*

Calculate the percentage of each impurity in the portion of Vinblastine Sulfate for Injection taken:

$$\text{Result} = [r_U/(\sum r_U + 25r_S)] \times 100$$

$r_{\bar{U}}$ peak response of each impurity appearing after the solvent peak from *Sample solution A*

$r_{\bar{S}}$ peak response of vinblastine from *Sample solution B*

Calculate the percentage of total impurities:

$$\text{Result} = [\sum r_U/(\sum r_U + 25r_S)] \times 100$$

$r_{\bar{U}}$ peak response of each impurity appearing after the solvent peak from *Sample solution A*

$r_{\bar{S}}$ peak response of vinblastine from *Sample solution B*

Acceptance criteria

Individual impurities: NMT 2.0%

Total impurities: NMT 5.0%

SPECIFIC TESTS

- **Bacterial Endotoxins Test** (85): It contains NMT 10.0 USP Endotoxin Units/mg of vinblastine sulfate.
- **Sterility Tests** (71): Meets the requirements

Delete the following:

- ~~● **Constituted Solution:** At the time of use, it meets the requirements for *Injections* $\langle 1 \rangle$, *Constituted Solutions*. ■ 2S (USP39)~~

Add the following:

- ● **Injections and Implanted Drug Products** $\langle 1 \rangle$, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and clarity of solutions:* At the time of use, it meets the requirements. ■ 2S (USP39)

Delete the following:

- ● ~~● **Completeness of Solution** $\langle 641 \rangle$: A 10 mg portion dissolves in 10 mL of Water for Injection to yield a clear solution. ■ 2S (USP39)~~

- **Other Requirements:** It meets the requirements for *Injections* $\langle 1 \rangle$, *Labeling*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described under *Injections* $\langle 1 \rangle$, *Containers for Sterile Solids*, and store in a refrigerator.
- **Labeling:** The label states: "For Intravenous Use Only–Fatal If Given By Other Routes." When dispensed, the container or syringe (holding the individual dose prepared for administration to the patient) must be enclosed in an overwrap bearing the statement: "Do Not Remove Covering Until Moment of Injection. For Intravenous Use Only–Fatal If Given By Other Routes."
- **USP Reference Standards** $\langle 11 \rangle$
 - USP Endotoxin RS
 - USP Vinblastine Sulfate RS
 - USP Vincristine Sulfate RS

[Note—No loss on drying determination is needed for USP Vincristine Sulfate RS.]

 - USP Vincristine Sulfate (Assay) RS

Stage 4 Harmonization

This section contains monographs or chapters undergoing harmonization by the Pharmacopoeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (**Stages 1** through **7**, as defined below). Stage 4 drafts are available for comments. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF*, this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

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This section may contain the following:

- reports or statements of Expert Committees
- original research reports
- evaluations of new and existing pharmacopeial methods
- commentaries
- articles relevant to compendial issues.

These items are published to stimulate discussion and continual review of Pharmacopeial standards. Generally, if an Expert Committee publishes an article on which they are specifically seeking comment, this will be clearly stated in the article. Readers may submit comments on issues raised in this section, but comment is not as critical as that for the *In-Process Revision* section. Readers interested in submitting comments should see *Instructions to Authors*.

Instructions to Authors

Contributions in the form of original research reports, evaluations of new and existing compendial methods, and other commentaries and articles relevant to drug standards or to *USP-NF* revision will be considered for publication in *Pharmacopeial Forum* under the section *Stimuli to the Revision Process*. Manuscripts are received with the explicit understanding that they have not been published previously in any language or medium and that they are not simultaneously under consideration by any other publication. All manuscripts are subject to review by USP headquarters staff, Committee members, or qualified outside referees, and if accepted for publication they will be subject to editing by USP staff. Accepted manuscripts become the property of the USP Convention (USPC) and may not be subsequently published elsewhere without written permission from the USPC. Authors are also responsible for obtaining permission for reprinting any illustrations that have been published elsewhere.

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A Rational Approach to Cascade Impactor Mensuration in a Good Cascade Impactor Practice Environment

Steven C Nichols^a and Jolyon P Mitchell^{b,c}

ABSTRACT This article was initially published in *PF* 40(1) [Jan.–Feb. 2014] and comments were received. The authors have revised the article to address comments received. The purpose of this *Stimuli* article is to provide the framework in support of a regimen of stage mensuration for both new and used cascade impactor systems as the principal means of assuring the suitability (aerodynamic particle size accuracy) of these apparatuses when used as a compendial method. The traceability chain is now uninterrupted from the determination of stage aerodynamic performance, as represented by its cut-point size, via the effective diameter of the nozzles of the stage in question to the international length standard. This part of the Good Cascade Impactor Practice regimen may also be used as a good laboratory practice by establishing a standard operating procedure, in which stage mensuration takes place both on receipt of a new cascade impactor and also periodically throughout its lifetime in use.

INTRODUCTION

The multi-stage cascade impactor (CI) is used widely to determine aerodynamic particle size distributions (APSDs) of orally inhaled products (OIPs) (1). The CI size-fractionating capability at a given volumetric flow rate depends primarily on the size of the nozzles at each stage (2). The accuracy of these particle size fractionating apparatuses is ultimately determined through calibration using monodisperse standards of known aerodynamic diameter that possess traceability to the international standard of length (3). The archival calibrations of the Next Generation Pharmaceutical Impactor (NGI), with stage nozzle diameters selected to be as close as possible to the nominal sizes at 15, 30, 60, and 100 L/min nominal flow rates, represent the current state of the art in this respect (4,5). Such calibrations, however, are extremely laborious and exacting to perform because they require specialized facilities capable of generating and delivering the calibration aerosols. The size range of these particles needs to encompass the overall operating range of the CI and provide a minimum of five data points from which the cut-point size at which a given stage is 50% efficient (d_{50}) can be determined as the calibration constant for that stage (4,5).

RECENT DEVELOPMENTS REGARDING STAGE MENSURATION

Since the mid-1990s, the United States Pharmacopeia (USP) and European Pharmacopoeia (Ph.Eur.) methods for APSD measurement have sanctioned the use of stage mensuration as a surrogate for formal recalibration. Currently, *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* { 601 } of the USP and monograph 2.9.18 of the Ph.Eur. provide wording to the effect that stage mensuration should be performed periodically, together with confirmation of other dimensions critical to the effective operation of the impactor (6,7).

Until recently, it was not possible to relate a measure of “average” nozzle diameter for a multi-

nozzle CI in an unambiguous way to d_{50} . However, in 2005, Roberts and Romay (8) showed that the "effective diameter, D_{eff} " could be used to describe the multi-nozzle set for the stage in question, where:

$$D_{eff} = (D^*)^{2/3} \times (D_{median})^{1/3} \quad [1]$$

D^* = area mean

D_{median} = area mean diameter

Later, Roberts (9) went on to show analytically that D_{eff} and d_{50} can be related through the expression of eq. 2 (with variables as defined in eq. 3, in numerator order):

$$D_{eff} = \left(\frac{Q}{n} \right)^{1/3} \left\{ \frac{4C_{50}\rho_p}{9\pi\eta St_{50}} \right\}^{1/3} (d_{50})^{2/3} \quad [2]$$

from which, by rearranging eq. [2]:

$$d_{50} = (D_{eff})^{3/2} \left\{ \frac{9\pi\eta n St_{50}}{4QC_{50}\rho_p} \right\}^{1/2} \quad [3]$$

d_{50} = a minimum of five data points from which the cut-point size at which a given stage is 50% efficient

D_{eff} = effective diameter

η = air viscosity, 1.807 Pa·s at 20° C

n = number of nozzles for the stage in question

St_{50} = dimensionless Stokes number at which the stage is 50% efficient at capturing incoming particles (typically close to 0.24 for round nozzles and neglecting the effect of gravity)

Q = volumetric rate through the CI

C_{50} = dimensionless slip correction term, which can be treated as equivalent to unity for particles that are >10 μm , increasing to 1.15 for particles that are 1.3 μm in diameter and to 2.0 for particles that are 0.13 μm in diameter

ρ_p = particle density, dependent upon the aerosol being sampled

Apart from Q , which can be set to within $\pm 5\%$ between 15 and 100 L/min, encompassing the range of flow rates associated with the impactor apparatuses defined in the pharmacopeial compendia, and using readily available laboratory equipment (10), the other terms are constants for particular measurement conditions and therefore need not be considered further when considering CI system verification. Nichols et al. (11) have since provided data for all of the multi-stage CIs that are designated apparatuses for OIP APSD measurement, linking the manufacturers' tolerances for the various stages to their equivalents in terms of deviations in stage d_{50} from the nominal value.

From the foregoing, it is possible to define the limits for D_{eff} in the compendia that relate to a

known accuracy for each stage in terms of its d_{50} size. The problem, however, has now become one of defining a meaningful specification for each stage and ultimately the overall accuracy for the CI apparatus as a whole in terms of stage d_{50} values. This goal is potentially achievable for the NGI, because this apparatus has a known "archival calibration". This calibration is based on a single representative instrument with stage nozzle diameters (and therefore its D_{eff} values) intentionally selected to have values as close as possible to nominal (4,5). Such rigor, however, is lacking with all other compendial apparatuses. There is currently little stakeholder support for undertaking archival calibrations of these CIs; therefore, the best that can be done is to relate mensurated stage D_{eff} values to the nominal mean diameters that are published in the pharmacopeias. The situation has been improved marginally by the addition of manufacturer-specified tolerances associated with the stage nozzle diameters for all apparatuses in the latest revision of $\langle 601 \rangle$ (7).

PROPOSED MENSURATION SCHEME

The complete manufacturing tolerances for the nozzles of all stages of all compendial apparatuses have been defined by Nichols et al. (11), with the intention that this information will, in due course, appear in future revisions to both $\langle 601 \rangle$ of the *USP* and monograph 2.9.18 of the *Ph.Eur*. It is important to note that although these tolerances are definitive in terms of the manufacturer's specifications, they need not necessarily be the same as those used to define internal "at mensuration" specification limits. It can be advantageous to set tighter limits to avoid the situation where a particular CI fails to meet the manufacturer's specifications at a given routine mensuration. Such a situation would result in uncertainty about the value of data that were obtained by that apparatus between the current mensuration and the elapsed time since the previous mensuration in which its performance was deemed to be acceptable. Given this reality, the scheme shown in *Figure 1* is a proposed revision to the current texts for both compendia. Guidance can be provided to the user on "practical" best practice to ensure that the quality of data produced by a given CI may not come into question as the result of a failed stage mensuration based on manufacturer tolerances.

The key parts of the mensuration process are as follows:

1. At receipt of a new CI, ensure, as part of installation qualification, that new CI apparatuses are compliant with manufacturers' dimensional specifications that ideally should be based on measurements for each and every nozzle, thereby confirming acceptance and establishing baseline mensuration data for each stage of each individual CI. As a minimum, this should include an assessment of mean diameter and its associated tolerance for the group of nozzles associated with each stage, as defined as pharmacopeial specifications in $\langle 601 \rangle$ of the *USP*. If a particular stage does not conform to specification, for instance because of missing or obstructed nozzles, reject the CI for remediation or replacement by the supplier/manufacturer. It is preferable to calculate D_{eff} from the mensuration data for each stage as the primary metric, against which its acceptance against the manufacturer-specified tolerance, as well as its future performance while in use, can be judged.
2. For stages that are acceptable in terms of the manufacturer's specifications, establish the in-use stage mensuration regimen. This process will always include measuring the individual impactor nozzle diameters and, for the reason given above, should normally be extended to determine D_{eff} at each mensuration after acceptance. Guidance on the

accuracy and precision of currently available optical microscopy-image analysis techniques that are recommended for stage mensuration has been provided by Chambers et al. (12). Note that the entity conducting the stage mensuration should be instructed to make these measurements before cleaning the stages so that the "as received" condition may be established. The manufacturer-established specifications will likely be used initially as benchmarks for the parameters that are used to judge whether the stage(s) affected requires remediation or replacement. Note that remediation could be as simple as careful cleaning to remove deposits that may be plugging nozzles. Acquisition of data from several subsequent mensuration exercises after exposure to the product(s) being evaluated in the typical testing environment may also provide the opportunity to develop meaningful in-service "at mensuration" limits that will likely be chosen to be tighter than those used to begin with. These internal limits can subsequently be used to indicate the need for remediation before the stage D_{eff} breaches either the upper or lower boundary of the tolerance range.

3. For those stages that are not acceptable in terms of the manufacturer's specifications, remove the CI from service and either recondition the CI or replace it altogether.
4. Establish the frequency between mensuration events. Although annual evaluation will be sufficient for most users, the duration between mensuration exercises should ideally be governed by the frequency of CI use, the type of compounds being collected, and the solvents/reagents used to recover the collected particulate matter.
5. Ensure that other non-nozzle dimensional data for the CI apparatus are checked and confirmed as being acceptable through a mensuration or appropriate visual inspection process. Guidance on the important dimensions for ancillary equipment, such as the induction port and pre-separator (if used), has been provided by Nichols et al. (11). If at any time the data are deemed to be unacceptable, it may be possible to take remedial action, for example by cleaning the affected component. Under such circumstances, discussion with the manufacturer to establish the most appropriate procedure beforehand is strongly recommended. However, if remedial action does not resolve the problem, then the individual component should be replaced before the apparatus is placed back into service.
6. If the stage or ancillary equipment fails specification, establish an appropriate remediation strategy; note that this process may include the supplier/manufacturer. A detailed description of what might be done to refurbish equipment that is out of specification cannot be provided here because there are many potential causes of changes to critical dimensions that are based on the usage regimen, for example solvent/reagent types used for cleaning and/or recovery of active pharmaceutical ingredient(s), as well as the physicochemical nature of the products themselves.

CONCLUSIONS

A regimen for CI apparatus stage mensuration has been provided to guide users in the continued assurance of CI suitability (aerodynamic particle size accuracy) for the routine assessment of OIP-generated aerosols through the apparatus life cycle. The underlying theory governing the operation of CIs has advanced to the point at which there is the potential for implementation in the pharmacopeial compendia of a robust calibration chain for these apparatuses. Such a scheme links the critical multi-nozzle dimensions using D_{eff} as the collective measure for a given size-fractionation stage to its size-fractionation performance based on stage d_{50} values that are directly relatable to APSD measurement accuracy.

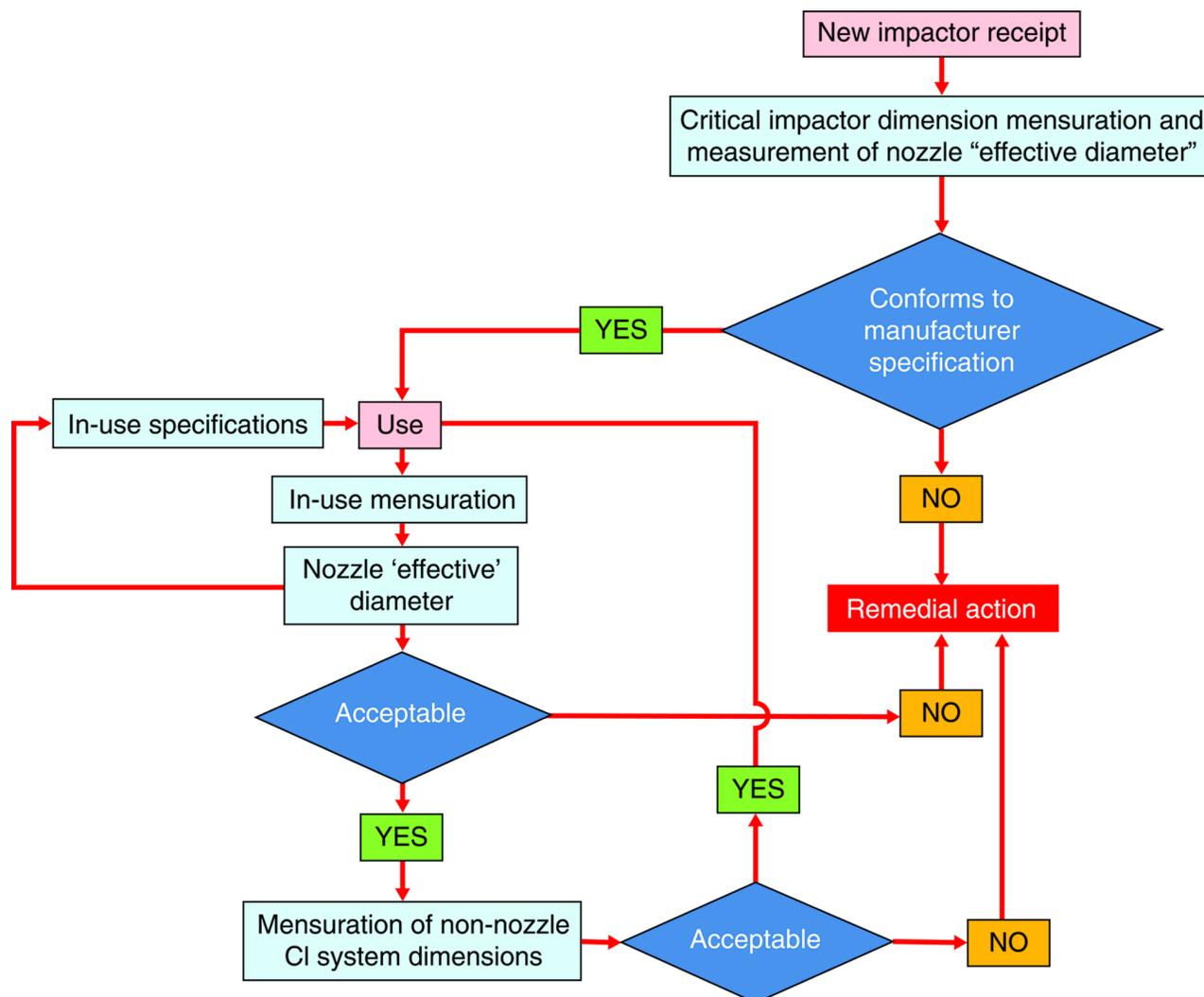


Figure 1. Scheme for the application of stage mensuration to confirm suitability for use of compendial CI apparatuses. [Note—The frequency of the "In-use mensuration" check is determined by the amount of impactor use and the product/solvent characteristics.]

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Total Parenteral Nutrition: Position of the USP Elemental Impurities Expert Panel^a

USP Elemental Impurities Expert Panel^a

ABSTRACT The work performed to revise *Elemental Impurities—Limits* 〈 232 〉 and *Elemental Impurities—Procedures* 〈 233 〉 has opened discussions about harmonization with The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Although much progress has been made toward harmonization, a divide remains regarding the classification and treatment of total parenteral nutrition (TPN). This *Stimuli* article describes and contrasts the USP approach and ICH approach to TPN and presents recommendations from the USP Elemental Impurities Expert Panel for accomplishing harmonization. USP is seeking feedback regarding the ICH Q3D approach to TPN and the Panel's recommendations regarding TPN. In particular, what impact will limits for doses of 2 L or more have on industry?

INTRODUCTION

As USP worked to finalize *Elemental Impurities—Limits* 〈 232 〉 and *Elemental Impurities—Procedures* 〈 233 〉, efforts were made to harmonize with The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Q3D—Elemental Impurities. Many areas required considerable discussion and negotiation between the two groups, and much progress has been made in harmonizing the approach to monitoring pharmaceuticals for elemental impurities.

One area where a divide remains is the treatment of total parenteral nutrition (TPN). Although the ICH approach is for TPN to remain within the scope of Q3D—Elemental Impurities and to treat TPN similarly to large-volume parenterals (LVPs), USP has chosen to remove TPN from the scope of 〈 232 〉.

There is clearly a difference between the USP approach and the ICH approach to TPN. The USP Elemental Impurities Expert Panel thinks that the 2-L reference volume for LVPs, as proposed by ICH, may be too onerous for some TPN products. Although the arguments presented by the Elemental Impurities Expert Panel in support of excluding TPN from the scope of 〈 232 〉 may also apply for LVPs, the nature of the matrix of most TPN solutions makes their analysis even more difficult than the analysis of LVPs. As a result, in an effort to harmonize as much as possible, the Elemental Impurities Expert Panel adopted the ICH requirements for LVPs but presents the following rationale for excluding TPN from the scope of 〈 232 〉.

THE CONCERNS OF THE USP ELEMENTAL IMPURITIES EXPERT PANEL REGARDING THE ICH APPROACH

The concerns of the USP Elemental Impurities Expert Panel about the ICH approach for including TPN within LVPs and the 2-L reference volume for LVPs focus on three main areas:

1. The nature of TPN
2. Dosing considerations
3. Analytical considerations

The Nature of TPN

TPN products are prepared from carbohydrates, amino acids, lipids, and electrolytes in various formulations consisting of single components or mixtures. Specific volumes and component concentrations vary, but from an analytical standpoint, TPN products are very highly dissolved solids, often with major concentrations of inorganic salts. Volumes range from 100 mL to several liters.

TPN solutions are classified by ICH as a subclass of all LVP solutions because:

1. Unlike the majority of therapeutic LVP solutions, TPN contains very high concentrations of dissolved salts, carbohydrates, amino acids, and lipids, either individually or in combinations, sometimes in excess of 50% of the total dissolved solids.
2. Many TPN units are produced in volumes of 2 L or more.

The parenteral permissible daily exposure (PDE) values for the elemental impurities are quite low for many elements. A general rule for introduction of a sample solution to an inductively coupled plasma (ICP)–mass spectrometer (MS) is that the dissolved solids are not in excess of a few tenths of a percentage. For example, in the case of a normal saline solution at 0.9% sodium chloride (NaCl) (w/w), an analyst would likely apply a 10-fold dilution to the test subject before analysis. For the purpose of this example, we will ignore any analytical considerations regarding the analysis of any specific target elements (e.g., analysis of arsenic in a TPN solution containing a chloride salt).

The applied 0.5J spiking concentrations, as required in § 233 to demonstrate accuracy and sensitivity, are displayed in *Table 1* for a 2-L daily dose and an applied 10-fold dilution. Note that many TPN formulations have inorganic salt concentrations far in excess of 0.9% and would correspondingly require higher rates of dilution. A complete digestion preparation would not lessen the amount of inorganic salts in the test material—only the carbohydrate/amino acid/lipid components. Mixtures of amino acids, carbohydrates, and electrolyte salts with total dissolved solids in excess of 20% are common. Some manufacturers' formulations have total dissolved solids that are much higher. Therefore, the required spiking limits at the 0.5J level are presented in *Table 1* for 25-, 50-, 100-, and 200-fold dilutions.

Table 1. Spiking Limits per § 233 for Demonstrating Accuracy/Sensitivity of an ICP–MS Method Based on a 2-L Daily Dose

Element	Parenteral PDE (µg/day)	2000-mL TPN Component Limit (µg/mL)	DF = 10	DF = 25	DF = 50	DF = 100	DF = 200
			0.5J (µg/L)				
Arsenic (As)	15	0.0075	0.375	0.15	0.075	0.0375	0.01875
Cadmium (Cd)	3	0.0015	0.075	0.03	0.015	0.0075	0.00375
Mercury (Hg)	3	0.0015	0.075	0.03	0.015	0.0075	0.00375
Lead (Pb)	5	0.0025	0.125	0.05	0.025	0.0125	0.00625
Cobalt (Co)	5	0.0025	0.125	0.05	0.025	0.0125	0.00625
Molybdenum (Mo)	1700	0.85	42.5	17	8.5	4.25	2.125

Element	Parenteral PDE (µg/day)	2000-mL TPN Component Limit (µg/mL)	DF = 10	DF = 25	DF = 50	DF = 100	DF = 200
			0.5J (µg/L)				
Selenium (Se)	85	0.0425	2.125	0.85	0.425	0.2125	0.10625
Vanadium (V)	12	0.006	0.3	0.12	0.06	0.03	0.015
Silver (Ag)	35	0.0175	0.875	0.35	0.175	0.0875	0.04375
Gold (Au)	130	0.065	3.25	1.3	0.65	0.325	0.1625
Iridium (Ir)	10	0.005	0.25	0.1	0.05	0.025	0.0125
Osmium (Os)	10	0.005	0.25	0.1	0.05	0.025	0.0125
Palladium (Pd)	10	0.005	0.25	0.1	0.05	0.025	0.0125
Platinum (Pt)	10	0.005	0.25	0.1	0.05	0.025	0.0125
Rhodium (Rh)	10	0.005	0.25	0.1	0.05	0.025	0.0125
Rubidium (Ru)	10	0.005	0.25	0.1	0.05	0.025	0.0125
Thallium (Tl)	8	0.004	0.2	0.08	0.04	0.02	0.01
Barium (Ba)	730	0.365	18.25	7.3	3.65	1.825	0.9125
Chromium (Cr)	1100	0.55	27.5	11	5.5	2.75	1.375
Copper (Cu)	130	0.065	3.25	1.3	0.65	0.325	0.1625
Lithium (Li)	280	0.14	7	2.8	1.4	0.7	0.35
Nickel (Ni)	20	0.01	0.5	0.2	0.1	0.05	0.025
Antimony (Sb)	600	0.3	15	6	3	1.5	0.75
Tin (Sn)	640	0.32	16	6.4	3.2	1.6	0.8

Dosing Considerations

TPN solutions are commonly administered through a bedside metering pump that mixes the various solutions and delivers them at a prescribed rate of infusion. Large-volume units of high-concentration solutions, for example a 2-L bag of 70% dextrose, may actually be administered to the patient for more than a 24-h period. This period of time should be considered when establishing limits.

Another consideration related to dosing is the acute nature of TPN administration. In most cases, TPN can be assumed to be an acute or short-term treatment. Most patients will receive TPN at relatively high volumes of solution over a relatively short period of time. PDEs are calculated as daily doses that would be acceptable with lifetime daily exposure. Different safety factors may therefore apply to pharmaceutical products administered for shorter treatment periods. This is already addressed in the ICH Q3D document: paragraph 3.3, Justification for Elemental Impurity Levels Higher Than an Existing PDE.

If the PDEs for parenteral administration are to be applied to TPN solutions, then the USP Elemental Impurities Expert Panel recommends that the volume limit for the maximum daily dose be set at 500 mL. Spiking concentrations at the 0.5J level are presented in *Table 2* below.

Table 2. Spiking Limits per $\langle 233 \rangle$ for Demonstrating Accuracy/Sensitivity of an ICP-MS Method Based on a 500-mL Daily Dose

Element	Parenteral PDE (µg/day)	500-mL TPN Component Limit (µg/mL)	DF = 10	DF = 25	DF = 50	DF = 100	DF = 200
			0.5J (µg/L)				
As	15	0.03	1.5	0.6	0.3	0.15	0.075
Cd	3	0.006	0.3	0.12	0.06	0.03	0.015
Hg	3	0.006	0.3	0.12	0.06	0.03	0.015
Pb	5	0.01	0.5	0.2	0.1	0.05	0.025
Co	5	0.01	0.5	0.2	0.1	0.05	0.025
Mo	1700	3.4	170	68	34	17	8.5

Element	Parenteral PDE (µg/day)	500-mL TPN Component Limit (µg/mL)	DF = 10	DF = 25	DF = 50	DF = 100	DF = 200
			0.5/ (µg/L)				
Se	85	0.17	8.5	3.4	1.7	0.85	0.425
V	12	0.024	1.2	0.48	0.24	0.12	0.06
Ag	35	0.07	3.5	1.4	0.7	0.35	0.175
Au	130	0.26	13	5.2	2.6	1.3	0.65
Ir	10	0.02	1	0.4	0.2	0.1	0.05
Os	10	0.02	1	0.4	0.2	0.1	0.05
Pd	10	0.02	1	0.4	0.2	0.1	0.05
Pt	10	0.02	1	0.4	0.2	0.1	0.05
Rh	10	0.02	1	0.4	0.2	0.1	0.05
Ru	10	0.02	1	0.4	0.2	0.1	0.05
Tl	8	0.016	0.8	0.32	0.16	0.08	0.04
Ba	730	1.46	73	29.2	14.6	7.3	3.65
Cr	1100	2.2	110	44	22	11	5.5
Cu	130	0.26	13	5.2	2.6	1.3	0.65
Li	280	0.56	28	11.2	5.6	2.8	1.4
Ni	20	0.04	2	0.8	0.4	0.2	0.1
Sb	600	1.2	60	24	12	6	3
Sn	640	1.28	64	25.6	12.8	6.4	3.2

Analytical Considerations

The USP Elemental Impurities Expert Panel thinks that the analytical limits that would be imposed on products with a 2-L daily dose (as illustrated in *Table 1*) would be arduous, even for laboratories that are well versed in the use of ICP-MS. Capabilities for routine detection in parts per trillion (ppt) will rely heavily on the cleanliness of the laboratory—perhaps more so than on the ICP-MS instrumentation itself. The average ICP-MS laboratory is not a class-100 clean room. For routine ppt detection, some laboratory facilities will need to be retrofitted for a manufacturer to perform the required analyses for LVPs using a 2-L daily dose. In such instances, it is essential to minimize metal surfaces in the laboratory. Although it is possible to coat metals with plastic, this is not the best option. Selection of hoods, cabinets, drawer pulls, benchtops, and other items must be made to eliminate as much metal as possible from the laboratory. Ceiling tiles must be removed, and sheetrock ceilings must be installed. An air lock between other areas and the ICP-MS laboratory is recommended, as is the use of a sticky pad at every entrance to the laboratory. Laboratory air should be brought in directly from outside, and access to the laboratory should be limited.

Beyond the actual laboratory facility, the reagents, labware, and other materials used must be of the best quality for trace-metals work. Ultra-pure acids must be used. To reduce the costs of such acids, laboratories may find it necessary to distill their own, thereby requiring additional safety considerations. Once the physical laboratory and equipment have been considered, the capabilities of the instrumentation must be considered.

On the basis of the limits presented in *Table 1*, it is clear that the 2-L dose limits—even for a 10-fold dilution and even if one regards all other analytical considerations—are very arduous for many of the elements.

USP ELEMENTAL IMPURITIES EXPERT PANEL RECOMMENDATIONS ON TPN

A more useful approach may be to change the PDE-per-dose paradigm and adopt limits similar to the aluminum (Al) rule that states that LVP TPN shall not contain >25 ppb of Al, irrespective

of dose or volume administered. The following URL provides additional details:
<https://www.federalregister.gov/articles/2001/01/26/01-2125/aluminum-in-large-and-small-volume-parenterals-used-in-total-parenteral-nutrition-delay-of-effective>.
 It would be prudent to apply these “fixed” limits to the elements with the lowest PDEs. Suggested applied fixed limits are presented in *Table 3*.

Table 3. Suggested “Fixed” Elemental Impurities Limits per the Aluminum Rule Paradigm

Element	TPN Limit (ppb)
As	30
Cd	6
Hg	6
Pb	10
Co	10
V	24
Ir	20
Os	20
Pd	20
Pt	20
Rh	20
Ru	20
Tl	16
Ni	40

The Panel is hopeful that USP will receive feedback regarding the ICH Q3D approach to TPN and the Panel’s recommendations regarding TPN. In particular, what impact will limits for doses of 2 L or more have on industry?

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The United States Pharmacopeial Convention comprises representatives from academic institutions; health practitioner and scientific associations; consumer organizations; manufacturer and trade associations; government bodies and associations; and non-governmental standards-setting and conformity assessment bodies. It publishes the U.S. Pharmacopeia and National Formulary, the legally recognized compendia of standards for drugs and products of other health care technologies. The USP and NF include assays and tests for the determination of strength, quality, and purity and requirements for packaging and labeling. **Pharmacopeial Forum's Public Review and Comment Process**

This section presents an overview of the public review and comment process, conducted through *Pharmacopeial Forum (PF)*, for the development of official standards in the *United States Pharmacopeia* and the *National Formulary (USP–NF)*.

USP publishes *PF* on a bimonthly basis to provide an opportunity to review and comment on new or revised standards.

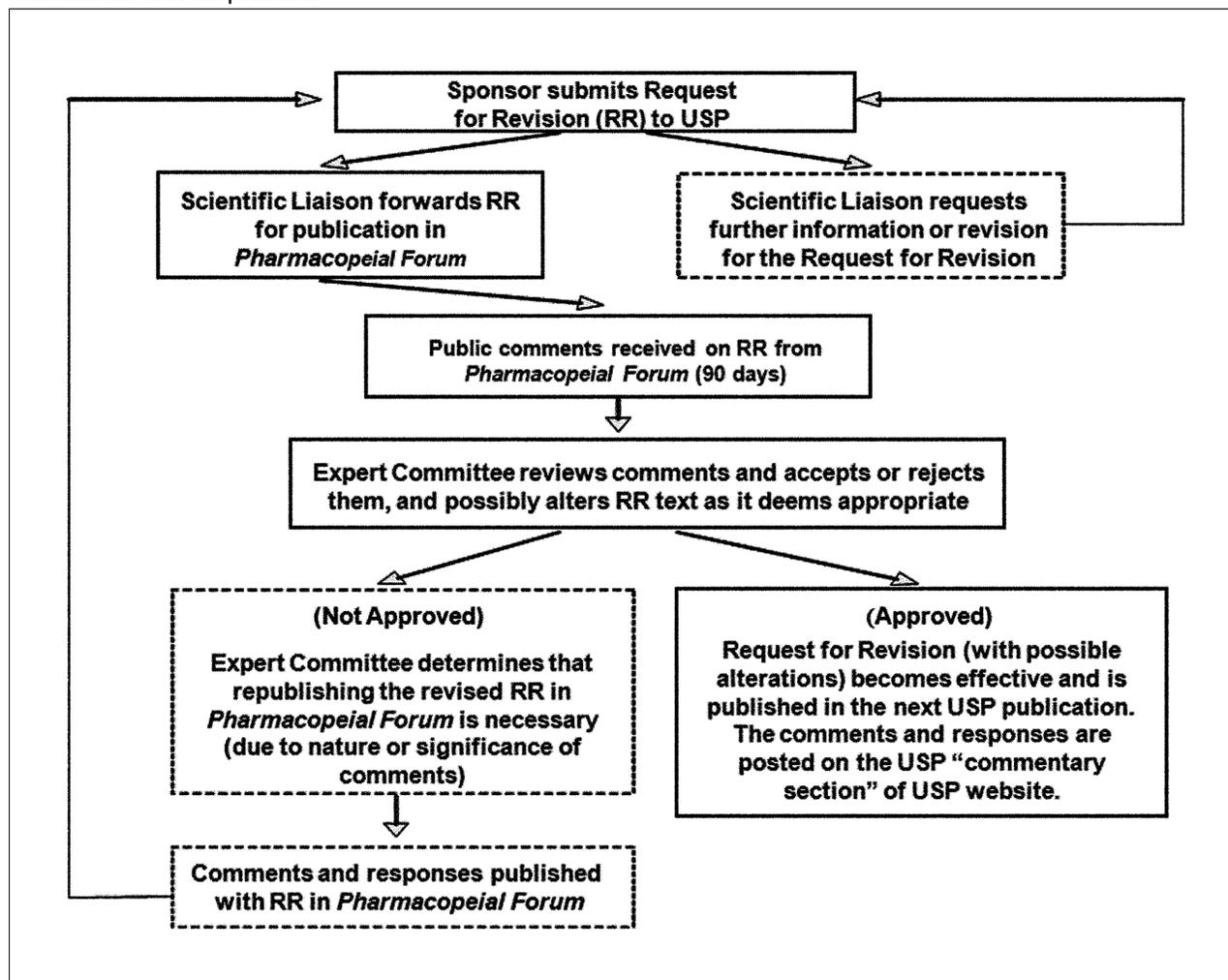
There are two types of proposed revisions in *PF*:

1. **Proposed Revisions**—New or revised standards targeted for adoption through USP's Standard Revision Process. USP's Revision Process calls for publication of a proposed revision in *PF* for a 90-day notice and comment period. After the comment period and subsequent review of comments and approval by the relevant USP Expert Committee, the official standard is published in the next available *USP–NF* or *Supplement*. If comments received are significant, or if the Expert Committee's consideration of comments results in significant additional changes, the Expert Committee may determine that republishing in *PF* is necessary prior to the revision becoming official. See the *In-Process Revision* section for current proposed revisions.
2. **Proposed Interim Revision Announcements**—New or revised standards that become official through an accelerated process in accordance with USP's Guideline on Accelerated Revisions (available on the USP Web site). *Interim Revision Announcements (IRAs)* allow for a revision to become official prior to the next *USP–NF* or *Supplement*. *IRAs* are first presented for a 90-day public comment period in the *Proposed Interim Revision Announcement* section of the *PF*. Note that final *IRAs*, as well as *Errata*, and *Revision Bulletins*, which also are defined in the Accelerated Revision Guideline, appear

only on the USP Web site.

USP welcomes comments and data on proposed revisions. A summary of comments received, along with USP's responses, will be published in the *Revisions and Commentary* section of the USP website (<http://www.usp.org/USPNF/revisions/>).

The chart below shows the public review and comment process and its relationship to standards development.



Questions on the process should be addressed to the USP Executive Secretariat, United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852 (e-mail: execsec@usp.org).

HOW TO USE PF

The various sections of *PF* are briefly described below. A more detailed description of each section is provided at the beginning of that section. A general description of the types and amount of information expected in a *Request for Revision* is available in the *Guideline for Submitting Requests for Revision to the USP-NF* on the USP Web site (www.usp.org/USPNF/submitMonograph/subGuide.html). Note that the Expert Committee listing and the Scientific Staff Directory also are located on the USP Web site (see below for links).

Proposed and Adopted Revisions to the *USP-NF*

Section	Content	How Readers Can Respond
<i>Proposed Interim Revision Announcements</i>	Proposals for <i>Interim Revision Announcements (IRAs)</i> that will be published as official <i>USP</i> or <i>NF</i> standards BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Expert Committee and Scientific Liaisons who handled the monograph or general chapter.	Review material and send comments within 90 days of the <i>PF</i> publication in which the standard was proposed (or per the comment deadline listed in the Briefing section). Direct comments to the USP Scientific Liaison using the contact information provided at the end of each <i>Proposed IRA</i>
<i>In-Process Revision</i>	Proposals for standards that will be published as official in a future <i>USP-NF</i> book or <i>Supplement</i> . BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Expert Committee and USP Scientific Liaisons who handle the monograph or general chapter.	Review material and send comments within 90 days of the <i>PF</i> publication in which the revision was proposed (or per the comment deadline listed in the Briefing section). Direct comments to the USP Scientific Liaison using the contact information provided at the end of each proposed revision. For general inquiries or in cases where a Scientific Liaison is not identified, use the general USP telephone number 301-881-0666 or fax number 301-998-6839 or stdsmonographs@usp.org .

Section	Content	How Readers Can Respond
Stage 4 Harmonization	<p>Revision proposals from the Pharmacopoeial Discussion Group (PDG), which comprises the European Pharmacopoeia, the Japanese Pharmacopoeia, and USP. The Stage 4 draft and the briefing are published in the forum of each pharmacopoeia for public comment. The draft is published in its entirety.</p> <p>BRIEFING: Scientific rationale for the potential inclusion or change or for the proposed change.</p>	<p>Review material and provide comments to the USP Scientific Liaison using the contact information provided at the end of each Stage 4 Harmonization. Individuals who wish to correspond with the European and Japanese Pharmacopoeias concerning monographs in the Stage 4 period of international harmonization should address their comments to the coordinating pharmacopoeia, with a copy to USP.</p> <p>PhEur Secretariat Ms. Lynn Kelso-Eleuterio Central Secretariat European Pharmacopoeia Department</p> <p>European Directorate for the Health Care Council of Europe 7, Allée Kastner CS 30026 67081 Strasbourg France Tel: +33 (3) 88 41 31 48 Fax: +33 (3) 88 41 27 71 lynn.kelso@edqm.eu</p> <p>JP Secretariat Mr. Issei Takayama Technical Officer Pharmaceuticals and Medical Devices Agency (PMDA) Shin-kasumigaseki Building 3-3-2, Kasumigaseki, Chiyoda-ku Tokyo, 100-0013 Japan Phone: +81-3-3506-9431 Fax: +81-3-3506-9440 takayama-issei@pmda.go.jp</p>
Stimuli to the Revision Process	<p>Articles on standards development topics authored by the USP Council of Experts, USP staff, or other interested parties on which USP desires public input prior to further development.</p>	<p>Review material and provide comments to the recipient indicated (usually footnoted in each <i>Stimuli</i> article).</p>

Other Sections

Expert Committees

A listing of the 2015–2020 Expert Committees that work on the development of USP compendial standards (<http://www.usp.org/expert-committees>)

Staff Directory

Names and contact information of key USP scientific staff members who work on the development of USP compendial standards

(<http://www.usp.org/USPNF/devProcess/contactScientists.html>)

Proposed Interim Revision Announcements

This section includes proposals for *Interim Revision Announcements (IRAs)* that will be published as official *USP* or *NF* standards. There is a 90-day comment period for these proposals (see <http://www.usp.org/USPNF/pf/pfRedesign.html> for the *PF* comment schedule). Readers should review material in this section and provide comments to the Scientific Liaison using the contact information appearing at the end of each proposed *IRA*. Information on how to comment can be found under the "Participation" section of www.usp.org. It is important to send comments promptly so that the Expert Committee members can consider reader's input as they are deciding whether to advance standards to official status. The approved official text will be published under *IRAs* in the "New Official Text" section of USP's Web site.

Each proposal is preceded by a Briefing that indicates the proposed revisions.

Symbols—New text is enclosed in symbols and set off from the current official text as shown in the following example: •new text•. Where the symbols appear together with no enclosed text, such as ••, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the date the proposal, if approved, will become official as an *IRA*. For example, •(IRA 1-Apr-2011)

BRIEFING

Dutasteride, *USP 38* page 3247. On the basis of a comment received, it is proposed to revise the monograph as indicated in item 1 below. Additional minor changes are also proposed (items 2–5).

1. Move dihydrodutasteride from *Organic Impurities, Procedure 1* to *Organic Impurities, Procedure 2*. USP has been informed that *Organic Impurities, Procedure 1* is not suitable for quantitating dihydrodutasteride and that *Organic Impurities, Procedure 2* should be used instead. Dihydrodutasteride has been removed from *Table 3* and added into *Table 4* along with its associated relative response factor.
2. Change the resolution between the dutasteride α -dimer and dutasteride β -dimer peaks in the *Suitability requirements* under *Organic Impurities, Procedure 2* from NLT 2.0 to NLT 1.5.
3. Include text in the *Identification* indicating that *Infrared Absorption (197A)* may be used.
4. Remove redundant text from the *Limit of quantitation* section under the *Limit of Platinum* test and change platinum to Dutasteride in the definition of C_S .
5. Remove the *Standard solution* from the *Organic Impurities* section, as it is not used.
6. The procedure in the *Water Determination* test has been clarified to correspond to that used by the sponsor.
7. Clarify the listing of the USP Dutasteride Resolution Mixture RS contents to indicate that it includes dutasteride.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

The comment period for this revision ends on November 30, 2015. In the absence of any significant adverse comments, it is proposed to implement this revision via an *Interim Revision*

Announcement to the Second Supplement to USP 39–NF 34, with an official date of March 1, 2016.

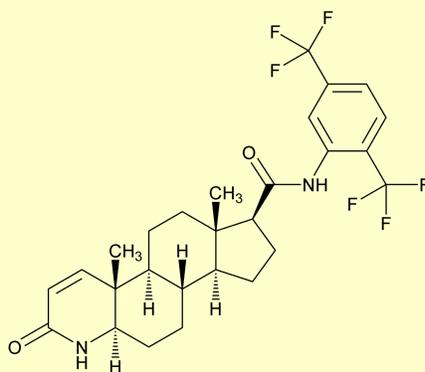
(CHM5: D. Porter.)

Correspondence Number—C157906

Comment deadline: November 30, 2015

Add the following:

▲ Dutasteride



$C_{27}H_{30}F_6N_2O_2$ 528.53

(5 α ,17 β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1-ene-17-carboxamide;
 $\alpha,\alpha,\alpha,\alpha',\alpha',\alpha'$ -Hexafluoro-3-oxo-4-aza-5 α -androst-1-ene-17 β -carboxy-2',5'-xylylide [164656-23-9].

DEFINITION

Dutasteride contains NLT 97.0% and NMT 102.0% of dutasteride ($C_{27}H_{30}F_6N_2O_2$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. Infrared Absorption** (197K) or (197M).
 - (197A) may be used. •(IRA 1-Mar-2016)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Diluent: *Acetonitrile* and water (60:40)

Mobile phase: *Acetonitrile*, water, and *trifluoroacetic acid* (52: 48: 0.025)

System suitability solution: 0.5 mg/mL of USP Dutasteride Resolution Mixture RS in *Diluent*. Sonicate to dissolve.

Standard solution: 0.5 mg/mL of USP Dutasteride RS in *Diluent*. Sonicate to dissolve.

Sample solution: 0.5 mg/mL of Dutasteride in *Diluent*. Sonicate to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 1.5 times the retention time of dutasteride

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 3* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between dutasteride 17α-epimer and dutasteride, *System suitability solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dutasteride (C₂₇H₃₀F₆N₂O₂) in the portion of Dutasteride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Dutasteride RS in the *Standard solution* (mg/mL)

C_U = concentration of Dutasteride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

Change to read:

- **Limit of Platinum**

[Note—Perform this test only if platinum is a known inorganic impurity of the manufacturing process.]

Diluent: *Hydrochloric acid* and *dimethyl sulfoxide* (2:98). Prepare in a plastic volumetric flask.

Standard stock solution: 10 µg/mL of platinum in *Diluent*. Prepare by diluting (1:100) a 1000-µg/mL commercially available platinum standard.

Standard solution 1: 1.0 µg/mL of platinum in *Diluent* from the *Standard stock solution*

Standard solution 2: 0.1 µg/mL of platinum in *Diluent* from *Standard solution 1*

Sample solution: 0.01 g/mL of Dutasteride in *Diluent*. Sonicate to dissolve.

Instrumental conditions

(See *Plasma Spectrochemistry* (730).)

Mode: ICP-OES

Analytical wavelength: 306.471 nm

Spectrophotometric system: Use an inductively coupled plasma-optical emission spectrophotometric system, and construct a calibration curve using the response from the *Diluent*, *Standard solution 1*, and *Standard solution 2*.

System suitability

Samples: *Diluent*, *Standard solution 1*, and *Standard solution 2*

Suitability requirements

Limit of quantitation: 3 µg/g for platinum. ~~Use the standard deviation, in µg/mL, of the platinum concentration obtained from the Diluent.~~

- (IRA 1-Mar-2016)

Calculate the limit of quantitation from the *Diluent*:

$$\text{Result} = 10 \times (SD/C_S)$$

SD = standard deviation of platinum from *Diluent* (µg/mL)

C_S = nominal concentration of platinum

- dutasteride•(IRA 1-Mar-2016)
in the *Sample solution* (g/mL)

Correlation coefficient: NLT 0.99 from the *Diluent*, *Standard solution 1*, and *Standard solution 2*

Analysis

Samples: *Diluent*, *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Plot the responses of the *Diluent*, *Standard solution 1*, and *Standard solution 2* versus their content (0, 0.1, and 1.0 µg/mL) of platinum. Determine the concentration, in µg/mL, of platinum in the *Sample solution* from the calibration curve.

Calculate the concentration, in µg/g, of platinum in the portion of Dutasteride taken:

$$\text{Result} = C_S/C_U$$

C_S = concentration of platinum in the *Sample solution* (µg/mL)

C_U = concentration of Dutasteride in the *Sample solution* (g/mL)

Acceptance criteria: NMT 5 µg/g

- **Limit of Residual Solvents**

Standard stock solution: 5 mg/mL each of *acetonitrile*, *ethyl acetate*, *pyridine*, *toluene*, *dioxane*, and *n-heptane* in *dimethyl sulfoxide*

Standard solution: 10 µg/mL each of acetonitrile, ethyl acetate, pyridine, toluene, dioxane, and *n*-heptane in dimethyl sulfoxide from the *Standard stock solution*

Sample solution: 10 mg/mL of Dutasteride in *dimethyl sulfoxide*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 30-m; capillary coated with 5-µm film of G1

Temperatures

Injection port: 180°

Detector: 260°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	—	50	3
50	10	200	2

Carrier gas: Helium

Flow rate: Head pressure at 12 psi

Split flow: 10 mL/min

Septum purge: 2 mL/min

Injector type: Headspace

Sample volume: 2 µL

Sample temperature: 85°

Equilibration time: 1 min

Thermostating time: 15 min

Needle temperature: 100°

Transfer line temperature: 110°

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between *n*-heptane and dioxane peaks

Relative standard deviation: NMT 5% for each solvent

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each solvent in the portion of Dutasteride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each solvent from the *Sample solution*

r_S = peak response of each solvent from the *Standard solution*

C_S = concentration of each solvent in the *Standard solution* (mg/mL)

C_U = concentration of Dutasteride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acetonitrile	0.30	0.3
Ethyl acetate	0.60	0.2
Dioxane	0.83	0.1
<i>n</i> -Heptane	0.85	0.5
Pyridine	0.92	0.2
Toluene	1.0	0.2

Change to read:

• **Organic Impurities, Procedure 1**

Diluent, Mobile phase, System suitability solution, Standard solution,

• (IRA 1-Mar-2016)

Sample solution, and Chromatographic system: Proceed as directed in the Assay.

System suitability

Sample: *System suitability solution*

[Note—See *Table 3* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between dutasteride 17 α -epimer and dutasteride

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Dutasteride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak area for each impurity from the *Sample solution*

r_T = sum of the peak areas from the *Sample solution*

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dutasteride acid ^a	0.10	1.0	0.2
Dutasteride dimethylamide ^b	0.11	1.4	0.2
Dutasteride methyl ester ^c	0.28	1.0	0.15
Dutasteride ethyl ester ^d	0.39	1.0	0.2
Dutasteride 17 α -5-ene ^e	0.90	1.0	0.2
Dutasteride 17 α -epimer ^f	0.93	1.0	0.3
Dutasteride	1.00	—	—
Chlorodutasteride ^g	1.15	0.33	0.4
Dihydrodutasteride ^h	1.19	1.0	0.15
Dutasteride 5-ene ⁱ	1.20	1.0	0.3
Any other individual impurity	—	—	0.1

^a (5 α ,17 β)-3-Oxo-4-azaandrost-1-ene-17-carboxylic acid.
^b (5 α ,17 β)-N,N-Dimethyl-3-oxo-4-azaandrost-1-ene-17-carboxamide.
^c Methyl (5 α ,17 β)-3-oxo-4-azaandrost-1-ene-17-carboxylate.
^d Ethyl (5 α ,17 β)-3-oxo-4-azaandrost-1-ene-17-carboxylate.
^e (17 α)-N-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1,5(6)-diene-17-carboxamide.
^f (5 α ,17 α)-N-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1-ene-17-carboxamide.
^g (1 α ,5 α ,17 β)-N-[2,5-Bis(trifluoromethyl)phenyl]-1-chloro-3-oxo-4-azaandrostane-17-carboxamide.
^h (5 α ,17 β)-N-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-17-carboxamide.
ⁱ (17 β)-N-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1,5(6)-diene-17-carboxamide.

•

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dutasteride acid ^a	0.10	1.0	0.2
Dutasteride dimethylamide ^b	0.11	1.4	0.2
Dutasteride methyl ester ^c	0.28	1.0	0.15
Dutasteride ethyl ester ^d	0.39	1.0	0.2
Dutasteride 17 α -5-ene ^e	0.90	1.0	0.2
Dutasteride 17 α -epimer ^f	0.93	1.0	0.3
Dutasteride	1.00	—	—
Chlorodutasteride ^g	1.15	0.33	0.4
Dutasteride 5-ene ^h	1.20	1.0	0.3
Any other individual impurity	—	—	0.1

^a (5 α ,17 β)-3-Oxo-4-azaandrost-1-ene-17-carboxylic acid.

^b (5 α ,17 β)-*N,N*-Dimethyl-3-oxo-4-azaandrost-1-ene-17-carboxamide.

^c Methyl (5 α ,17 β)-3-oxo-4-azaandrost-1-ene-17-carboxylate.

^d Ethyl (5 α ,17 β)-3-oxo-4-azaandrost-1-ene-17-carboxylate.

^e (17 α)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1,5(6)-diene-17-carboxamide.

^f (5 α ,17 α)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1-ene-17-carboxamide.

^g (1 α ,5 α ,17 β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-1-chloro-3-oxo-4-azaandrostane-17-carboxamide.

^h (17 β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1,5(6)-diene-17-carboxamide.

•(IRA 1-Mar-2016)

Change to read:

• **Organic Impurities, Procedure 2**

Diluent: Acetonitrile and water (60:40)

Mobile phase: Acetonitrile and water (80:20)

System suitability solution: 0.5 mg/mL of USP Dutasteride Resolution Mixture RS in *Diluent*. Sonicate to dissolve.

Sample solution: 0.5 mg/mL of Dutasteride in *Diluent*. Sonicate to dissolve.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L11

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 5 times the retention time of dutasteride

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.0

•1.5•(IRA 1-Mar-2016)

between dutasteride α-dimer and dutasteride β-dimer peaks

Analysis

Sample: *Sample solution*

Integrate the dutasteride peak and all drug-related peaks eluting after the dutasteride peak.

Calculate the percentage of each impurity in the portion of Dutasteride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

•

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100 \bullet(\text{IRA 1-Mar-2016})$$

r_U = peak area of each impurity from the *Sample solution*

r_T = sum of the peak areas from the *Sample solution*

= relative response factor (see *Table 4*)•(IRA 1-Mar-2016)

• F

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dutasteride	1.0	—
Dutasteride α-dimer ^a	3.7	0.3
Dutasteride β-dimer ^b	4.3	0.5
Any other individual impurity	—	0.1
Total impurities ^c	—	2.0

a-COC(=O)C1=CC=C(C=C1)C2=CC(=C(C=C2)C(F)(F)F)C3=CC(=O)C4=CC(=O)C5=C(C=C4)C(F)(F)F
 b-COC(=O)C1=CC=C(C=C1)C2=CC(=C(C=C2)C(F)(F)F)C3=CC(=O)C4=CC(=O)C5=C(C=C4)C(F)(F)F
 c- Sum of impurities from *Tables 3* and *Table 4*.

•

Table 4

TABLE 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dutasteride	1.0	—	—
Dihydrodutasteride ^a	1.19	0.38	0.15
Dutasteride α -dimer ^b	3.7	1.0	0.3
Dutasteride β -dimer ^c	4.3	1.0	0.5
Any other individual impurity	—	1.0	0.1
Total impurities ^d	—	—	2.0

^a (5 α ,17 β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-17-carboxamide.
^b {[(5 α ,17 β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1-ene-17-carboxamide-]4-yl}{[(5 α ,17 α)-3-oxo-4-azaandrost-1-ene]-17-yl}methanone.
^c {[(5 α ,17 β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1-ene-17-carboxamide-]4-yl}{[(5 α ,17 β)-3-oxo-4-azaandrost-1-ene]-17-yl}methanone.
^d Sum of impurities from Table 3 and Table 4.

•(IRA 1-Mar-2016)

SPECIFIC TESTS

Change to read:

- **Water Determination** (921), *Method Ic*

Sample: 100 mg

Analysis: Heat at ~~180°~~ for 4 min.

- The sample is heated in a tube at 180° for 4 min in a stream of dry inert gas. •(IRA 1-Mar-2016)

Acceptance criteria: NMT 0.50%

- **Optical Rotation** (781S), *Specific Rotation*

Sample solution: 10 mg/mL in chloroform and alcohol (98:2)

Acceptance criteria: +15.0° to +25.0°

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store below 30°.

Change to read:

- **USP Reference Standards** (11)

USP Dutasteride RS

USP Dutasteride Resolution Mixture RS

The mixture contains

- Dutasteride and •(IRA 1-Mar-2016)

the following impurities (other impurities may also be present):

Dutasteride 17 α -epimer: (5 α ,17 α)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1-ene-17-carboxamide.

$C_{27}H_{30}F_6N_2O_2$ 528.53

Dutasteride α -dimer: {[[(5 α ,17 β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1-ene-17-carboxamide-]4-yl]}{[(5 α ,17 α)-3-oxo-4-azaandrost-1-ene]-17-yl}methanone.

$C_{46}H_{55}F_6N_3O_4$ 827.94

Dutasteride β -dimer: {[[(5 α ,17 β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1-ene-17-carboxamide-]4-yl]}{[(5 α ,17 β)-3-oxo-4-azaandrost-1-ene]-17-yl}methanone.

$C_{46}H_{55}F_6N_3O_4$ 827.94

▲USP38

BRIEFING

Methylphenidate Hydrochloride Extended-Release Tablets, USP 38 page 4348. On the basis of comments received, it is proposed to revise the monograph as follows:

1. Replace the existing HPLC procedure in the *Assay* with the procedure used in the test for *Organic Impurities*. The proposed method does not require the use of an internal standard. Internal standards are not typically used in LC analyses unless the sample preparation involves extensive isolation techniques. The procedure is based on analyses using the Waters Symmetry C18 brand of L1 column. The typical retention time for methylphenidate is about 12 min.
2. Expand the brief cross reference to the current *Assay* in *Dissolution Test 1* to include the procedure details.
3. Replace the reference to the *Organic Impurities* test in *Identification test B* with a reference to the proposed *Assay*.
4. Include the approved storage condition in the *Packaging and Storage* section.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

The comment period for the above revision ends November 30, 2015. In the absence of significant adverse comments, it is proposed to implement this revision via an *Interim Revision Announcement* in the *Second Supplement to USP 39-NF 34*, with an official date of March 1, 2016.

(CHM4: R. Ravichandran.)

Correspondence Number—C158482

Comment deadline: November 30, 2015

Methylphenidate Hydrochloride Extended-Release Tablets

DEFINITION

Methylphenidate Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$).

IDENTIFICATION

• A. Infrared Absorption

Sample: Place a portion of powdered Tablets, equivalent to 100 mg of methylphenidate hydrochloride, in a 100-mL beaker. Add 20 mL of chloroform, stir for 5 min, and filter, collecting the filtrate. Evaporate the filtrate to about 5 mL. Add ethyl ether slowly, with stirring, until crystals form. Filter the crystals, wash with ethyl ether, and dry at 80° for 30 min.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the crystals so obtained exhibits maxima only at the same wavelengths as those of a similar preparation of USP Methylphenidate Hydrochloride RS.

Change to read:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *test for Organic Impurities*
 - *Assay.* • (IRA 1-Mar-2016)

ASSAY

Change to read:

- **Procedure**

~~**Buffer:** Dissolve 1.64 g of anhydrous sodium acetate in 900 mL of water. Adjust with acetic acid to a pH of 4.0, and dilute with water to 1000 mL.~~

~~**Mobile phase:** Methanol, acetonitrile, and *Buffer* (4:3:3)~~

~~**Internal standard solution:** 0.4 mg/mL of phenylephrine hydrochloride in *Mobile phase*~~

~~**Standard stock solution:** 0.2 mg/mL of USP Methylphenidate Hydrochloride RS in *Mobile phase*~~

~~**Standard solution:** Transfer 10.0 mL of the *Standard stock solution* to a glass-stoppered, 25-mL conical flask, add 5.0 mL of the *Internal standard solution*, and mix.~~

~~**Sample stock solution:** Nominally 0.2 mg/mL of methylphenidate hydrochloride in *Mobile phase*, prepared as follows. Finely powder NLT 20 Tablets. Transfer a suitable amount of the powder to a suitable volumetric flask to obtain the nominal concentration. Add 70% of the flask volume of *Mobile phase*, and sonicate for 15 min. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable membrane filter, discarding the first portion of the filtrate. [Note—Avoid the use of glass filters. Polypropylene filters are suitable for use.]~~

~~**Sample solution:** Transfer 10.0 mL of the clear filtrate to a glass-stoppered, 25-mL conical flask, and add 5.0 mL of the *Internal standard solution*.~~

~~**Chromatographic system**~~

~~(See *Chromatography* (621), *System Suitability*.)~~

~~**Mode:** LC~~

~~**Detector:** UV 210 nm~~

~~**Column:** 4.6 mm × 25 cm; packing L10~~

~~**Flow rate:** 1.5 mL/min~~

~~**Injection volume:** 50 µL~~

~~**System suitability**~~

Sample: ~~Standard solution~~

~~[Note—The relative retention times for phenylephrine hydrochloride and methylphenidate hydrochloride are 0.8 and 1.0, respectively.]~~

Suitability requirements

Resolution: ~~NLT 2.0 between the analyte and internal standard peaks~~

Relative standard deviation: ~~NMT 2.0% for the peak response ratios of the analyte to the internal standard~~

Analysis

Samples: ~~Standard solution and Sample solution~~

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of methylphenidate hydrochloride to the internal standard from the ~~Sample solution~~

R_S = peak response ratio of methylphenidate hydrochloride to the internal standard from the ~~Standard solution~~

C_S = concentration of USP Methylphenidate Hydrochloride RS in the ~~Standard solution~~ (mg/mL)

C_U = nominal concentration of methylphenidate hydrochloride in the ~~Sample solution~~ (mg/mL)

•Mobile phase: Dissolve 2 g of octanesulfonic acid sodium salt in 730 mL of water. Adjust with phosphoric acid to a pH of 2.7. Mix with 270 mL of acetonitrile.

Solution A: Acidified water; adjusted with phosphoric acid to a pH of 3

Diluent A: Acetonitrile and Solution A (25:75)

Diluent B: Acetonitrile and methanol (50:50)

System suitability solution: 80 µg/mL of USP Methylphenidate Hydrochloride RS, 1 µg/mL of methylphenidate hydrochloride erythro isomer from USP Methylphenidate Hydrochloride Erythro Isomer Solution RS, and 2 µg/mL of USP Methylphenidate Related Compound A RS in Diluent A

Standard solution: 0.1 mg/mL of USP Methylphenidate Hydrochloride RS in Diluent A

Sample stock solution: Nominally 1 mg/mL of methylphenidate hydrochloride prepared as follows. Dissolve NLT 10 Tablets in a suitable volumetric flask with 20% of the total flask volume of Diluent B. [Note—Alternatively, a portion of powder from NLT 10 Tablets may be transferred to a suitable volumetric flask and suspended in 20% of the total flask volume of Diluent B.] Stir for 4 h. Dilute with Solution A to volume.

Sample solution: Nominally 0.1 mg/mL of methylphenidate hydrochloride in Solution A from the Sample stock solution. [Note—Centrifuge before chromatographic analysis.]

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 15-cm; 5-µm packing *L1*

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 25 µL

Run time: 2 times the retention time of methylphenidate

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 6* for relative retention times.]

Suitability requirements

Resolution: NLT 4.0 between methylphenidate related compound A and methylphenidate hydrochloride erythro isomer; NLT 6.0 between the methylphenidate and erythro isomer peaks, *System suitability solution*

Tailing factor: NMT 2.0 for the methylphenidate peak, *Standard solution*

Relative standard deviation: NMT 2.0% for the methylphenidate peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methylphenidate hydrochloride in the *Sample solution* (mg/mL)

•(IRA 1-Mar-2016)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **Dissolution** <711>

Test 1

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Times: 1, 2, 3.5, 5, and 7 h

Sample solution: Use portions of the solution under test passed through a suitable filter of 0.45-µm pore size. Do not use glass fiber filters.

•**Buffer:** Dissolve 1.6 g of *anhydrous sodium acetate* in 900 mL of water. Adjust with *acetic acid* to a pH of 4.0 and dilute with water to 1000 mL.

Mobile phase: Methanol, acetonitrile, and *Buffer* (40:30:30)

Internal standard solution: 0.4 mg/mL of phenylephrine hydrochloride in *Mobile phase*

Standard stock solution: $(1.5 \times [L/500])$ mg/mL of USP Methylphenidate Hydrochloride RS in *Mobile phase* where *L* is the label claim of methylphenidate hydrochloride in mg/Tablet

Standard solution: Transfer 10.0 mL of the *Standard stock solution* to a glass-stoppered, 25-mL conical flask, add 5.0 mL of the *Internal standard solution*, and mix.

Sample stock solution: Use portions of the solution under test passed through a suitable filter of 0.45- μ m pore size. Do not use glass fiber filters.

Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a glass-stoppered, 25-mL conical flask, add 5.0 mL of the *Internal standard solution*, and mix. •(IRA 1-Mar-2016)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 25-cm; packing *L10*

Flow rate: 1.5 mL/min

Injection volume: 50 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times for phenylephrine hydrochloride and methylphenidate hydrochloride are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the analyte and internal standard peaks

Relative standard deviation: NMT 2.0% for the peak response ratios of the analyte to the internal standard

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved by using the procedure in the *Assay*, making any necessary volumetric adjustments.

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
1	25–45
2	40–65
3.5	55–80
5	70–90
7	NLT 80

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

For products labeled for dosing every 24 h

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Acidified water; adjusted with phosphoric acid to a pH of 3; 50 mL at $37 \pm 0.5^\circ$

Apparatus 7 (see *Drug Release* (724)): 30 cycles/min; 2–3 cm amplitude. Follow *Sample Preparation A* using a metal spring sample holder (*Figure 5d*). (ERR 1-Jun-2015) Place one Tablet in the holder with the Tablet orifice facing down, and cover the top of the holder with Parafilm™. At the end of each specified test interval, the systems are transferred to the next row of new test tubes containing 50 mL of fresh *Medium*.

Times: 1-h intervals for a duration of 10 h

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved by using the following method.

Solution A: Dissolve 2.0 g of *sodium 1-octanesulfonate* in 700 mL of water, mix well, and adjust with *phosphoric acid* to a pH of 3.0.

Mobile phase: Acetonitrile and *Solution A* (30:70)

Diluent: Acetonitrile and *Medium* (25:75)

Standard stock solution: 0.3 mg/mL of USP Methylphenidate Hydrochloride RS in *Diluent*

Standard solutions: Prepare at least six solutions by making serial dilutions of the *Standard stock solution* in *Diluent* to bracket the expected drug concentration range.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.2-mm \times 5-cm; 5- μ m packing *L1*

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 25 μ L

System suitability

Sample: Middle range concentration of the *Standard solutions*

Suitability requirements**Capacity factor:** NLT 2

• (IRA 1-Mar-2016)

Tailing factor: NMT 2**Relative standard deviation:** NMT 2% for the peak response of the analyte; NMT 2% for the retention time of the analyte**Analysis****Samples:** *Standard solutions* and the solution under test

Construct a calibration curve by plotting the peak response versus the concentration of the *Standard solutions*. Determine the amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in each interval by linear regression analysis of the standard curve.

Tolerances: See *Table 2*.**Table 2**

Time (h)	Amount Dissolved (%)
1	12–32
4	40–60
10	NLT 85
3–6 (avg)	9–15 (/h)

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution (711)*, *Acceptance Table 2*.

Calculate the average percentage released from 3–6 h:

$$\text{Result} = (Y - X) / 3$$

Y = cumulative drug released from 0–6 h

X = cumulative drug released from 0–3 h

For products labeled for dosing every 24 h**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.**Medium:** pH 6.8 phosphate buffer (6.8 g/L of *monobasic potassium phosphate* in water; adjusted with 2 N sodium hydroxide or 10% phosphoric acid to a pH of 6.80); 900 mL**Apparatus 1:** 100 rpm**Times:** 0.75, 4, and 10 h**Buffer:** pH 4.0 phosphate buffer (2.72 g/L of *monobasic potassium phosphate* in water; adjusted with 2 N sodium hydroxide or 10% phosphoric acid to a pH of 4.00)**Mobile phase:** Acetonitrile and *Buffer* (17.5: 82.5)**Standard solution:** 0.06 mg/mL of USP Methylphenidate Hydrochloride RS in 0.1 N

hydrochloric acid

Sample solution: Pass a portion of the solution under test through a suitable polytetrafluoroethylene (PTFE) filter of 0.45- μm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.0-mm \times 5-cm; 2.5- μm packing *L1*

Column temperature: 50°

Flow rate: See *Table 3*.

Table 3

Time (min)	Flow Rate (mL/min)
0.0	0.75
2.5	0.75
3.0	2.00
6.0	2.00
6.5	0.75
7.0	0.75

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for methylphenidate related compound A, the erythro isomer, and methylphenidate are 0.47, 0.65, and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of methylphenidate hydrochloride ($\text{C}_{14}\text{H}_{19}\text{NO}_2 \cdot \text{HCl}$) in the sample withdrawn from the vessel at each time point (i) shown in *Table 4*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = sum of the peak responses of methylphenidate and methylphenidate related compound A from the *Sample solution*

r_S = peak response of methylphenidate from the *Standard solution*

C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution*

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($\text{C}_{14}\text{H}_{19}\text{NO}_2 \cdot \text{HCl}$) dissolved at each time point (i) shown in *Table 4*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S] \times (1/L) \times 100$$

C_i = concentration of methylphenidate hydrochloride in the portion of sample withdrawn at time point (i) (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (i)	Time (h)	Amount Dissolved (%)
1	0.75	12–30
2	4	55–80
3	10	NLT 80

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: 0.001 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Times: 1, 2, 6, and 10 h

Mobile phase: Acetonitrile and water (20:80). For every L of *Mobile phase* add 1.0 mL of *formic acid* and 0.2 mL of *trifluoroacetic acid*.

Standard solution: 0.02 mg/mL of USP Methylphenidate Hydrochloride RS in *Mobile phase*

Sample solution: Pass a portion of the solution under test through a suitable PTFE filter of 0.45- μ m pore size. Do not use glass fiber filters.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.0-mm \times 15-cm; 3- μ m packing *L1*

Column temperature: 40°

Flow rate: 0.75 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution and Sample solution*

Calculate the concentration (C_i) of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i) shown in *Table 5*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of methylphenidate from the *Sample solution*

r_S = peak response of methylphenidate from the *Standard solution*

C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution*

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at each time point (i) shown in *Table 5*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S] \times (1/L) \times 100$$

$$\text{Result}_4 = \{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S] \times (1/L) \times 100$$

C_i = concentration of methylphenidate hydrochloride in the portion of sample withdrawn at time point (i) (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 5*.

Table 5

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	20–40
2	2	35–55
3	6	65–85
4	10	NLT 80

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

•(RB 1-Jun-2014)

- **Uniformity of Dosage Units** (905): Meet the requirements

IMPURITIES

Change to read:

- **Organic Impurities**

Mobile phase: Dissolve 2 g of *sodium-1-octanesulfonate* in 730 mL of water. Adjust with *phosphoric acid* to a pH of 2.7. Mix with 270 mL of acetonitrile.

Solution A: Acidified water; adjusted with *phosphoric acid* to a pH of 3

Diluent A: Acetonitrile and *Solution A* (25:75)

Diluent B: Acetonitrile and methanol (50:50)

System suitability solution: 80 µg/mL of USP Methylphenidate Hydrochloride RS, 1 µg/mL of methylphenidate hydrochloride erythro isomer from USP Methylphenidate Hydrochloride Erythro Isomer Solution RS, and 2 µg/mL of USP Methylphenidate Related Compound A RS in *Diluent A*

Standard solution: 0.2 µg/mL of USP Methylphenidate Hydrochloride RS, 0.5 µg/mL of methylphenidate hydrochloride erythro isomer from USP Methylphenidate Hydrochloride Erythro Isomer Solution RS, and 1.5 µg/mL of USP Methylphenidate Related Compound A RS in *Diluent A*

Sample stock solution: Nominally 1 mg/mL of methylphenidate hydrochloride prepared as follows. Dissolve NLT 10 Tablets in a suitable volumetric flask with 20% of the total flask volume of *Diluent B*. [Note—Alternatively, a portion of powder from NLT 10 Tablets may be transferred to a suitable volumetric flask and suspended in 20% of the total flask volume of *Diluent B*.] Stir for 4 h. Dilute with *Solution A* to volume.

Sample solution: 0.1 mg/mL of methylphenidate hydrochloride in *Solution A* from the *Sample stock solution*. [Note—Centrifuge before chromatographic analysis.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 15-cm; 5-µm packing *L1*

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 25 µL

Run time: 2 times the retention time of methylphenidate

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 6.0 between the methylphenidate and erythro isomer peaks

Tailing factor: NMT 2.0 for the methylphenidate peak

Relative standard deviation: NMT 2.0% for the methylphenidate peak; NMT 4.0% each for the methylphenidate related compound A and erythro isomer peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylphenidate related compound A or erythro isomer in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methylphenidate related compound A or erythro isomer from the *Sample solution*

r_S = peak response of methylphenidate related compound A or erythro isomer from the *Standard solution*

C_S = concentration of USP Methylphenidate Related Compound A RS or methylphenidate hydrochloride erythro isomer in the *Standard solution* (mg/mL)

C_U = nominal concentration of methylphenidate hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of USP Methylphenidate Hydrochloride RS from the *Standard solution*

C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methylphenidate hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 6.

Table 6

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methylphenidate related compound A • (RB 1-Jun-2014)	0.47	1.5
Erythro isomer • ^a • (RB 1-Jun-2014)	0.65	0.5
Methylphenidate hydrochloride • (IRA 1-Mar-2016)	1.0	—
Any unspecified degradation product	—	0.2
Total • degradation products • (RB 1-Jun-2014)	—	2.5
• ^a Methyl (<i>RS,SR</i>)-2-phenyl-2-(piperidin-2-yl) acetate. • (RB 1-Jun-2014)		

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers.
 - Store at controlled room temperature. • (IRA 1-Mar-2016)
- **Labeling:** The labeling states the *Dissolution* test with which the product complies if other than *Test 1*.

Change to read:

- **USP Reference Standards** <11>

USP Methylphenidate Hydrochloride RS

USP Methylphenidate Hydrochloride Erythro Isomer Solution RS

This solution contains 0.5 mg of methylphenidate hydrochloride erythro isomer per mL in

methanol.

USP Methylphenidate Related Compound A RS

α -Phenyl-2-piperidineacetic acid hydrochloride.

$C_{13}H_{17}NO_2 \cdot HCl$ •255.74•(ERR 1-Jun-2015)

In-Process Revision

This section contains proposals for adoption as official *USP* or *NF* standards (either proposed *new* standards or proposed *revisions* of current *USP* or *NF* standards). These may be any of the following: (1) proposed revisions placed directly under *In-Process Revision*, (2) modifications of revisions previously proposed under *In-Process Revision*, or (3) proposed revisions for articles awaiting approval by FDA. Readers should review material in this section and provide comments to the Scientific Liaison using the contact information appearing at the end of each proposal. Information on how to comment can be found under the "Participation" section of www.usp.org. It is important to send comments promptly, before the comment deadline listed after each title.

Briefings Each Proposal is preceded by a Briefing in the following format:

Briefing
Name of Item , citations of the most recent USP publications in which this item appeared. Rationale for the revision. Other relevant information. (For example, if a chromatographic method is being proposed, column specifications and retention times for compounds of interest.) Finally, the Expert Committee acronym (the name of the Scientific Liaison who handled the particular monograph or general chapter, and the USP tracking correspondence number, as shown in the example below:
(Expert Committee Acronym: Liaison Name.)
Correspondence Number—CXXXXX

Symbols Proposed revisions are shown with language proposed for deletion or replacement crossed out. All *USP-NF* revisions use the following symbols that indicate the final destination of the official text: •new text• if slated for an *IRA*; ▲new text▲ if slated for *USP-NF*; ■new text■ if slated for a *Supplement* to *USP-NF*; ►new text◄ for articles awaiting approval by FDA. The same symbols indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as ••, or ■■, or ▲▲, or ►◄, it means that text has been deleted and no new text was proposed to replace it.

In revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA*, *Supplement*, or the *USP* or *NF* as the publication where the revision will appear if approved. For example, ■_{2S} (*USP 34*) indicates that the proposed revision is slated for the *Second Supplement to USP 34*, and ▲_{USP35} and ▲_{NF30} indicates that the revisions are proposed for *USP 35* and *NF 30*, respectively. In the case of revisions for articles awaiting approval by FDA, the symbols are followed by TBD indicating that the official publication is To Be Determined. For example, ◄(TBD) does not yet have a target official publication.

Official Title Changes Where the specification "**Monograph title change**" is found, it indicates that the official title stated after that specification will be substituted for the former title in the appropriate places throughout that monograph once this revision becomes official.

BRIEFING

⟨1⟩ **Injections and Implanted Drug Products (Parenterals)—Product Quality Tests**, *USP* 38 page 53 and *PF* 39(5) [Sept.–Oct. 2013]. There are five *USP* general chapters that link with *USP*'s taxonomy for pharmaceutical dosage forms: *Injections and Implanted Drug Products* ⟨1⟩, *Oral Drug Products—Product Quality Tests* ⟨2⟩, *Topical and Transdermal Drug Products—Product Quality Tests* ⟨3⟩, *Mucosal Drug Products—Product Quality Tests* ⟨4⟩, and *Inhalation and Nasal Drug Products—General Information and Product Quality Tests* ⟨5⟩. This taxonomy describes five routes of administration: injection or parenteral; gastrointestinal or oral; topical (dermal and transdermal); mucosal; and lungs or inhalation (for more information, see the *Stimuli* article titled *Product Quality and Product Performance General Chapters* in *PF* 39(3) [May–June 2013]). The five general chapters provide critical product quality attributes for the various dosage forms by the various routes of administration. In addition to these five chapters, *Ophthalmic Ointments* ⟨771⟩ outlines the critical product quality attributes for the various dosage forms that can be applied to the eye. Product quality tests assess physical, chemical, and microbial attributes. Taken together, the tests help ensure identity, strength, quality, and purity of a dosage form. The General Chapters—Dosage Forms Expert Committee is proposing the following revision to ⟨1⟩, ⟨2⟩, ⟨3⟩, ⟨4⟩, ⟨5⟩, and ⟨771⟩, to clarify the applicability of these chapters.

Additionally, minor editorial changes have been made to update this chapter to current *USP* style.

(GCDF: D. Hunt.)

Correspondence Number—C161336; C128347

Comment deadline: November 30, 2015

Change to read:

⟨1⟩ INJECTIONS ▲ AND IMPLANTED DRUG PRODUCTS (PARENTERALS)—PRODUCT QUALITY TESTS

(Chapter to become official May 1, 2016)

(Current chapter name is ⟨1⟩ Injections)

Change to read:

INTRODUCTION

Parenteral drug products include both injections and implanted drug products that are injected through the skin or other external boundary tissue, or implanted within the body to allow the direct administration of the active drug substance(s) into blood vessels, organs, tissues, or lesions. Injections may exist as either immediate- or extended-release dosage forms. Implanted parenteral drug products are long-acting dosage forms that provide continuous release of the active drug substance(s), often for periods of months to years. For systemic delivery, they may be placed subcutaneously; for local delivery, they may be placed in a specific region of the body. Routes of administration for parenteral drug products include intravenous, intraventricular, intra-arterial, intra-articular, subcutaneous, intramuscular, intrathecal, intracisternal, and intraocular.

Parenteral dosage forms include solutions, suspensions, emulsions, sterile powders for solutions and suspensions (including liposomes), implants (including microparticles), and products that consist of both a drug and a device such as drug-eluting stents. The reader is directed to *Pharmaceutical Dosage Forms* (1151) and to the later sections of this chapter for additional descriptions of dosage forms that fall into the general category of parenteral drug products. *Nomenclature* (1121) provides information on nomenclature used to establish USP names and monograph titles for parenteral drug products. [Note—All references to chapters above 1000 are for informational purposes only, for use as a helpful resource. These chapters are not mandatory unless explicitly called out for application.]

~~Chapter (1) provides a framework to support the revision and the development of individual monographs, and is not meant to replace individual monographs.~~

■ **2S (USP39)**

Chapter (1) provides lists of

■ **consolidated 2S (USP39)**

common product quality test requirements in a concise and a coherent fashion. The chapter is divided into four main sections: 1) universal product quality tests that are applicable to parenteral dosage forms; 2) specific product quality tests, which are tests that should be considered in addition to *Universal Tests*; 3) product quality tests for specific dosage forms, which lists all the applicable tests (Universal and Specific) for the specific dosage form; and 4) product performance tests.

~~If a monograph exists, it will reference (1) or indicated chapter parts. If a specific drug product monograph is missing (not in existence), the general chapters provide the quality tests that can be used by manufacturers until the dosage form monograph is developed by USP.~~

■ This chapter applies, in part or in its entirety, when referenced in a drug product monograph and includes the quality tests for the specific route of administration. The quality tests listed can be used, as appropriate, by manufacturers toward the development of new drug product monographs for submission to USP. ■ **2S (USP39)**

The Pharmacopeial definitions for sterile preparations for parenteral use may not apply to some biologics because of their special nature and licensing requirements (see *Biologics* (1041)). However, some biological finished drug products containing "Injection" in the monograph title must meet the requirements of (1) or indicated chapter subparts, where it is specified in the monograph.

Drug Product Quality and Drug Product Performance Tests

Procedures and acceptance criteria for testing parenteral drug products are divided into two categories: 1) those that assess product quality attributes, e.g., identification, sterility, and particulate matter, and are contained in this chapter; and 2) those that assess product performance, e.g., in vitro release of the drug substance from the drug product. Whereas quality tests assess the integrity of the dosage form, the performance tests assess performance (bioavailability) after the product has been administered to the patient. A product performance test, i.e., drug release test for suspensions, emulsions, powder for suspension (including microparticles and liposomes), and drug-eluting stents, should be carried out using appropriate test procedures.

PRODUCT QUALITY TESTS COMMON TO PARENTERAL DOSAGE FORMS

Universal Tests

Universal tests are listed below and are applicable to parenteral dosage forms.

DESCRIPTION

A qualitative description of the dosage form should be provided. The acceptance criteria should include the final acceptable appearance. If color changes during storage, a quantitative or a semiquantitative procedure may be appropriate. This section specifies the content or the label claim of the article (see *Labeling* (7)). Additional information about commonly used terms and definitions can be found in (1121).

IDENTIFICATION

Identification tests are discussed in *General Notices and Requirements 5.40*. Identification tests should establish the identity of the drug or drugs present in the article and should discriminate between compounds of closely related structure that are likely to be present. The most conclusive test for identity is the infrared (IR) absorption spectrum (see *Spectrophotometry and Light-Scattering* (851) and *Spectrophotometric Identification Tests* (197)). If no suitable IR spectrum can be obtained, other analytical methods can be used. Near-infrared or Raman spectrophotometric methods also could be acceptable for the sole identification of the drug product formulation (see *Near-Infrared Spectroscopy* (1119) and *Raman Spectroscopy* (1120)). Identification solely by a single chromatographic retention time is not regarded as specific. However, the use of two chromatographic procedures in which the separation is based either on different principles or a combination of tests in a single procedure can be acceptable (see *Chromatography* (621) and *Thin-Layer Chromatographic Identification Test* (201)). Additional information regarding identification tests can be found in *Identification Tests—General* (191) and *Mass Spectrometry* (736).

ASSAY

A specific and stability-indicating test should be used to determine the strength (content) of the drug product. In cases where the use of a nonspecific assay is justified, other supporting analytical procedures should be used to achieve overall specificity. A specific procedure should be used when there is evidence of excipient interference with the nonspecific assay.

IMPURITIES

Tests for impurities are discussed in *General Notices and Requirements 5.60*. All articles should be tested to ensure that they meet the requirements.

FOREIGN AND PARTICULATE MATTER

Articles intended for parenteral administration should be prepared in a manner designed to exclude particulate matter as defined in *Subvisible Particulate Matter in Therapeutic Protein Injections* (787), *Particulate Matter in Injections* (788), and *Particulate Matter in Ophthalmic Solutions* (789), as well as excluding other foreign matter as appropriate for the dosage form. Each final container of all parenteral preparations should be inspected to the extent possible for

the presence of observable foreign and particulate matter (hereafter termed visible particulates) in its contents. The inspection process should be designed and qualified to ensure that every lot of all parenteral preparations is essentially free from visible particulates, as defined in *Visible Particulates in Injections* (790). Qualification of the inspection process should be performed with reference to particulates in the visible range and those particulates that might emanate from the manufacturing or filling process. Every container in which the contents show evidence of visible particulates must be rejected. The inspection for visible particulates may take place during examination for other critical defects such as cracked or defective containers or seals or when characterizing the appearance of a lyophilized product. When the nature of the contents or the container-closure system permits only limited inspection of the total contents, the 100% inspection of a lot should be supplemented with the inspection of constituted (e.g., dried) or withdrawn (e.g., from a dark amber container) contents of a sample of containers from the lot.

Large-volume injections for single-dose infusion, small-volume injections, and pharmacy bulk packages (PBP) are subject to the light obscuration or microscopic procedures and limits for subvisible particulate matter set forth in (788), unless otherwise specified in the chapter or in the individual monograph. An article packaged as both a large-volume and a small-volume injection meets the requirements set forth for small-volume injections where the container is labeled as containing 100 mL or less. It meets the requirements set forth for large-volume injections for single-dose infusion where the container is labeled as containing more than 100 mL.

STERILITY

The sterility of all drug products intended for parenteral administration should be confirmed by the use of methods described in *Sterility Tests* (71) or by an approved alternative method.

BACTERIAL ENDOTOXINS

All articles intended for parenteral administration should be prepared in a manner designed to limit bacterial endotoxins as defined in *Bacterial Endotoxins Test* (85) or *Pyrogen Test* (151).

CONTAINER CONTENT

Container contents should be determined when appropriate (see the proposed general chapter *Container Content for Injections* (697)).

LEACHABLES AND EXTRACTABLES

The packaging system should not interact physically or chemically with the preparation to alter its strength, quality, or purity beyond the official or established requirements. The packaging system should meet the requirements in *Elastomeric Closures for Injections* (381), *Packaging and Storage Requirements* (659), *Containers—Glass* (660), *Containers—Plastics* (661), *Plastic Materials of Construction* (661.1), and *Plastic Packaging Systems for Pharmaceutical Use* (661.2). Further information regarding packaging systems testing may be found in *Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems* (1663) and *Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems* (1664).

CONTAINER-CLOSURE INTEGRITY

The packaging system should be closed or sealed in such a manner as to prevent contamination or loss of contents. Validation of container integrity must demonstrate no penetration of microbial contamination or gain or loss of any chemical or physical parameter deemed necessary to protect the product (see *Sterile Product Packaging—Integrity Evaluation* (1207)).

LABELING

All articles intended for parenteral administration should meet the labeling requirements defined in (7).

Specific Tests

In addition to the *Universal Tests* listed above, the following *Specific Tests* may be considered on a case-by-case basis and, when appropriate, are referenced in the *USP–NF* monograph.

PHYSICOCHEMICAL PROPERTIES

These include such properties as *Osmolality and Osmolarity* (785), *pH* (791), *Specific Gravity* (841), and *Viscosity—Capillary Methods* (911).

UNIFORMITY OF DOSAGE UNITS

This test is applicable for parenteral drug products and dosage forms packaged in single-unit containers. It includes both the mass of the dosage form and the content of the active substance in the dosage form (see *Uniformity of Dosage Units* (905)).

VEHICLES AND ADDED SUBSTANCES

There are other vehicles, both aqueous and nonaqueous, beyond those that are discussed below. All vehicles should be suitable for their intended use and not impact drug product quality.

Aqueous vehicles: Aqueous vehicles must meet the requirements of (151) or (85), whichever is specified in the monograph. *Water for Injection* is generally used as the vehicle. Sodium chloride or dextrose may be added to render the resulting solution isotonic, and Sodium Chloride Injection or Ringer's Injection may be used in whole or in part instead of *Water for Injection*.

Nonaqueous vehicles: Fixed oils are classified under *Nonaqueous vehicles*. Fixed oils used as vehicles are of vegetable origin and are odorless. They meet the requirements in the test for *Solid Paraffin* in the *Mineral Oil* monograph with the cooling bath maintained at 10°.

Also meet the requirements of the following tests:

- *Saponification Value* (see *Fats and Fixed Oils* (401), *Saponification Value*): Between 185 and 200
- *Iodine Value* (see *Fats and Fixed Oils* (401), *Iodine Value*): Between 79 and 141
- *Unsaponifiable Matter* (see *Fats and Fixed Oils* (401), *Unsaponifiable Matter*): NMT 1.5%
- *Acid Value* (see *Fats and Fixed Oils* (401), *Acid Value (Free Fatty Acids)*): NMT 0.2
- *Peroxide Value* (see *Fats and Fixed Oils* (401), *Peroxide Value*): NMT 5.0
- *Water, Method Ic* (from *Water Determination* (921), *Method Ic (Coulometric Titration)*): NMT 0.1%
- *Limit of Copper, Iron, Lead, and Nickel*: [Note—The test for nickel is not required if the

oil has not been subjected to hydrogenation, or a nickel catalyst has not been used in processing.] Proceed as directed in *Fats and Fixed Oils* (401), *Trace Metals or Elemental Impurities—Procedures* (233). Meet the requirements in *Elemental Impurities—Limits* (232).

Synthetic mono- or diglycerides of fatty acids may be used provided they are liquid and remain clear when cooled to 10° and have an *Iodine Value* of NMT 140.

Added substances: Suitable substances may be added to preparations to increase stability or usefulness unless they are proscribed in the monograph. No coloring agent may be added to a preparation solely for the purpose of coloring the finished preparation (see *General Notices and Requirements 5.20* and *Antimicrobial Effectiveness Testing* (51)).

Observe special care in the choice and use of added substances in preparations with volumes that exceed 5 mL. The following limits prevail unless otherwise directed:

- Mercury and cationic surface-active agents: NMT 0.01%
- Chlorobutanol, cresol, phenol, and similar substances: NMT 0.5%
- Sulfur dioxide or an equivalent amount of sulfite, bisulfite, or metabisulfite of potassium or sodium: NMT 0.2%

ANTIMICROBIAL PRESERVATIVES

Antimicrobial agents must be added to preparations intended for injection that are packaged in multiple-dose containers unless one of the following conditions prevails: 1) there are different directions in the individual monograph; 2) the substance contains a radionuclide with a physical half-life of less than 24 h; or 3) the active ingredients are themselves antimicrobial. Substances must meet the requirements of (51) and *Antimicrobial Agents—Content* (341).

WATER CONTENT

The water content of freeze-dried (lyophilized) products should be determined when appropriate (see (921)).

BIOLOGICAL REACTIVITY

Implantable and combination drug products (drug/device) that contain a polymeric material should meet the requirements of *Biological Reactivity Tests, In Vivo* (88).

GLOBULE SIZE DISTRIBUTION

Emulsions should meet the requirements of *Globule Size Distribution in Lipid Injectable Emulsions* (729).

ALUMINUM CONTENT

Large-volume parenterals used to make total parenteral nutrition therapy (TPN) are limited to 25 µg/L. Small-volume parenterals and pharmacy bulk packages used to make TPN must state on the immediate container label the maximum level of aluminum at expiry if the maximum level exceeds 25 µg/L.

COMPLETENESS AND CLARITY OF SOLUTIONS

The following tests are performed to demonstrate suitability of constituted solutions prepared before administration. Constitute the solution as directed in the labeling supplied by the manufacturer:

- The solid dissolves completely, leaving no undissolved matter.
- The constituted solution is not significantly less clear than an equal volume of the diluent or of *Purified Water* contained in a similar vessel and examined similarly. Protein solutions may exhibit an inherent opalescence.

The constituted solution is free from particulate matter that can be observed on visual inspection (see <790>).

PRODUCT QUALITY TESTS FOR SPECIFIC PARENTERAL DOSAGE FORMS

Product quality tests for the specific dosage forms are listed below. Specific chapter(s) referenced for the test can be found in the *Universal Tests* and *Specific Tests* sections. When there is no compendial test available, a validated procedure with acceptance criteria should be used.

Solutions

A solution is a clear, homogeneous liquid dosage form that contains one or more chemical substances (e.g., drug substances or excipients) dissolved in a solvent (aqueous or nonaqueous) or a mixture of mutually miscible solvents. Solutions intended for parenteral administration (e.g., by injection or for irrigation) must be sterile and biocompatible with the intended administration site. This includes consideration of factors such as tonicity, pH, pyrogenicity, extraneous particulate matter, and physicochemical compatibility, among others. Unless otherwise justified, the following tests are required for solutions for injection:

- *Universal Tests*
- *Specific Tests*
 - *Physicochemical Properties*: Specific Gravity, Viscosity, pH, Osmolarity and Osmolality
 - *Antimicrobial Preservatives*

Sterile Powders for Solution

Sterile powders for solution (also referred to as sterile powders for injection) consist of drug substances and other components as dry-formulation ingredients to ensure the chemical and physical stability of the presentation within a final-use container. Companion sterile diluent or diluent compartments may be provided to facilitate constitution to the desired final volume. The sterile article for injection may be presented in several forms: lyophilized powder intended for final solution, powdered solids intended for final solution, or dry solids that form viscous liquids upon constitution.

The description should include a section that deals with ease of dispersion and reconstitution. The dosage form is a homogeneous solid that is readily constituted to the final form with the specified diluent, and dispersion is completed with gentle agitation.

Unless otherwise justified, the following tests apply to sterile powders for injection:

- *Universal Tests*
- *Specific Tests*
 - *Physicochemical Properties*: Specific Gravity, Viscosity, pH, Osmolarity and Osmolality

The following applies to constituted solutions:

- (905): To ensure the consistency of dosage units, each unit in a batch should have a drug substance content within a narrow range around the label claim. Dosage units are defined as dosage forms that contain a single dose or a part of a dose of drug substance in each unit. For liquid dosage forms, analysts should conduct the assay on an amount of well-mixed constituted material that is removed from an individual container under conditions of normal use, should express the results as delivered dose, and should calculate the acceptance value.
- *Loss on Drying* (731): The procedure set forth in this chapter determines the amount of volatile matter of any kind that is driven off under the conditions specified.
- (921): Water or solvent content may have important effects on reconstitution and stability. For articles that require water or solvent content control, analysts should perform one of the following methods or a suitable replacement.
- *Appearance*: Analysts should assess the level of and the unit variation for the following parameters:
 - Color of cake: Varies within target parameters
 - Texture and homogeneity of cake: Varies within target parameters
 - Presence of foreign material: All units with visible foreign material must be rejected
 - Particle size and distribution (dry powder): Evaluation of the powder solids for proper solid form and crystallinity is a measure of process control and consistency.
- *Particle Size Distribution Estimation by Analytical Sieving* (786): This chapter can be used for loose powders.

CRYSTALLINITY

The crystallinity of a material can be characterized to determine compliance with the crystallinity requirement where stated in the individual monograph of a drug substance.

- *Optical Microscopy* (776): Crystallinity can be characterized by polarized light microscopy for qualitative evaluation of the size, shape, and crystallinity of solids. Unless otherwise specified in the individual monograph, analysts should mount a solid specimen in mineral oil on a clean glass slide or cover slip and should examine the mixture using a polarizing microscope. The particles show birefringence (interference colors) and extinction positions when the microscope stage is revolved.

VEHICLES AND DILUENTS

Guidelines for constitution and suspension of dry powders are found in the specific monographs. If there is a specific packaged diluent for use with a particular product that is not included in a monograph, then the final article is prepared with that diluent.

Suspensions

Parenteral suspensions are liquid dosage forms that contain solid particles in a state of uniform dispersion. Suspensions for parenteral administration must be sterile and compatible with the administration site. Consideration should be given to pH and pyrogenicity, and appropriate limits should be identified. Physical stability evaluations of parenteral suspension preparations should include evaluations to confirm that the particle size range of suspended matter does not change with time and to confirm that the solids in the preparation can be readily resuspended to yield a uniform preparation.

In addition to the tests for injectable solutions, the following tests are required for suspensions for injection unless otherwise justified:

- *Universal Tests*
- *Specific Tests*
 - *Physicochemical Properties: pH*
 - *Uniformity of Dosage Units*
 - *Antimicrobial Preservatives*

Liposomes

Liposomes are unique drug products with unique properties that can be either solutions or suspensions. Liposomes are aqueous dispersions of amphiphilic lipids and have low water solubility. They are organized as a bilayer sheet that encloses an internal aqueous compartment and are known as lipid bilayer vesicles. Liposomes can have a single lipid bilayer (unilamellar vesicle) or can have an onion-like multilayered structure (multilamellar vesicle). The amphiphilic lipids comprise a hydrated head group at the water interface of the bilayer attached to a hydrophobic group that forms the interior of the bilayer by association with the hydrophobic group of lipids from the opposite leaflet of the bilayer. The physical properties of the liposome and its bilayer can vary widely and depend on lipid composition, aqueous composition, and temperature relative to the acyl components' phase transition points. Because of the central aqueous compartment, a simple test for the presence of liposomes in a lipid dispersion is to determine the presence of an entrapped aqueous phase.

A liposome drug product consists of the drug substance, liposome components, and other inactive but critical ingredients such as an aqueous dispersion unless the contents are a lyophilized product.

Unless otherwise justified, the following tests are required for liposomes:

- *Universal Tests*
- *Specific Tests*
 - *Physicochemical Properties: pH*
 - *Lipid and Fatty Acid Composition, including degree of unsaturation and positional specificity in acyl side chains and critical degradation products such as lysolipids¹*
 - *Particle Size¹*
 - *Particle Size Distribution of Liposomal Vesicles¹*
 - *Lamellarity¹*
 - *Phospholipid Composition¹*
 - *Percent Free vs. Percent Encapsulated Lipids¹*
 - *Free Drug vs. Encapsulated Drug*
 - *Ionic Strength and Osmotic Strength¹*

Sterile Powders for Suspension

Sterile powders for suspension consist of drug substances and other components as dry-formulation ingredients to ensure the chemical and physical stability of the presentation within a final-use container. Companion sterile diluent or diluent compartments may be provided to facilitate constitution to the desired final volume.

The sterile article for injection may be presented in several forms: lyophilized powder intended for final suspension, powdered solids intended for final suspension, and microparticles that retain their integrity and are delivered as a sterile suspension. The description should include a section that deals with ease of dispersion and reconstitution. The dosage form is a homogeneous solid that is readily constituted to the final form with the specified diluent, and dispersion is completed with gentle agitation.

Unless otherwise justified, the following tests apply to sterile powders for injection:

- *Universal Tests*
- *Specific Tests*
- *Bacterial Endotoxins*
 - *Physicochemical Properties: pH, Osmolarity and Osmolality*

The following applies to constituted suspensions:

- (905): To ensure the consistency of dosage units, each unit in a batch should have a drug substance content within a narrow range around the label claim. Dosage units are defined as dosage forms that contain a single dose or a part of a dose of drug substance in each unit. For liquid dosage forms, analysts should conduct the assay on an amount of well-mixed constituted material that is removed from an individual container under conditions of normal use, should express the results as delivered dose, and should calculate the acceptance value.
- (731): The procedure set forth in this chapter determines the amount of volatile matter of any kind that is driven off under the conditions specified.
- (921): Water or solvent content may have important effects on reconstitution and stability. For articles that require water or solvent content control, analysts should perform one of the following methods or a suitable replacement.
- *Appearance*: Analysts should assess the level of and the unit variation for the following parameters:
 - Color of cake: Varies within target parameters
 - Texture and homogeneity of cake: Varies within target parameters
 - Presence of foreign material: All units with visible foreign material must be rejected.
 - Particle size and distribution (dry powder): Evaluation of the powder solids for proper solid form and crystallinity is a measure of process control and consistency.
- (786): This chapter can be used for loose powders.

CRYSTALLINITY

The crystallinity of a material can be characterized to determine compliance with the crystallinity requirement where stated in the individual monograph of a drug substance.

- (776): Crystallinity can be characterized by polarized light microscopy for qualitative evaluation of the size, shape, and crystallinity of solids. Unless otherwise specified in the individual monograph, analysts should mount a solid specimen in mineral oil on a

clean glass slide or cover slip and should examine the mixture using a polarizing microscope: The particles show birefringence (interference colors) and extinction positions when the microscope stage is revolved.

VEHICLES AND DILUENTS

Guidelines for constitution and suspension of dry powders are found in the specific monographs. If there is a specific packaged diluent for use with a particular product that is not included in a monograph, then the final article is prepared with that diluent.

MICROPARTICLES

Some microparticles are provided as a sterile powder to be reconstituted as a suspension before injection. Major microparticle preparations are for reconstitution as a suspension for injection. For quality test requirements, please refer to *Implants*.

Emulsions

Emulsions for parenteral dosage forms are liquid preparations of drug substances dissolved or dispersed in a suitable emulsion medium. Oil-in-water or water-in-oil emulsions typically entrap the drug substance.

Emulsions typically are white, turbid, homogeneous liquid dosage forms that contain one or more chemical substances (e.g., drug substances and excipients) dissolved in a solvent (aqueous or nonaqueous) or mixture of mutually miscible solvents. Emulsions intended for intravenous administration must be sterile and must be compatible with the intended administration site.

Unless otherwise justified, the following tests are required for emulsions for injection:

- *Universal Tests*
- *Specific Tests*
 - *Physicochemical Properties*: pH, Osmolarity and Osmolality
 - *Globule Size Distribution*
 - Amount of Fat Globules (lipids)¹
 - Percent Free vs. Percent Encapsulated Lipids¹
 - Free Drug vs. Encapsulated Drug

Implants

Implants for extended release consist of a matrix of drug substance and polymeric excipient that may or may not have an outer rate-controlling membrane. The polymeric excipient must be biocompatible but may or may not be bioresorbable. Some implants are made from medical-grade metal with an osmotic pump inside that effects the extended release of the drug substance. Implants must be sterile and usually are formed in the shape of a cylinder, although other shapes are used. Solvents used to dissolve the formulation can lead to sterilization, and thus the internal sterility test method should demonstrate that the sample preparation does not lead to sterilization of the test sample.

Cylindrically shaped implants for systemic delivery usually are provided in an inserter for subcutaneous or local administration such as local ocular delivery. Implants also can be surgically implanted for local delivery, e.g., ocular delivery.

Unless otherwise justified, the following tests are required for implants:

- *Universal Tests*
- *Specific Tests*
 - *Uniformity of Dosage Units*

Depending on the drug product, the following tests may be required:

- *Water Content*

IN SITU GELS

Sterile in situ gels are liquid preparations that are intended for injection into specific therapeutic targets. Typically they consist of polymers in organic solvents, and upon injection the solvents migrate away from the site, leaving a gelled mass. The preparations may be injected as-is, upon reconstitution, from in situ formation, or from chemically initiated catalysis that results in the final form.

Unless otherwise justified, the following tests are required for in situ gels:

- *Universal Tests*
- *Specific Tests*
 - *Physicochemical Properties: Viscosity*
 - *Antimicrobial Preservatives*
 - *Catalyst Initiator Content and Activity¹*
 - *Residual Monomer¹*
 - *Time to Gel Formation¹*

MICROPARTICLES

Injectable, resorbable microparticles for extended release generally range from 20 to 100 µm in diameter. They consist of drug substances embedded within a biocompatible, bioresorbable polymeric excipient, e.g., polyester excipients. Microparticles are provided as a sterile powder in a vial or syringe.

Just before intramuscular or subcutaneous administration, the microparticle powder should be suspended in an aqueous injection vehicle (diluent). The injection vehicle usually consists of *Water for Injection*, surfactant, and a viscosity enhancer, and the vehicle may contain a compound that adjusts osmolality, e.g., a sugar with or without a compound that controls pH, e.g., an acid. The injection vehicle must be sterile and must be tested according to requirements for solutions that are intended for parenteral administration.

Unless otherwise justified, the following tests are required for microparticles for injection:

- *Universal Tests*
- *Specific Tests*
 - *Uniformity of Dosage Units*
 - *Water Content*
 - *Residual Solvents*

Depending on the drug product, the following tests may be required:

- *Particle Size Distribution*
- *Physicochemical Properties: pH, Osmolality and Osmolarity*

Drug-Eluting Stents

Drug-eluting stents are tiny metal or polymer scaffolds used to keep arteries open following a

medical intervention, in which a drug substance is incorporated into or onto the stent platform. Drug-eluting stents typically have two components of testing: 1) functional tests that generally are American Society for Testing and Materials (ASTM) International methods that fall outside the scope of this chapter, and 2) analytical tests.

Unless otherwise justified, the following tests are required for drug-eluting stents:

- *Universal Tests*
- *Specific Tests*
 - *Uniformity of Dosage Units*: The content of the active substance in the dosage form is applicable for drug-eluting stents packaged in single-unit containers. The test can be performed by either content uniformity or weight variation (see <905>). With appropriate justification, the number of stents needed for this test may be fewer than the recommended number of stents in <905>.
 - Biological Reactivity
 - Particulates
 - Coating Thickness(es)¹
 - Coating Robustness and Susceptibility to Cracking on Expansion¹
 - Free Drug vs. Encapsulated Drug¹
 - Morphology (surface and cross section)¹
 - Polydispersity¹
 - Copolymer Ratio¹
 - Glass Transition Point¹
 - Coating Adhesion to Stent Surface¹
 - Tissue Reaction Tests¹
 - Polymer Molecular Weight¹

PRODUCT PERFORMANCE TESTS

A performance test for injection and implanted products must have the ability to measure drug release from the manufactured pharmaceutical dosage forms. It must be reproducible and reliable, and although it is not a measure of bioavailability, the performance test must be capable of detecting changes in drug release characteristics from the finished product. These changes have the potential to alter the biological performance of the drug in the dosage form. They may be related to active or inactive/inert ingredients in the formulation, physical or chemical attributes of the finished formulation, manufacturing variables, shipping and storage effects, aging effects, and other formulation factors critical to the quality characteristics of the finished drug product.

Please refer to *The Dissolution Procedure: Development and Validation* <1092> while developing the drug release test, selecting the drug release medium, apparatus or procedure, and analytical method. Product performance tests can serve many useful purposes in product development and in post-approval drug product monitoring. They provide assurance of equivalent performance for products that have undergone post-approval raw material changes, relocation or change in manufacturing site, and other changes as detailed in the FDA SUPAC Guidances for Industry (SUPAC-IR, SUPAC-MR, and SUPAC-SS; available at www.fda.gov/cder/guidance). In this chapter, a USP performance test for injection and implanted products forms to support batch release is to be considered. ▲USP38

¹ No compendial test available; a validated procedure with acceptance criteria should be used.

BRIEFING

⟨2⟩ **Oral Drug Products—Product Quality Tests**, *USP 38* page 66 and *PF 38(4)* [Jul.–Aug. 2012]. There are five *USP* general chapters that link with *USP*'s taxonomy for pharmaceutical dosage forms: *Injections and Implanted Drug Products* ⟨1⟩, *Oral Drug Products—Product Quality Tests* ⟨2⟩, *Topical and Transdermal Drug Products—Product Quality Tests* ⟨3⟩, *Mucosal Drug Products—Product Quality Tests* ⟨4⟩, and *Inhalation and Nasal Drug Products—General Information and Product Quality Tests* ⟨5⟩. This taxonomy describes five routes of administration: injection or parenteral; gastrointestinal or oral; topical (dermal and transdermal); mucosal; and lungs or inhalation (for more information, see the *Stimuli* article titled *Product Quality and Product Performance General Chapters* in *PF 39(3)* [May–June 2013]). The five chapters provide critical product quality attributes for the various dosage forms by the various routes of administration. In addition to these five chapters, *Ophthalmic Ointments* ⟨771⟩ outlines the critical product quality attributes for the various dosage forms that can be applied to the eye. Product quality tests assess physical, chemical, and microbial attributes. Taken together, the tests help ensure identity, strength, quality, and purity of a dosage form. The General Chapters—Dosage Forms Expert Committee is proposing the following revision to ⟨1⟩, ⟨2⟩, ⟨3⟩, ⟨4⟩, ⟨5⟩, and ⟨771⟩, in this issue of *PF*, to clarify the applicability of these chapters.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCDF: A. Hernandez-Cardoso.)

Correspondence Number—C160057

Comment deadline: November 30, 2015

⟨2⟩ ORAL DRUG PRODUCTS—PRODUCT QUALITY TESTS

Change to read:

INTRODUCTION

Oral delivery is the most common route of administration for drug products. All oral drug products lead to systemic and/or local action in the oral cavity and/or gastrointestinal tract. Oral drug products fall into two main categories: solids and liquids. Solid oral drug products include but are not restricted to capsules, tablets, granules, and powders. Similarly, liquid oral drug products include but are not restricted to solutions, suspensions, and emulsions. The definitions and descriptions of these dosage forms and brief information about their composition and manufacturing process are found in *Pharmaceutical Dosage Forms* ⟨1151⟩. [Note—All references to chapters above ⟨1000⟩ are for informational purposes only, for use as a helpful resource. These chapters are not mandatory unless explicitly called out for application.] Chapter *Oral Drug Products—Product Quality Tests* ⟨2⟩ focuses on the product quality tests that are generally necessary for oral drug products for a single or combination of small molecules of active ingredients. Biologics in solid dosage forms are not considered. In this chapter, the terms “drug substance” and “active ingredient” are used interchangeably. The contents of this chapter do not necessarily apply to drug products that are intended for use other than by oral administration. For example, the chapter does not address oromucosal

dosage forms. Some of the tests indicated in this chapter may be performed on an in-process basis or omitted as routine tests based on process validation. However, the product must meet USP compendial requirements when sampled and tested, once the product is on the market.

~~Chapter (2) provides a framework to support new individual monographs; these are “moving forward” documents, and are not intended to move away from individual monographs (not replacing individual monographs).~~

■ **2S (USP39)**

~~Chapter (2) provides lists of consolidated common product quality test requirements in a concise and coherent fashion. If a monograph exists, it will contain all of the tests required for the dosage form. If a specific drug product monograph is missing (not in existence), the general chapter provides specific quality tests that are available to manufacturers as a resource until particular monographs needed for the dosage form are developed by USP.~~

■ The general chapter applies, in part or whole, when referenced in a drug product monograph. The general chapter includes the quality tests for the specific route of administration. The quality tests listed can be used as appropriate by manufacturers toward the development of new drug product monographs for submission to the USP. ■ **2S (USP39)**

If a validated performance test procedure is available for the specific drug product, it is identified in a general chapter below (1000). In cases where a validated procedure cannot be recommended, but if the information is available for a product quality and/or product performance test, it is described in an informational chapter above (1000).

Drug Product Quality Tests and Performance Tests

Monograph tests, analytical procedures, and acceptance criteria for testing oral drug products are divided into two categories: 1) those that assess general product quality attributes, and 2) those that assess product performance, which is a specific quality attribute typically linked to bioavailability and bioequivalence studies (see *Assessment of Drug Product Performance—Bioavailability, Bioequivalence, and Dissolution* (1090)). Drug product quality tests are intended to assess attributes such as identification, strength (assay), impurities (universal tests), dose content uniformity, pH, minimum fill, alcohol content, volatile content, and microbial content (specific tests). Drug product performance tests are designed to assess in vitro drug release from dosage forms, e.g., *Dissolution* (711) and *Drug Release* (724). For liquid oral drug products in solution, performance is considered optimal, and a monograph performance test is not included.

Each of these attributes is important for a primary understanding of the quality and performance of a drug product. Thus, they form the basis for the monograph. A compendial product should meet all drug product quality tests and drug product performance tests contained in its monograph.

[Note—Dissolution tests, specifically dissolution profile similarity between higher strengths and lower strengths of a given manufacturer’s product and dissolution profile similarity between the generic product and the reference product, are used for granting biowaivers. See (1090).]

PRODUCT QUALITY TESTS FOR ORAL DRUG PRODUCTS

Drug product quality tests for oral drug products fall into two categories: 1) universal tests that are applicable to all oral drug products and should be included in the monograph, and 2)

specific tests that should be considered for inclusion for specific types of oral products.

Universal Tests

Product quality attributes for oral dosage forms are important to ensure that commercialized products meet minimum quality requirements. Universal tests should be applied to all oral dosage forms and include *Description*, *Identification*, *Strength (Assay test)*, and *Impurities* (organic, inorganic, and residual solvents).

DESCRIPTION

Description is general in nature and is not a standard in itself. It communicates the appearance of an article that complies with monograph standards.

IDENTIFICATION

The identification test is defined in *General Notices*, 5.40 *Identity*. It is included in a monograph as an aid to confirm that the article contains the labeled drug substance by providing a positive identification of the drug substance or substances in a drug product.

One method of confirming the identity is to compare the retention time of the sample with that obtained for the standard injections in a chromatographic assay procedure. Other methods often used to orthogonally confirm the identity of the active ingredient are: *Thin-Layer Chromatographic Identification Test* (201), *Spectrophotometric Identification Tests* (197), *Nuclear Magnetic Resonance Spectroscopy* (761), *Near-Infrared Spectroscopy* (1119), and *Raman Spectroscopy* (1120), among others. The analytical procedure must be able to distinguish the active ingredient from all excipients that are present or from potential degradation products likely to be present. Care must also be taken to ensure that the chromatographic system separates the article from other closely related drug substances, impurities, and additives. Infrared and ultraviolet absorption also can be used for identification (see (197)), if the procedure has been demonstrated to be selective for the drug substance via an appropriate validation or verification study. The results of the identification test must be compared to the results obtained from a similarly prepared, suitable reference standard.

ASSAY

The assay is a specific and stability-indicating test to determine the potency (content) of the drug product. When a nonspecific assay (e.g., titration) is justified, other supporting analytical procedures should ensure that any interfering species can be detected. In general the *a priori* acceptance of $\pm 10\%$ variation in limits of a quality attribute (e.g., assay) from the target label claim (100%) in most cases is intended to account for manufacturing variability and shelf-life stability and is primarily based on the notion that such variation in a quality attribute is less likely to have any noticeable adverse impact on the desired clinical outcome. Acceptance criteria of 95.0%–105.0% are used with justification (e.g., for drug products with narrow therapeutic index). Activity assays and absolute content assays also are acceptable when justified.

IMPURITIES

Process impurities, synthetic by-products, and other inorganic and organic impurities may be

present in the drug substance and in the excipients used in the manufacture of the drug product. These impurities are limited by drug substance and excipient monographs. During product manufacture and over the shelf life of the product, degradation products can form. These can be a result of degradation of the drug substance or from interactions between the drug substance and excipient(s), among other factors. The procedures and acceptance criteria should specifically limit toxic materials. See specific requirements in the *General Notices 5.60 Impurities and Foreign Substances*, . [Note—For additional information, see *Impurities in Drug Substances and Drug Products* (1086).]

Specific Tests for Tablets

In addition to the *Universal Tests* described above, the following specific tests for tablets should be considered, depending upon the nature of the drug substance and formulation.

VOLATILE CONTENT

The test and the specific method depend on the nature of the article. Special consideration should be given to dosage forms for which water content has been shown to be a potential quality attribute and to products where solvent is used in the manufacture of the drug product. When the presence of moisture or other volatile material may become critical, analysts must determine the amount of unbound volatile solvents or volatile matter of any kind that is driven off by *Loss on Drying* (731) or another suitable technique (e.g., water activity). For substances that appear to contain water as the only volatile constituent, the procedure given in *Water Determination* (921) may be appropriate. For drug products, analysts also should consult *Residual Solvents* (467).

DISINTEGRATION

Disintegration is an essential attribute of oral solids, except for those intended to be chewed before being swallowed and for delayed- or extended-release products. This test measures the time it takes for the dosage unit to disintegrate in an aqueous medium and is described in detail in *Disintegration* (701). For certain dosage forms, e.g., effervescent tablets, disintegrating tablets, soluble tablets, and others, the *European Pharmacopoeia* describes the disintegration test in great detail. The disintegration test for some of the dosage forms in this chapter is included for completeness. For detailed procedures, please refer to (701) or the *European Pharmacopoeia*. The disintegration test, if included, is used only as a quality control test and not as a product performance test and should conform with the specifications in the monograph. Only when disintegration has been correlated with dissolution of a dosage form can a disintegration test be used as a product performance test (ICH Guidance Q6A, available at www.ich.org). In all other instances, a dissolution test should be considered as a product performance test.

TABLET FRIABILITY

The test procedure is applicable to most compressed, uncoated tablets. Friability determines the ability of tablets to withstand mechanical stresses and their resistance to chipping and surface abrasion. [Note—For additional information, see *Tablet Friability* (1216).]

TABLET BREAKING FORCE

Tablet breaking force measures the mechanical integrity of tablets, which is the force required to cause them to fail (i.e., break) in a specific plane. [Note—For additional information, see *Tablet Breaking Force* (1217).]

UNIFORMITY OF DOSAGE UNITS

Uniformity of dosage units must be demonstrated by either content uniformity or weight variation. Content uniformity is based on the assay of the individual content of drug substance(s) in a number of dosage units to determine whether the individual contents are sufficiently close to label claim. Weight variation can be used as an alternative to estimate content uniformity under certain conditions (see *Uniformity of Dosage Units* (905)).

Uncoated Tablets

Uncoated tablets include single-layer tablets that result from a single compression of particles and multilayer tablets that consist of concentric or parallel layers obtained by successive compression of particles of different composition. The excipients used generally are not specifically intended to modify the release of the active substance in the digestive fluids. Uncoated tablets include but are not limited to: effervescent tablets, buccal tablets, sublingual tablets, chewable tablets, disintegrating tablets, orally disintegrating tablets, boluses, soluble tablets, tablets for oral solution, and tablets for oral suspension. Boluses, large elongated tablets intended for administration to animals, should be considered uncoated tablets and must be in compliance with the product quality requirements. For uncoated tablets, disintegration should be tested as directed in (701).

BUCCAL, SUBLINGUAL, AND ORALLY DISINTEGRATING (ORODISPERSIBLE) TABLETS

These dosage forms will be discussed in a future new chapter that appears in this *PF, Mucosal Drug Products—Product Quality Tests* (4). They are listed here for informational purposes and completeness.

CHEWABLE TABLETS

Chewable tablets are not required to comply with the disintegration test. Chewable tablets (intact) should undergo dissolution testing, as a product performance test (if cited in the monograph), because they might be swallowed without proper chewing by a patient. In general, the dissolution test conditions for chewable tablets should be the same as for nonchewable tablets of the same active ingredient or moiety.

DISINTEGRATING OR DISPERSIBLE TABLETS

These are tablets intended to be dispersed in water before administration, giving a homogeneous dispersion.

Dispersion fineness: Place 2 tablets in 100 mL of water, and stir until completely dispersed. A smooth dispersion that passes through a No. 25 sieve (710 μm) is obtained.

TABLETS FOR ORAL SOLUTION AND TABLETS FOR ORAL SUSPENSION

Dispersion fineness: Place 2 tablets in 100 mL of water, and stir until completely dispersed. A smooth dispersion that passes through a No. 25 sieve (710 µm) is obtained.

Coated Tablets

Coated tablets are tablets covered with one or more layers of mixtures of various substances such as natural or synthetic resins, gums, gelatin, inactive and insoluble fillers, sugars, plasticizers, polyols, waxes, coloring matter authorized by the competent authority, and sometimes flavoring substances and active substances. Tablets coated by sugar or film include but are not limited to: plain coated tablets, extended-release tablets, and delayed-release tablets. A disintegration test, when applicable, should be performed as directed in <701>. There are no additional specific quality tests for extended-release tablets and delayed-release tablets. Universal quality tests should be applied to these products.

Specific Tests for Capsules

In addition to the *Universal Tests* described above, the following *Specific Tests for Capsules* should be considered, depending on the nature of the drug substance and formulation. Product quality tests that are considered specific to the type of capsule include those for volatile content (<731> and <921>). One-piece capsules typically are used to deliver a drug substance as a solution or suspension. Two-piece capsules consist of two telescoping cap-and-body pieces in a range of standard sizes and are used to deliver solid material as powder, granules, or small tablets. Modified-release capsules include but are not limited to: delayed-release capsules and extended-release capsules.

Disintegration: Proceed as directed in *Dissolution* <701>, *Soft Gelatin Capsules*, for one-piece capsules and *Dissolution* <701>, *Hard Gelatin Capsules* for two-piece capsules. Disintegration for modified-release capsules is described in great detail in the *European Pharmacopoeia*. There are no additional specific quality tests for extended-release capsules and delayed-release capsules. Universal quality tests should be applied to these products.

Specific Tests for Granules

In addition to the *Universal Tests* described above, the following *Specific Tests for Granules* should be considered, depending on the nature of the drug substance and formulation. Granules are solid dosage forms that are composed of agglomerations of smaller particles. Granules include but are not limited to: effervescent granules, coated granules, extended-release granules, and delayed-release granules. Tests that are considered specific to the type of granules include volatile content (<731> and <921>). Disintegration for effervescent granules is described in great detail in the *European Pharmacopoeia*. On the basis of the nature of the article and scientific criteria, additional tests may apply, including powder fineness and others.

Specific Tests for Powders

Oral powders should indicate: "For Oral Use Only". Tests that are considered specific to the

type of powders include: *Minimum Fill* (755) and volatile content ((731) and (921)). Chapter (755) has specifications that apply to oral powders.

On the basis of the nature of the article and scientific criteria, additional tests may apply, including pH in an aqueous solution, powder fineness, microbial limits, and others.

Specific Tests for Liquids

The recommended product quality tests for a liquid drug product include the *Universal Tests* described above and the specific tests included below. Most of the quality tests for liquids require the evaluation of single-dose products to estimate the quality attribute. Specific directions to perform the quality tests for either single-dose or multiple-dose products are provided in the monograph or the general chapter. For example, weight variation may be used when adequacy of mix for the active substance(s) and excipients in the blend is well controlled to ensure their uniform distribution, as in solutions.

DELIVERABLE VOLUME

When the liquid formulation is packaged in a multiple-dose container, compliance with *Deliverable Volume* (698) is required.

ALCOHOL DETERMINATION

If the liquid formulation contains a quantity of alcohol, *Alcohol Determination* (611) should be included. The limits may be an absolute concentration, in percentage, or relative to a labeled content.

PH

Liquid oral products typically are aqueous formulations that are susceptible to pH changes from exposure to atmospheric CO₂. The uptake of atmospheric CO₂ and pH change of oral liquid products is only relevant to aqueous-based products. Although it is less critical than in ophthalmic preparations, the pH of a liquid formulation can affect flavor and stability. The pH range as outlined in *pH* (791) is indicated in the monograph.

MICROBIAL CONTENT

The presence of certain microorganisms in nonsterile preparations may have the potential to reduce or even inactivate the therapeutic activity of the product and has a potential to adversely affect the health of the patient. Some liquid oral products can be subject to extreme microbiological control, and others require none. The needed microbial specification for a given liquid oral product depends on its formulation and use and is indicated in the monograph.

[Note—For additional information, see *Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use* (1111).]

ANTIOXIDANT

Release testing should be performed. Shelf-life testing may be unnecessary where justified by

development and stability data (ICH Guidance Q6A).

EXTRACTABLES

Where development and stability data show no significant evidence of extractables, elimination of this test may be proposed. Where data demonstrate the need and acceptance criteria for oral solutions—rubber stopper, cap liner, plastic bottle—data should be collected as early in the development process as possible (ICH Guidance Q6A).

Types of Liquid Dosage Forms

Specific quality tests for these dosage forms are provided in their respective monographs.

ORAL SOLUTIONS AND POWDERS AND GRANULES FOR SOLUTION

Tests of “for Solution” formulations are conducted on a well-mixed solution of the drug product constituted as described in the labeling.

EMULSIONS, SUSPENSIONS, AND POWDERS AND GRANULES FOR SUSPENSION

Tests of “for Suspension” formulations are conducted on a well-mixed suspension of the drug product constituted as described in the labeling. Product quality tests for suspensions should include a test of suspendability.

POWDERS AND GRANULES FOR SYRUPS AND POWDERS FOR ORAL DROPS

After dissolution or suspension, they comply with monograph requirements for the final dosage form. Volatile content (<731) and (<921) may be an additional quality test for powders and granules for reconstitution.

Specific Tests for Miscellaneous Oral Dosage Forms

LYOPHILIZED ORAL PRODUCTS

Chapter *Water Determination* (<921), *Method Ia*: Lyophilized oral products comply with the test. The limits are approved as indicated in the specific monograph.

BRIEFING

<3> **Topical and Transdermal Drug Products—Product Quality Tests**, *USP* 38 page 71 and *PF* 41(2) [Mar.–Apr. 2015]. There are five USP general chapters that link with USP’s taxonomy for pharmaceutical dosage forms: *Injections and Implanted Drug Products* (1), *Oral Drug Products—Product Quality Tests* (2), *Topical and Transdermal Drug Products—Product Quality Tests* (3), *Mucosal Drug Products—Product Quality Tests* (4), and *Inhalation and Nasal Drug Products—General Information and Product Quality Tests* (5). This taxonomy describes five routes of administration: injection or parenteral; gastrointestinal or oral; topical (dermal and transdermal); mucosal; and lungs or inhalation (for more information, see the *Stimuli* article titled *Product Quality and Product Performance General Chapters* in *PF* 39(3) [May–June 2013]). The five chapters provide critical product quality attributes for the various dosage

forms by the various routes of administration. In addition to these five chapters, *Ophthalmic Ointments* (771) outlines the critical product quality attributes for the various dosage forms that can be applied to the eye. Product quality tests assess physical, chemical, and microbial attributes. Taken together, the tests help ensure identity, strength, quality, and purity of a dosage form. The General Chapters—Dosage Forms Expert Committee is proposing the following revision to (1), (2), (3), (4), (5), and (771), in this issue of *PF*, to clarify the applicability of these chapters.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCDF: M. Marques.)

Correspondence Number—C160868; C148324

Comment deadline: November 30, 2015

(3) TOPICAL AND TRANSDERMAL DRUG PRODUCTS—PRODUCT QUALITY TESTS

Change to read:

INTRODUCTION

Topically applied drug products fall into two general categories: those applied to achieve local action and those applied to achieve systemic effects after absorption through the skin into the blood circulation. Local action can occur at or on the surface of the application site (e.g., stratum corneum-ocular epithelium ■ 2S (USP39)); in the underlying tissues (e.g., epidermis and/or dermis); and on ■ in ■ 2S (USP39) subcutaneous tissues (e.g., muscle or joint). Topically applied drug products include, but are not limited to, creams, gels, ointments, pastes, suspensions, lotions, foams, sprays, aerosols, solutions, and transdermal delivery systems (TDS, also known as patches ■ 2S (USP39)). The definitions and descriptions of these dosage forms, as well as brief information on their composition and/or manufacturing processes, can be found in *Pharmaceutical Dosage Forms* (1151).

Procedures and acceptable criteria for testing topically applied drug products can be divided into those that assess general product quality attributes and those that assess product performance. The product quality attributes include the following: description, identification, assay (strength), impurities, physicochemical properties, uniformity of dosage units, water content, pH, apparent viscosity, microbial limits, antimicrobial preservative content, antioxidant content, sterility (if applicable), and other tests that may be product specific. Product performance testing assesses drug release and other attributes that affect drug release from the finished dosage form.

■ Chapter *Topical and Transdermal Drug Products—Product Quality Tests* (3) provides lists of consolidated common product quality test requirements in a concise and coherent fashion. This chapter applies, in whole or in part, when referenced in a drug product monograph and includes the quality tests for the specific route of administration. The quality tests listed can be used as appropriate by manufacturers toward the development of new drug product monographs for submission to *USP*.

■ 2S (USP39)

Although most topically applied drug products are semisolids, liquids, or suspensions, TDS are physical devices that are applied to the skin and vary in their composition and method of fabrication. ■ ■2S (USP39) TDS release their active ingredients by different mechanisms. They can be passive or active. This chapter covers only the tests related to passive TDS.

Change to read:

**PRODUCT QUALITY TESTS FOR TOPICALLY APPLIED ■ TOPICAL AND TRANSDERMAL
■2S (USP39) DRUG PRODUCTS**

Universal Tests

Universal tests (see *ICH Guidance Q6A—Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances*, available at www.ich.org) are listed as follows and are applicable to all topically applied drug products.

DESCRIPTION

A qualitative description of the drug product should be provided. The acceptance criteria should include the final acceptable appearance of the finished dosage form and packaging. A visual examination should identify changes in color, adhesive migration (i.e., cold flow; see *Cold Flow Test*) for TDS, separations, crystallization, and others that are specific to the drug product. The description should specify the content or the label claim of the article. ■ For TDS, a visual examination should also be done to assess potential use issues with the product. The examination should include an assessment of the difficulty of removing the TDS from the pouch (e.g., due to adhesive migration adhering the system to the pouch); inability to remove the TDS from the pouch without damage to the system; and adhesive residue remaining on the pouch after removal of the TDS. ■2S (USP39) This is not a compendial test but is part of the manufacturer's specification for the drug product.

IDENTIFICATION

Identification tests are discussed in *General Notices and Requirements, Monograph Components, Identity 5.40*. Identification tests should establish the identity of the drug or drugs present in the article and should discriminate between compounds of closely related structures that are likely to be present. Identity tests should be specific for the drug substance(s) (e.g., infrared spectroscopy). Near-infrared (NIR) or Raman spectrophotometric methods also could be acceptable for the identification of the drug product (for additional information, see *Near-Infrared Spectroscopy* (1119) and *Raman Spectroscopy* (1120)). Identification solely by a single chromatographic retention time is not specific.

ASSAY

A specific and stability-indicating test should be used to determine the strength (content) of the drug product. ■ This assay requirement can be satisfied for topical products containing antibiotics by a standard microbiological method (see *Antibiotics—Microbial Assays* (81)). ■2S (USP39) In cases when the use of a nonspecific assay (e.g., *Titrimetry* (541)) is justified, other supporting analytical procedures should be used to achieve overall specificity.

IMPURITIES

Process impurities, synthetic byproducts, impurities associated with the adhesive (e.g., residual monomers), residual solvents (see *Residual Solvents* (467)), heavy metals (see *Heavy Metals* (231) (Official 1-Dec-2015)), and other inorganic and organic impurities may be present in the drug substance and in the excipients used in the manufacture of the drug product and should be assessed and controlled. Impurities arising from the degradation of the drug substance and those arising during the manufacturing process of the drug product also should be assessed and controlled.

Specific Tests

In addition to the *Universal Tests* listed previously, the following *Specific Tests* should be considered on a case-by-case basis:

UNIFORMITY OF DOSAGE UNITS

This test is applicable for TDS and for ■ topical ■ 2S (USP39) dosage forms packaged in single-dose ■ unit ■ 2S (USP39) containers, ■ such as sachets/packets ■ 2S (USP39) (see *Uniformity of Dosage Units* (905)).

WATER CONTENT

A test for water content should be included when appropriate (see *Water Determination* (921)). This test is generally formulation dependent. Therefore, it is not included in the compendial drug product monograph but is part of the manufacturer's specification for the drug product.

MICROBIAL LIMITS

Microbial examination of nonsterile drug products is performed according to the methods given in *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* (61) and *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* (62), unless the formulation itself is demonstrated to have antimicrobial properties. Acceptance criteria for nonsterile pharmaceutical products based on total aerobic microbial count and total combined yeasts and molds count are given in *Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use* (1111).

ANTIMICROBIAL PRESERVATIVE CONTENT

Acceptance criteria for antimicrobial preservative content in ■ multidose ■ multi-unit ■ 2S (USP39) products should be established. They should be based on the levels of antimicrobial preservative necessary to maintain the product's microbiological quality at all stages throughout its proposed usage and shelf life (see *Antimicrobial Effectiveness Testing* (51)).

ANTIOXIDANT CONTENT

If antioxidants are present in the drug product, tests of their content should be established unless oxidative degradation can be detected by another test method such as impurity testing. Acceptance criteria for antioxidant content should be established. They should be based on the levels of antioxidant necessary to maintain the product's stability at all stages throughout the product's proposed usage and shelf life.

STERILITY

Depending on the use of the dosage form (e.g., ~~ophthalmic preparations,~~ ^{■ 2S (USP39)} products that will be applied to open wounds or burned areas), sterility of the product should be demonstrated as appropriate (see *Sterility Tests* (71)).

PH

When applicable, topically applied drug products should be tested for pH. ~~at the time of batch release and at designated stability time points for batch-to-batch monitoring.~~ ^{■ 2S (USP39)} Because some topically applied drug products contain very limited quantities of water or aqueous phase, pH measurements may not always be warranted. This test is generally formulation dependent. Therefore, it is not included in the compendial drug product monograph but is part of the manufacturer's specification for the drug product.

PARTICLE SIZE

■ When the finished product contains a suspended solid drug substance, the product should be examined for particle size. ^{■ 2S (USP39)} The particle size of the active drug substance(s) in topically applied drug products is usually ~~determined~~ ^{■ established} ^{■ 2S (USP39)} and controlled at the formulation development stage. However, topically applied drug products should be examined for evidence of particle ^{■ size} ^{■ 2S (USP39)} alteration (i.e., ^{■ drug polymorphic form,} ^{■ 2S (USP39)} ~~appearance of particles, changes in particle form,~~ ^{■ 2S (USP39)} ~~size, shape, habit, or~~ ^{■ morphology, agglomeration, or} ^{■ 2S (USP39)} ~~aggregation~~) of the drug substance that may occur during the course of product processing and storage. ~~Such examinations should be conducted at the time of batch release and at designated stability test time points for batch-to-batch monitoring because changes that are visually (macro and microscopically) observable would likely compromise the integrity and/or performance of the drug product.~~ ^{■ 2S (USP39)} These types of tests are generally formulation dependent. Therefore, such tests are not included in compendial monographs but are part of the manufacturer's specification for the drug product.

CRYSTAL FORMATION

When ^{■ the drug substance is dissolved in} ^{■ 2S (USP39)} ~~the finished product, does not contain a suspended active drug substance but rather contains a dissolved active drug substance~~ ^{■ 2S (USP39)} the product should be ^{■ microscopically} ^{■ 2S (USP39)} ~~examined for evidence of crystal formation of the active drug substance. Such examinations should be done microscopically and conducted at the time of batch release and at designated stability test time points.~~ ^{■ 2S (USP39)} This test is generally formulation dependent. Therefore, it is not included in the compendial drug product monograph but is part of the manufacturer's specification for the drug product. It is recommended that the potential for the drug product to form crystals of drug substance be examined during product development using conditions of stress. ^{■ 2S (USP39)}

Change to read:

SPECIFIC TESTS FOR OPHTHALMIC DRUG PRODUCTS

~~Ophthalmic dosage forms must meet the requirements of *Sterility Tests* (71). If the specific ingredients used in the formulation do not lend themselves to routine sterilization techniques,~~

ingredients that meet the sterility requirements described in (71), along with aseptic manufacture, may be used. Multiple-use ophthalmic preparations must contain a suitable substance or mixture of substances to prevent growth of, or to destroy, microorganisms accidentally introduced during the use of the product (see *Added Substances in Ophthalmic Ointments* (771)), unless otherwise directed in the individual monograph or unless the formula itself is bacteriostatic and/or the delivery system promotes bacteriostasis. The finished ophthalmic preparation must be free from large particles and must meet the requirements for *Leakage* and for *Metal Particles* in (771). The immediate containers for ophthalmic preparations shall be sterile at the time of filling and closing. It is mandatory that the immediate containers for ophthalmic preparations be sealed and tamper proof so that sterility is ensured at the time of first use.

■ See *Ophthalmic Ointments* (771). ■ 2S (USP39)

Change to read:

SPECIFIC TESTS FOR TOPICALLY APPLIED SEMISOLID DRUG PRODUCTS

■

Minimum Fill

This test applies only to multiple-dose containers, such as tubes and jars (see *Minimum Fill* (755)). ■ Single- and multiple-unit containers must meet minimum fill requirements as established by testing described in *Minimum Fill* (755). ■ 2S (USP39) For single-dose ■ unit ■ 2S (USP39) containers, such as sachets, see chapter ■ where the test for ■ 2S (USP39) (905) is applied, the test for (755) is not required. ■ 2S (USP39)

Apparent Viscosity

Viscosity is a measure of a formulation's resistance to flow and is an assessment of the rheological properties of the dosage form (e.g., semisolid dosage form). Because only Newtonian fluids possess a measurable viscosity that is independent of shear rate, semisolid pharmaceutical dosage forms that are non-Newtonian products exhibit an apparent viscosity. ■ of a rheological property of a semisolid dosage form. The term "apparent viscosity" applies to non-Newtonian fluids, which comprise the majority of semisolid pharmaceutical dosage forms. ■ 2S (USP39)

The apparent viscosity of semisolid drug products should be tested at the time of batch release and initially at designated stability test time points to set specifications for batch-to-batch and shelf-life monitoring. ■ ■ 2S (USP39) Measurement procedures should be developed as outlined in • *Viscosity—Capillary Methods* (911) • (CN 1-May-2015) , ■ *Viscosity—Rotational Methods* (912), and *Viscosity—Rolling Ball Method* (913). ■ 2S (USP39) For semisolids that show thixotropy and/or irreversible changes in viscosity after shearing, specific attention should be given to sample preparation procedures to minimize variability in the measurement of apparent viscosity caused by variable shear histories (e.g., mixing speed and temperature, filling operation, and sample handling). Furthermore, for some products it may be warranted to have apparent viscosity specifications at more than ■ one stage of the manufacturing process or with more than ■ 2S (USP39) one set of ■ test ■ 2S (USP39) conditions (e.g., bulk in-process stage, final packaged

product, high and low shear rates, and different temperatures).

~~Apparent viscosity specifications based on data obtained during product development and shelf-life testing should be established for batch release and throughout the proposed shelf life.~~

~~■ An acceptable range for apparent viscosity should be established based on data obtained during product development and based on statistical assessment of multiple product batches over the product's shelf life. ■ 2S (USP39) The apparent viscosity test is formulation and/or process dependent. Therefore, it is not included in compendial drug product monographs but is part of the manufacturer's specification for the drug product. Furthermore, the specifications for apparent viscosity of semisolid dosage forms at batch release and during stability testing may be different. Although the apparent viscosity of the finished drug product at the time of batch release must conform to the product development specifications, for stability testing, the apparent viscosity specifications for the drug product should be based on statistical assessment of the product over its shelf life. ■ 2S (USP39)~~

■ Apart from single-point viscosity measurements, more advanced rheological techniques (flow, oscillatory, creep, and stress relaxation testing) can be applied to develop a mechanistic understanding of a formulation and its structure. These techniques may be useful for product development using the principles of quality by design or for comparative physicochemical characterization of the test and reference formulations in the case of a biowaiver argument in a generic drug application. However, these techniques are not generally suitable for routine quality testing. Common parameters derived from rheological testing of semisolid pharmaceutical dosage forms that may be useful for characterization and comparison are the storage modulus, loss modulus, relaxation modulus, compliance, thixotropic index, and yield stress. Acceptance criteria are product specific and defined to ensure that the apparent viscosity of each batch of semisolid drug product is within the range defined by the product design and is consistent between batches based on the product development specifications and statistical assessment of multiple product batches over the product's shelf life. ■ 2S (USP39)

Uniformity in Containers

Typically applied semisolid drug products may show physical separation during manufacturing processes and during their shelf life. To ensure the integrity of the drug product, it is essential to evaluate the uniformity of the finished product. ~~at the time of batch release and throughout its assigned shelf life.~~ ■ This test applies only to multiple-dose ■ unit ■ 2S (USP39) containers, such as tubes and jars. ~~For single-dose containers, such as sachets, see chapter *Uniformity of Dosage Units* (905).~~ ■ 2S (USP39) This test does not apply to more fluid topical drug products in multiple-unit containers, such as emulsions, lotions, two-phase gels, or topical suspensions, in which the labeling directs the user to mix the product (e.g., shake well) before use. ■ 2S (USP39)

PRODUCTS PACKAGED IN TUBES

~~Within-tube content uniformity can be assessed in the following manner.~~

~~Carefully remove or cut off the bottom tube seal and make a vertical cut from the bottom to the top of the tube. Carefully cut the tube around the upper rim, open the two flaps, and lay the flaps open to expose the product.~~

~~Inspect the product visually for the presence of phase separation, change in physical appearance and texture, and other properties described in the product test for *Description*. If~~

~~there is no observable phase separation or change in physical appearance and texture, and if the product meets the *Description* acceptance criteria, proceed as described in the following sections. If the product exhibits phase separation and/or change in physical appearance or texture, the product fails the tube content uniformity test.~~

~~The procedures described as follows can be modified depending on the sensitivity of the quantitative procedure used to determine the amount of the drug substance(s) present in the formulation.~~

~~For multiple-dose products that contain 5 g or more~~

~~Procedure 1~~

- ~~1. Using a single tube, after visually inspecting the product, remove an appropriate amount of product from the top, middle, and bottom portions of the tube. The sample size should be sufficient for at least one quantitative determination of the active ingredient(s). Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results using *Acceptance criteria A*.~~
- ~~2. If the product fails *Acceptance criteria A*, test three additional tubes from the same batch following step 1 described above, and evaluate all 12 test results using *Acceptance criteria B*.~~

~~Procedure 2~~

- ~~1. Using two tubes, after visually inspecting the product, remove an appropriate amount of product from the top, middle, and bottom portions of each tube. The sample size should be sufficient for at least one quantitative determination of the active ingredient(s). Determine the amount of the active ingredient(s) in each portion of the tube using any appropriate validated quantitative procedure, and evaluate the test results using *Acceptance criteria A*.~~
- ~~2. If the product fails *Acceptance criteria A*, test two additional tubes from the same batch following step 1 described above, and evaluate all 12 test results using *Acceptance criteria B*.~~

~~For multiple-dose products that contain less than 5 g of product~~

- ~~1. Test the top and bottom portions of two tubes using *Procedure 1* or *Procedure 2* as described above. Evaluate the test results using *Acceptance criteria A*.~~
- ~~2. If the product fails *Acceptance criteria A*, test two additional tubes from the same batch following step 1 described above, and evaluate all eight test results using *Acceptance criteria B*.~~

~~Tube (container) content uniformity test acceptance criteria:~~ ~~In determining the relative standard deviation (RSD) from multiple tubes, first determine the variance from the three measurements for each tube and average across the tubes. The RSD is calculated using this average variance.~~

~~*Acceptance criteria A*—All results are within the product assay range, and the RSD is NMT 6% or as specified in the product specification or in the compendial monograph. If the RSD is greater than 6%, use *Acceptance Criteria B*.~~

~~Acceptance criteria B—All results are within the product assay range, and the RSD of the 12 assay results is NMT 6% or as specified in the product specification or in the compendial monograph.~~

■

VISUAL UNIFORMITY

Carefully remove or cut off the bottom tube seal and make a vertical cut from the bottom to the top of the tube. Carefully cut the tube around the upper rim, open the two flaps, and lay the flaps open to expose the product. Repeat this procedure for a total of three tubes. ■

■_{2S (USP39)}

Inspect the product visually for the presence of phase separation, change in physical appearance and texture (e.g., color change, crystallization, lumping), and other properties described in the product specification for *Description*. If there is no observable ■ significant ■_{2S (USP39)} phase separation or significant ■ ■_{2S (USP39)} change in physical appearance and texture, and if the product meets the *Description* criteria, the product passes the test. If the product exhibits ■ significant ■_{2S (USP39)} phase separation and significant ■ or ■_{2S (USP39)} change in physical appearance or texture, the product fails the test.

UNIFORMITY OF ACTIVE INGREDIENT(S)

The following procedures can be modified depending on the sensitivity of the quantitative procedure used to determine the amount of the drug substance(s) present in the formulation.

For multiple-dose ■ unit ■_{2S (USP39)} tubes that contain 5 g or more of product:

Stage 1:

1. Using three tubes ■ a single tube, ■_{2S (USP39)} after performing the test for *Visual Uniformity*, remove an appropriate amount of the product from the top (i.e., cap end), middle, and bottom (i.e., seal end) portions of the tube. The sample size should be sufficient for at least one quantitative determination of the active ingredient(s) ■ and should not exceed the maximum dose recommended by the product labeling for a single application. ■_{2S (USP39)}
2. Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the single tube using the *Stage 1* acceptance criteria outlined in number 3.
3. The *Stage 1* acceptance criteria are met if:
 - None of the nine results (i.e., three each from three tubes) are outside of the product assay range by $\pm 5.0\%$ (e.g., if the product assay range is 90.0%–120.0%, the range will be 85.0%–125.0%), ■ three results are outside of the product assay range, ■_{2S (USP39)} and
 - The maximum difference in the amount of active ingredient(s) determined within each tube, for each of the three tubes tested, is NMT 10.0%. For example, if the three measurements within a tube are 87.0%, 95.2%, and 89.7%, the maximum difference would be 8.2% (i.e., $95.2\% - 87.0\% = 8.2\%$). ■ the tube is NMT 10.0%. For example, if the three measurements within the tube are 97.0%, 95.2%, and 99.7%, the maximum difference would be 4.5% (i.e., $99.7\% - 95.2\% = 4.5\%$). ■_{2S (USP39)}
4. Proceed to *Stage 2* testing if NMT one of the nine test results is outside of the product assay range by $\pm 5.0\%$, and none are outside the product assay range by $\pm 15.0\%$, and

the maximum difference of the amount of active ingredient(s) measured within each tube is NMT 15.0%, none of the test results are outside the product assay range by NMT 5.0% (e.g., if the product assay range is 90.0%–120%, the range will be 85.0%–125.0%), and the maximum difference in the amount of active ingredient(s) measured within the tube is NMT 10.0%.

5. Proceed to *Stage 3* testing if NMT one of the three test results is outside of the product assay range by $\pm 5.0\%$, none are outside the product assay range by $\pm 15.0\%$, and the maximum difference of the amount of active ingredient(s) measured within the tube is NMT 15.0%.

■ 2S (USP39)

Stage 2:

1. Test an additional seven ■ two ■ 2S (USP39) tubes for *Visual Uniformity* and *Uniformity of Active Ingredient(s)* for a total of three samples from 10 ■ each from three ■ 2S (USP39) tubes.
2. ■ Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the three tubes using the *Stage 2* acceptance criteria. ■ 2S (USP39)
3. *Stage 2* acceptance criteria are met if:
 - The *Visual Uniformity* test is met for all tubes;
 - 29 of 30 test results are within the product assay range by $\pm 5.0\%$, and none are outside of the product assay range by $\pm 15.0\%$; and ■ None of the nine results (i.e., three each from three tubes) are outside of the product assay range by NMT 5.0%, and ■ 2S (USP39)
 - The maximum difference of the amount of active ingredient(s) measured within each tube, for each of the 10 tubes tested, is NMT 15.0% ■ samples tested, is NMT 10.0%.
4. Proceed to *Stage 3* testing if NMT one of the nine test results is outside of the product assay range by $\pm 5.0\%$, none are outside the product assay range by $\pm 15.0\%$, and the maximum difference of the amount of active ingredient(s) measured within each tube is NMT 15.0%.

Stage 3:

1. Test an additional seven tubes for *Visual Uniformity* and *Uniformity of Active Ingredient(s)* for a total of three samples each from 10 tubes.
2. Determine the amount of active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the 10 tubes using the *Stage 3* acceptance criteria as outlined in number 3..
3. *Stage 3* acceptance criteria are met if:
 - The *Visual Uniformity* test is met for all tubes; and
 - 29 of the 30 test results are within the product assay range by $\pm 5.0\%$, and none are outside of the product assay range by $\pm 15.0\%$; and
 - The maximum difference of the amount of active ingredient(s) measured within each tube, for each of the 10 tubes tested, is NMT 15.0%.

■ 2S (USP39)

For multiple-dose ■ unit ■_{2S} (USP39) tubes that contain less than 5 g of product:

Stage 1:

1. Using ~~three tubes~~ ■ a single tube, ■_{2S} (USP39) after performing the test for *Visual Uniformity*, remove an appropriate amount of product from the top (i.e., cap end) and bottom (i.e., seal end) portions of the tube. The sample size should be sufficient for at least one quantitative determination of the active ingredient(s) ■ and should not exceed the maximum dose recommended by the product labeling for a single application. ■_{2S} (USP39)
2. Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the tube using the *Stage 1* acceptance criteria outlined in number 3.
3. *Stage 1* acceptance criteria are met if:
 - ~~None of the six test results (i.e., two each from three tubes) are outside of the product assay range by ±5.0% (e.g., if the product assay range is 90.0%–120.0%, the product assay range ±5% is 85.0%–125.0%), and~~ ■ Neither result is outside of the product assay range; and ■_{2S} (USP39)
 - The difference between the amount of active ingredient(s) determined for the two samples within ~~each tube, for each of the three tubes~~ ■ the tube ■_{2S} (USP39) tested is NMT 10.0%. For example, if the two measurements within a tube were 95.2% and 89.7%, the difference would be 5.5%.
4. Proceed to *Stage 2* testing if ~~NMT one of the six tests results is outside of the product assay range by ±5.0%, and none are outside of the product assay range by ±15.0%, and the maximum difference of the amount of active ingredient(s) measured within each tube is NMT 15.0%~~ ■ neither of the test results are outside the product assay range by ±5.0% (e.g., if the product assay range is 90.0%–120.0%, the range will be 85.0%–125.0%), and the difference between the amounts of active ingredient(s) measured within the tube is NMT 10.0%.
5. Proceed to *Stage 3* testing if NMT one of the test results is outside of the product assay range by ±5.0%, and none are outside the product assay range by ±15.0%, and the difference between the amounts of active ingredient(s) measured within the tube is NMT 15.0%. ■_{2S} (USP39)

Stage 2:

1. Test an additional ~~seven~~ ■ two ■_{2S} (USP39) tubes for *Visual Uniformity* and *Uniformity of Active Ingredient(s)* for a total of two samples each from ~~±0~~ ■ three ■_{2S} (USP39) tubes ■
2. Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the three tubes using the *Stage 2* acceptance criteria as outlined in number 3. ■_{2S} (USP39)
3. *Stage 2* acceptance criteria are met if:
 - The *Visual Uniformity* test is met for all tubes; and
 - ~~19 of 20 test results are within the product assay range by ±5.0%, and none are outside of the product assay range by ±15.0%; and~~ ■ None of the six test results (i.e., two each from three tubes) are outside of the product assay range by ±5.0%; and ■_{2S} (USP39)

- The difference between the amount of active ingredient(s) determined for the two samples within each tube, for each of the ~~10 tubes tested~~, is NMT 15.0% ■
three tubes tested is NMT 10.0%.
4. Proceed to *Stage 3* testing if NMT one of the six test results is outside of the product assay range by $\pm 5.0\%$, none are outside of the product assay range by $\pm 15.0\%$, and the difference between the amounts of active ingredient(s) measured within each tube is NMT 15.0%.

Stage 3:

1. Test an additional 7 tubes for *Visual Uniformity* and *Uniformity of Active Ingredient(s)* for a total of 20 samples, 2 samples each from 10 tubes.
2. Determine the amount of active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the 10 tubes using the *Stage 3* acceptance criteria as outlined in number 3.
3. *Stage 3* acceptance criteria are met if:
 - The *Visual Uniformity* test is met for all tubes; and
 - 19 of 20 test results are within $\pm 5.0\%$ of the product assay range, and none are outside of the product assay range by $\pm 15.0\%$; and
 - The difference between the amount of active ingredient(s) determined for the two samples within each tube, for each of the 10 tubes tested, is NMT 15.0%.

■ 2S (USP39)

■ 2S (USP39)

PRODUCTS PACKAGED IN CONTAINERS OTHER THAN TUBES

For semisolid products packaged in a container other than a tube when the sampling method presented previously cannot be used, other sampling methods are acceptable, such as this method described for a jar:

1. Select a suitable syringe of sufficient length to extend to the bottom of the container.
2. Remove and set aside the syringe plunger, and cut off the bottom of the syringe barrel. Sampling should take place from a location to the left or right of the mid-line of the jar surface to preserve an undisturbed region on the other side for any additional investigation (see *Figure 1*).

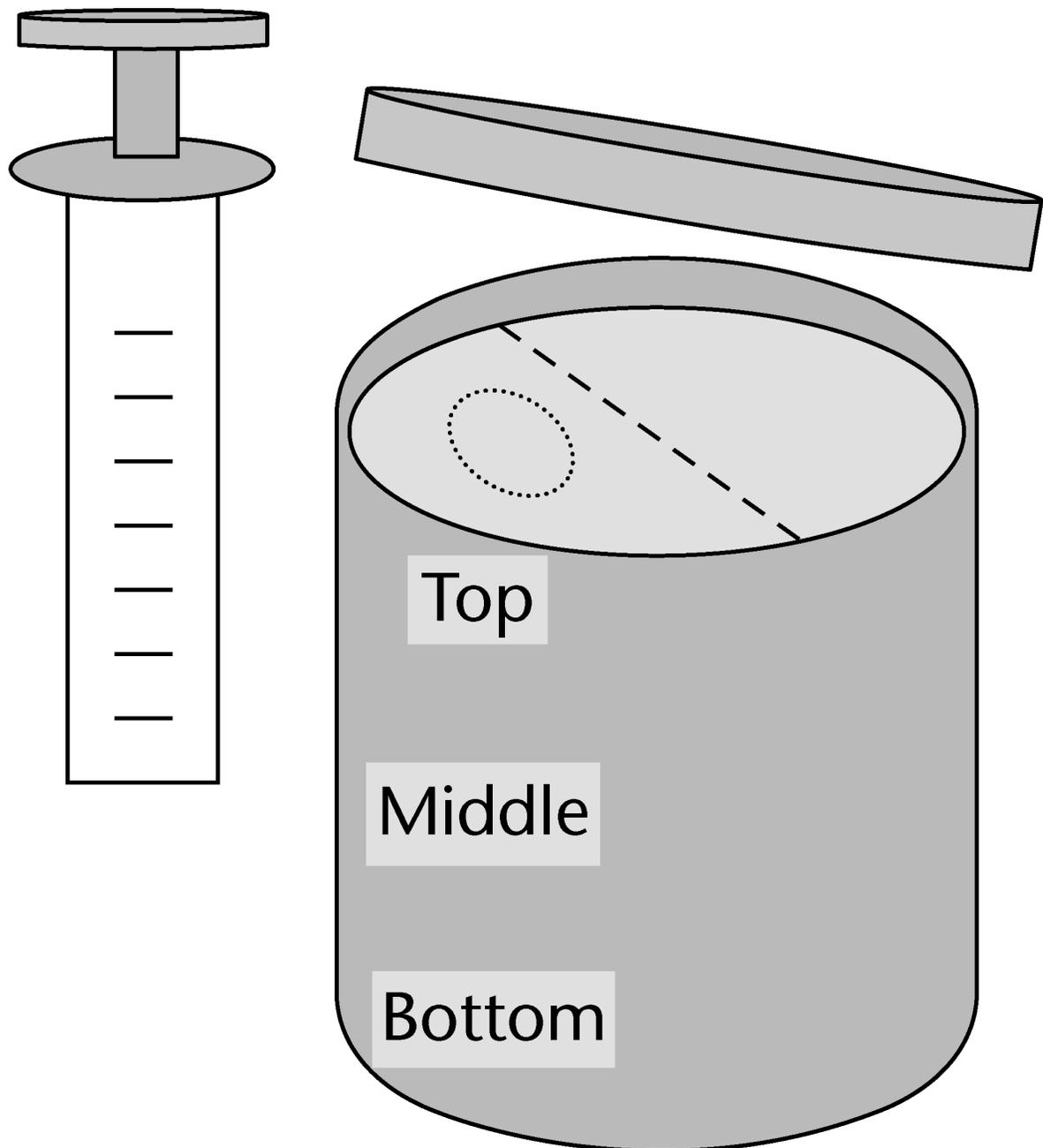


Figure 1. Sampling from a jar container.

3. Slowly push the syringe barrel into the container until it reaches the bottom. Then twist the syringe barrel containing the sample core, and remove the syringe from the container.
4. Insert the syringe plunger into the barrel, and carefully extrude the sample core onto a clean surface in three equal portions to represent the top, middle, and bottom portions of the container.
5. Remove an appropriate sample representative of the top, middle, and bottom portions of the container samples, and test according to the instructions outlined in *Products Packaged in Tubes*.

Change to read:

SPECIFIC TESTS FOR TDS

~~TDS or patches~~ ■ TDS ■ 2S (USP39) are formulated with an adhesive layer to ensure intimate contact with the skin and allow the delivery of the desired dose of the drug. Adhesives in TDS must permit easy removal of the release liner before use, adhere properly to human skin upon application, maintain adhesion to the skin during the prescribed period of use, and permit easy removal of the TDS at the end of use without leaving a residue or causing damage to the skin or other undesirable effect(s). Additionally, adhesives must be able to maintain the performance of the TDS throughout the shelf life of the drug product.

~~Three types of TDS adhesion tests are generally used: peel adhesion test (from a standard substrate), release liner peel test, and tack test.~~

■ Testing of the physical properties of the TDS generally include peel adhesion, release liner peel, tack, cold flow, shear, and crystal formation (see *Crystal Formation*). The peel adhesion, release liner peel, and tack tests measure the adhesion properties of the TDS. Each of these tests measures the force required to separate the TDS from another surface. The cold flow and shear tests measure the cohesive properties of the TDS. These latter tests measure the resistance to flow of the adhesive matrix. ■ 2S (USP39)

Acceptance criteria are product specific and defined to ensure that adhesion of each batch of TDS is within the range defined by the product design and is consistent between batches based on the product development specifications or ■ and ■ 2S (USP39) statistical assessment of multiple product batches over the product's shelf life.

■ In addition to physical testing, this section also discusses the *Leak Test* applicable to form-fill-seal-type (reservoir or pouched) TDS. ■ 2S (USP39)

Peel Adhesion Test

This test measures the force required to remove (peel away) a TDS attached to a standard substrate surface (e.g., polished stainless steel). The TDS is applied to the substrate using specified techniques for application and is conditioned at a specified temperature and time. Then the TDS is peeled away from the substrate with an instrument that allows for control of the peel angle (e.g., 90° or 180°) and peel rate (e.g., 300 mm/min), and the peel force is recorded. This procedure is repeated using a minimum of five independent samples. The product fails the test if the mean peel force is outside the acceptable range determined during product development and/or ■ ■ 2S (USP39) based on statistical assessment of multiple product batches over the product's shelf life.

Release Liner Peel Test

This test measures the force required to separate the release liner from the adhesive layer of the TDS. The test is performed with a finished product sample. The test sample is conditioned using specific procedures (temperature and time). Then, the release liner is pulled away from the TDS with an instrument that allows for control of the peel angle (e.g., 90° or 180°) and peel rate (300 mm/min), and the peel force is recorded. This procedure is repeated using a minimum of five independent samples. The product fails the test if the mean peel force is outside the acceptable range determined during product development and/or ■ ■ 2S (USP39) based on statistical assessment of multiple product batches over the product's shelf life.

Tack Test

Several methods of tack tests have been developed. Examples include the *Probe Tack Method* and the *Rolling Ball Method*. It is up to the TDS manufacturer to decide which ~~one~~ **tack test** ~~is more~~ **most** ~~is~~ **2S (USP39)** appropriate for each drug product.

PROBE TACK METHOD

This test measures the force required to separate the tip of the test probe from the adhesive layer of the TDS. This test uses an instrument designed to create a bond between the tip of the stainless steel test probe (of defined geometry) and the TDS using a controlled force (light pressure) and specified test conditions (i.e., rate, contact time, contact pressure, and temperature). Then while controlling the rate of probe removal, the test measures the profile of force required to separate the probe tip from the TDS and the maximum force required to break the bond (tack). This procedure is repeated using a minimum of five independent samples. The product fails the test if the mean test result [force profile(s) and/or tack] is outside the acceptable range determined during product development and/or **2S (USP39)** based on statistical assessment of multiple product batches over the product's shelf life.

ROLLING BALL METHOD

This test measures the distance traveled by a defined ball, on the adhesive layer of the TDS under defined conditions, as a parameter dependent on the tack properties of the adhesive layer. This test uses a setup designed to roll a ball (of defined material, weight, size, and surface) from a ramp (of defined angle and length) onto the adhesive layer (with defined orientation) under specified test conditions (e.g., temperature; see *ASTM D3121* for more details). The distance traveled by the ball on the adhesive layer is measured using a suitable measuring device. This procedure is repeated using a minimum of five independent samples. The product fails the test if the mean distance traveled is outside the acceptable range determined during product development and/or **2S (USP39)** based on statistical assessment of multiple product batches over the product's shelf life.

■

Cold Flow Test

Cold flow is the migration of the adhesive matrix beyond the edge of the TDS backing, ~~and through the slit in the release liner,~~ **2S (USP39)** which may occur during the course of product processing and storage. Cold flow is an inherent property of TDS due to the use of pressure-sensitive adhesives that flow when force is applied (i.e., if the adhesive matrix did not flow, the TDS would not stick). The magnitude of the cold flow is generally dependent on the product formulation, storage conditions, and storage time. Cold flow ~~can be~~ **should be** ~~quantitatively and~~ **2S (USP39)** ~~quantitatively measured as an amount (i.e., the region of cold flow can be carefully isolated from the transdermal system and weighed or assayed), an area (i.e., the region of cold flow can be imaged, with suitable magnification, and the area of the cold flow measured using image analysis software), or, most commonly, as a distance (i.e., the distance the adhesive has migrated beyond the edge of the TDS backing, typically NMT a few millimeters, can be measured with suitable magnification; this method is described in further detail below).~~ **by a** ~~suitable method.~~ **It is up to the TDS manufacturer to determine the most suitable cold flow test for each drug product. Acceptance criteria are product specific and defined to ensure that the cold flow of each batch of TDS is within the range defined by the product design and is consistent between batches based on the product development specifications and statistical**

assessment of multiple batches over the product's shelf life. ■ 2S (USP39)

Assessing cold flow through a combination of qualitative and quantitative methods may be needed. The method(s) chosen to assess cold flow must demonstrate discerning capabilities and be scientifically justified throughout the shelf life of the drug product.

DISTANCE METHOD

Samples to be tested should be carefully handled so as not to disturb the cold flow (if present). If the TDS to be tested is not adhered to the pouching material, carefully remove the TDS without disturbing the cold flow. If the TDS to be tested is adhered to the pouching materials, carefully cut around the perimeter of the pouch so that all edges of the pouch are open. Then carefully peel the front and back of the pouch apart, exposing the TDS, which may be adhered to the inside of the front or back pouch film. A minimum of three TDS should be prepared for testing.

With the aid of magnification (~20–100×) and appropriate lighting, a measurement of the distance the adhesive matrix has migrated beyond the edge of the TDS backing should be taken. A minimum of four measurements should be taken from each TDS. The positions on the TDS where the measurements are to be taken should be evenly spaced and predefined. For example, with the TDS oriented with the printing, measurements could be taken at the 3:00, 6:00, 9:00, and 12:00 positions. Measurements may be taken with the use of a graduated eye piece, through the use of a camera with suitable software, or other appropriate approach.

The product fails if the overall average measured cold flow is outside the acceptable range determined during product development and based on statistical assessment of multiple product batches over the product's shelf life.

Note that the test for cold flow provides a direct measure of the cohesive properties of the adhesive matrix, which is a quality attribute that is correlated to shear. Although the shear test has some value during product development as a rudimentary predictor of the potential for cold flow, with the inclusion of cold flow as a product quality test, shear is not recommended as a test to control the quality of the finished product.

■ ■ 2S (USP39)

Shear Test

The *Shear Test* measures the cohesive strength of a TDS. It can be measured under static (see *Static Shear Test*) or dynamic conditions. Shear testing may not be feasible for all TDS because the presence of multiple layers of adhesive in the system, the presence of a membrane or scrim, or the use of an emulsion adhesive system may result in the inability to achieve cohesive failure. TDS that are constructed with a peripheral adhesive ring or form-fill-seal TDS may not be suitable for this test. ■ It is up to the TDS manufacturer to decide if a shear test is appropriate, and if so, which shear test is most appropriate for each drug product. Acceptance criteria are product specific and defined to ensure that the shear of each batch of TDS is within the range defined by the product design and is consistent between batches based on the product development specifications and statistical assessment of multiple product batches over the product's shelf life. ■ 2S (USP39)

Static Shear Test

For the *Static Shear Test*, the time required to remove a standard area of the TDS from the substrate (i.e., stainless steel test panel) under a standard load (e.g., 250 g) is measured. The TDS is applied to a test panel, that is at an angle 2° from the vertical ■ ■2S (USP39) and the sample is subjected to a shearing force by means of a given weight suspended from the TDS. The test apparatus holds the test panels at ■ 0°–■2S (USP39) 2° from vertical to ensure that the TDS will not experience peeling action when the weight is attached. Dwell time, weight used, test panel type, mode of failure, and sample size should be noted; the time taken for the TDS sample to detach from the test panel is reported. This procedure is repeated using a minimum of five independent samples yielding valid results (see *Determination of the Validity of Static Shear Test*). The product fails the test if the mean shear force ■ (i.e., arithmetic mean or geometric mean as determined by the manufacturer) ■2S (USP39) is outside the acceptable range determined during product development and based on statistical assessment of multiple product batches over the product's shelf life.

DETERMINATION OF THE VALIDITY OF STATIC SHEAR TEST

Cohesion is the preferred ■ desired ■2S (USP39) failure mode for the static shear test. Cohesive failure is indicated when adhesive is left on the TDS and on the stainless steel test panel. In contrast, an adhesive failure occurs when: 1) the TDS strips cleanly from the stainless steel panel, leaving no visible adhesive on the stainless steel panel; 2) the adhesive transfers to the stainless steel test panel, leaving no adhesive on the TDS; or 3) the TDS delaminates at an interface (e.g., between a membrane and an adhesive layer, or between the two different adhesive layers of a bilayer product). If adhesive failure is indicated, the sample test is invalid. In addition, if the TDS breaks or tears before detaching from the stainless steel test panel or the TDS slides out of the clamp before detaching from the stainless steel test panel, ■ ■2S (USP39) or the hanging weight does not hang freely, the sample test is invalid.

■2S (USP39)

Leak Test

This test is applicable only for form-fill-seal-type (reservoir or pouched) TDS. Form-fill-seal TDS must be manufactured with zero tolerance for leaks because of their potential for dose dumping if leaking occurs.

In-process control methods to examine TDS for leaks or potential leaks are needed and require considerable development on the part of TDS manufacturers.

IN-PROCESS TESTING

During the manufacturing process, the presence of leakage (or potential for leakage) due to TDS perforation, cuts, and faulty seals resulting from failures such as air bubbles, gel splash, or misalignment of a TDS backing and release liner layers must be examined. Unless automated process analytical technology is implemented, in-process testing to identify these defects should be performed using the following test procedures.

Visual inspection:

- A specified number of TDS, defined on the basis of batch size, should be examined randomly.
- Each sampled TDS should be thoroughly visually inspected for leakage.
- The product fails if any of the TDS examined are detected with a leak.

Seal integrity: TDS seals should be stress tested to ensure that the application of pressure does not force seals to open, thereby leading to leakage.

- A specified number of TDS, defined on the basis of batch size, should be randomly examined.
- Each sampled TDS should be thoroughly visually inspected for leakage.
- Each sampled TDS is placed on a hard, flat surface and overlaid with a weight so that it is subjected to 13.6 kg. The weight should be left in place for 2 min. Upon removal of the weight, the TDS should be visually inspected for leakage.
- The product fails if the number of TDS detected with a leak is greater than the acceptable limit established by the manufacturer.

Packaged product testing: TDS may leak after they have been individually placed in the primary packaging material as a result of the packaging operation itself or by a user opening the packaging. Therefore, TDS should be tested for leakage after they have been manufactured and packaged in their primary packaging material.

- A specified number of TDS, defined on the basis of batch size, should be randomly examined after they have been placed in their primary packaging material.
- The sampled TDS should be removed from their packaging and thoroughly visually inspected for leakage.
- Each sampled TDS should then be uniformly wiped with a solvent-moistened swab. Both the backing side and the release liner side of the TDS should be wiped. The inside surface of the pouch should also be wiped. The swab(s) is then extracted and assayed for the drug.
- The product fails if the total amount of drug from the TDS, and the corresponding pouch, exceed the acceptable limit established by the manufacturer.

BRIEFING

⟨4⟩ **Mucosal Drug Products—Product Quality Tests**, *USP* 38 page 76 and *PF* 40(1) [Jan.–Feb. 2014]. There are five *USP* general chapters that link with *USP*'s taxonomy for pharmaceutical dosage forms: *Injections and Implanted Drug Products* ⟨1⟩, *Oral Drug Products—Product Quality Tests* ⟨2⟩, *Topical and Transdermal Drug Products—Product Quality Tests* ⟨3⟩, *Mucosal Drug Products—Product Quality Tests* ⟨4⟩, and *Inhalation and Nasal Drug Products—General Information and Product Quality Tests* ⟨5⟩. This taxonomy describes five routes of administration: injection or parenteral; gastrointestinal or oral; topical (dermal and transdermal); mucosal; and lungs or inhalation (for more information, see the *Stimuli* article titled *Product Quality and Product Performance General Chapters* in *PF* 39(3) [May–June 2013]). The five general chapters provide critical product quality attributes for the various dosage forms by the various routes of administration. In addition to these five chapters, *Ophthalmic Ointments* ⟨771⟩ outlines the critical product quality attributes for the various dosage forms that can be applied to the eye. Product quality tests assess physical, chemical, and microbial attributes. Taken together, the tests help ensure identity, strength, quality, and purity of a dosage form. The General Chapters—Dosage Forms Expert Committee is proposing the following revision to ⟨1⟩, ⟨2⟩, ⟨3⟩, ⟨4⟩, ⟨5⟩, and ⟨771⟩, in this issue of *PF*, to clarify the applicability of these chapters.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCDF: W. Brown.)

Correspondence Number—C159266

Comment deadline: November 30, 2015

Add the following:**▲ (4) MUCOSAL DRUG PRODUCTS—PRODUCT QUALITY TESTS****Change to read:****INTRODUCTION**

The mucosal route of drug administration is subdivided into seven membrane surfaces for the purposes of taxonomic distinction of dosage forms by route of administration. These membrane surfaces are characterized as otic, ophthalmic, nasal, oropharyngeal, urethral, vaginal, and rectal. This grouping does not include the pulmonary mucosal route addressed in *Inhalation and Nasal Drug Products—General Information and Product Quality Tests* (5). A drug product is administered to any of these seven mucosal surfaces to effect either local action or systemic absorption. Local action is to the area proximate to application. Where local action is intended, systemic absorption is not typically desired and is unnecessary for therapeutic effect. In some cases, however, the mucosal delivery of a drug for systemic absorption is used because it avoids first-pass metabolism, it provides more rapid systemic delivery, or it provides an alternative when oral delivery (to the gastrointestinal tract) is not possible due to a disease state. A large number of the dosage forms listed in *Pharmaceutical Dosage Forms* (1151) can be delivered by way of the various membrane surfaces in the mucosal category. [Note—All references to chapters above 1000 are for informational purposes only, for use as a helpful resource. These chapters are not mandatory unless explicitly called out for application.] Analytical procedures and acceptance criteria for testing drug products are divided into two categories: those that assess general product quality attributes and those that assess product performance. Drug product quality tests assess attributes such as identification, assay (strength), dose content uniformity, and impurities and are usually part of the compendial monograph. Product performance tests include the dissolution test for a solid oral dosage form, *Dissolution* (711), and the drug release test, *Drug Release* (724). Taken together, quality and performance tests ensure the identity, strength, quality, and purity of a mucosal drug product.

~~This chapter also provides lists of consolidated, common product quality tests and certain specific tests based on route of drug administration requirements. Existing monographs contain all the tests required for the article. For new, specific drug product monographs or where a monograph is not available, the chapter provides specific quality tests as a resource for manufacturers until particular monographs for the specific product are developed by USP.~~

■ Chapter (4) provides lists of consolidated common product quality test requirements in a concise and coherent fashion. This chapter applies, in part or in its entirety, when referenced in a drug product monograph, and includes the quality tests for the specific route of administration. The quality tests listed can be used, as appropriate, by manufacturers toward the development of new drug product monographs for submission to the USP. ■2S (USP39)

Change to read:**PRODUCT QUALITY TESTS FOR MUCOSAL DRUG PRODUCTS**

This chapter provides product quality tests that are generally necessary, tests that apply to specific products, and tests that apply to one or more of the specific mucosal routes. Quality tests listed under a specific mucosal route in this chapter represent expectations for any dosage form administered by that specific route.

Generally Necessary Tests

Product quality attributes for mucosal dosage forms should reflect acceptable requirements for marketed products. The following tests should be generally applied to all dosage forms intended for mucosal delivery. Tests that are generally necessary for any article include: *Definition*, *Identification*, *Assay*, and *Impurities* (organic, inorganic, and residual solvents). *Uniformity of Dosage Units* (905) is typically included in a *USP* product monograph.

DEFINITION

The *Definition* section (see *General Notices and Requirements 4.10*) in a *USP* monograph describes the drug product and specifies the range of acceptable assayed content of the drug substance(s) present in the dosage form. For certain products, the *Definition* includes any relevant additional information, such as the presence or absence of other components, excipients, or adjuvants, and cautionary statements on toxicity and stability. Appearance information is used in a regulatory submission to aid in product identification. Because the size, shape, color, etc.,

■ and other attributes ■ 2S (*USP39*)

are attributes of individual marketed products, a qualitative description is typically not required as part of a *USP* monograph (see (1151)).

IDENTIFICATION

Identification is included in a monograph as an aid in verifying the identity of the article and to provide a positive identification of the drug substance or substances in a drug product (see *General Notices and Requirements 5.40*).

ASSAY

The assay is used to determine the strength (content) of the drug product. Typically, the assay is specific and stability-indicating. When a nonspecific assay is justified, other supporting analytical procedures should ensure that any interfering species will be detected and can be limited. Assay results are often reported as a percentage of the label claim, with acceptance criteria that are typically in the range from 90.0% to 110.0%. For some antibiotic products, the range may be wider. The width of these limits is intended to allow for manufacturing variability, including changes in stability, as well as analytical variation. The narrower acceptance range of 95.0%–105.0% is used less often and with justification.

IMPURITIES

Process impurities include those arising from starting materials, synthetic byproducts, and other inorganic and organic impurities that may be present in the drug substance and in the excipients used in the manufacture of the drug product. These impurities are controlled by using the appropriate test, as specified within the drug substance and excipient monographs. Impurities in the drug product may also result from degradation of the drug substance or

excipients, from interactions between the drug substance and an excipient, or from interactions between the drug substance and the packaging components. The procedures and acceptance criteria should specifically limit toxic degradation products as well as degradation products that endanger

■ compromise ■ 2S (USP39)

the quality of the article if they exceed certain levels. Limits should be provided for process impurities that are found to be present during the test for degradation products. A more complete discussion of impurities is provided in *Impurities in Drug Substances and Drug Products* (1086)¹ and in ICH Q3B Impurities in New Drug Products.¹

UNIFORMITY OF DOSAGE UNITS

Chapter (905) is used to ensure the consistency of drug substance content in dosage units within a narrow range around the label claim. The test is applied only to dosage forms containing a single dose or a part of a dose of the drug substance in each unit. Uniformity of dosage units may be demonstrated by one of two methods: content uniformity or weight variation. Content uniformity is based on the assays of a number of individual dosage units. Weight variation can be used to estimate content uniformity under certain conditions.

Dosage Forms by Specific Mucosal Route and Product-Specific Tests

In addition to the generally necessary product quality tests already discussed, the dosage form may require specific quality tests that are common across routes of administration. *Injections and Implanted Drug Products* (1) provides testing requirements common to injectable and implantable products. *Oral Drug Products—Product Quality Tests* (2) provides testing requirements for tablets and lozenges. *Topical and Transdermal Drug Products—Product Quality Tests* (3) provides testing requirements common to semi-solids (creams, ointments, and gels). Chapter (5) presents testing requirements for sprays and aerosols. Where a dosage form has no specific test given in this chapter, no additional test is required unless included in the individual monograph specification.

OTIC ROUTE

The otic route is characterized by administration of a preparation into, or by way of, the ear. Demonstration of sterility (see *Sterility Tests* (71)) is not always required for products delivered to the ear. Typically, sterility is required where the product is administered to the inner ear or where the eardrum is damaged. Where sterility is not required, the quantitative enumeration of mesophilic bacteria and fungi that grow under anaerobic conditions, *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* (61), or the determination of the absence or limited occurrence of specified organisms, *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* (62), may be required.

If an antimicrobial preservative is used, *Antimicrobial Effectiveness Testing* (51) and *Antimicrobial Agents—Content* (341) may be required.

Dosage forms given by the otic route include liquids, solutions, and suspensions.

OPHTHALMIC ROUTE

The ophthalmic route is to the eye. In addition to the generally necessary tests, the following specific tests for ophthalmic drug products should be considered (see *Table 1*). For products

that are injected or implanted into the eye, see (1). Some of the important product quality tests for products administered by the ophthalmic route are listed below. See *Ophthalmic Ointments* (771) for details and other product quality information.

- Foreign and Particulate Matter
- Sterility
- Particle Size and Particle Size Distribution
- Antimicrobial Preservative

Table 1. Drug Products Administered by the Ophthalmic Route, with Product-Specific Tests

Ophthalmic Route	
Dosage Form	Product-Specific Tests
Gels	<i>Minimum Fill</i> (755)
	<i>Topical and Transdermal Drug Products—Product Quality Tests</i> (3)
Emulsions	<i>Osmolality and Osmolarity</i> (785)
	<i>pH</i> (791)
	Surface tension
	<i>Viscosity—Capillary Methods</i> (911)
	<i>Viscosity—Rotational Methods</i> (912)
	Zeta potential
Inserts	<i>Bacterial Endotoxins Test</i> (85)

Ophthalmic Route	
Dosage Form	Product-Specific Tests
Ointments	<i>Minimum Fill (755)</i>
	<i>Topical and Transdermal Drug Products—Product Quality Tests (3)</i>
Solutions	<i>Particulate Matter in Ophthalmic Solutions (789)</i>
	<i>pH (791)</i>
	<i>Viscosity—Capillary Methods (911)</i>
	<i>Viscosity—Rotational Methods (912)</i>
	<i>Viscosity—Rolling Ball Method (913)</i>
	<i>Osmolality and Osmolarity (785)</i>
Strips	Currently no specific tests (additional specific monograph requirements may apply)
	<i>pH (791)</i>
	<i>Osmolality and Osmolarity (785)</i>

	Ophthalmic Route
Dosage Form	Product-Specific Tests
Suspensions	Particle size and particle size distribution
	<i>Viscosity—Capillary Methods</i> (911)
	<i>Viscosity—Rotational Methods</i> (912)
	<i>Viscosity—Rolling Ball Method</i> (913)

NASAL ROUTE

The nasal route is administration to the nose, or by way of the nose, for local or systemic effect (see *Table 2*).

Table 2. Drug Products Administered by the Nasal Route, with Product-Specific Tests

Nasal Route	
Dosage Form	Product-Specific Tests
Aerosols	<i>Inhalation and Nasal Drug Products—General Information and Product Quality Tests (5)</i>
Gels (Jelly)	<i>Minimum Fill (755)</i>
	<i>Topical and Transdermal Drug Products—Product Quality Tests (3)</i>
Ointments	<i>Minimum Fill (755)</i>
	<i>Topical and Transdermal Drug Products—Product Quality Tests (3)</i>
Sprays	<i>Inhalation and Nasal Drug Products—General Information and Product Quality Tests (5)</i>
Solutions	<i>Inhalation and Nasal Drug Products—General Information and Product Quality Tests (5)</i>

OROPHARYNGEAL ROUTE

The oropharyngeal route is into the oral cavity and/or pharyngeal region. The oropharyngeal route is subclassified by the specific intra-oral surfaces, such as buccal or sublingual. Buccal and sublingual administrations are typically intended to promote systemic absorption by permeation through the respective mucosa. However, in this context, oral administration may mean topical application for local action (see *Table 3*). Product quality tests for products administered to oropharyngeal surfaces often conform to those for oral administration to the gastrointestinal tract (see (2)).

Table 3. Drug Products Administered by the Oropharyngeal Route, with Product-Specific Tests

Oropharyngeal Route	
Dosage Form	Product-Specific Tests
Buccal patches	See <i>Topical and Transdermal Drug Products—Product Quality Tests</i> (3) for testing requirements common to patches.
Films	Currently no specific tests (additional specific monograph requirements may apply)
Gels	<i>Minimum Fill</i> (755)
	<i>Topical and Transdermal Drug Products—Product Quality Tests</i> (3)
Gums	Currently no specific tests (additional specific monograph requirements may apply)
Lozenges	Currently no specific tests (additional specific monograph requirements may apply)
Ointments	<i>Topical and Transdermal Drug Products—Product Quality Tests</i> (3)
	<i>Minimum Fill</i> (755)
Solutions (Rinses)	Currently no specific tests (additional specific monograph requirements may apply)
Sprays	<i>Inhalation and Nasal Drug Products—General Information and Product Quality Tests</i> (5)
Tablets	<i>Oral Drug Products—Product Quality Tests</i> (2)

URETHRAL ROUTE

The urethral route is into the urethra, typically for local action, but systemic distribution is also possible. Chapters (61) and chapter

- 2S (USP39)

(62) may apply. Drug products in this category include urethral inserts.

VAGINAL ROUTE

The vaginal route is into the vagina, typically for local action, but systemic distribution is also possible. Chapters (61) and chapter

■ 2S (USP39)

(62) may apply (see Table 4).

Relative foam density: Determine relative foam density by weighing a mass of foam (*m*) and a mass of the same volume of water (*e*) in a flat-bottom dish. Relative foam density = *m/e*.

Volume of foam expansion: Estimate the volume of foam expansion at 25° using a graduated buret and a foam-generating container equipped with a dose-actuating device and fitted to the buret.

Table 4. Drug Products Administered by the Vaginal Route, with Product-Specific Tests

Vaginal Route	
Dosage Form	Product-Specific Tests
Creams	Minimum Fill (755)
	Topical and Transdermal Drug Products—Product Quality Tests (3)
Foams	Minimum Fill (755)
	Physical appearance (of the foam and of the collapsed foam)
	Relative foam density
	Volume of foam expansion
Gels	Minimum Fill (755)
	Topical and Transdermal Drug Products—Product Quality Tests (3)
Inserts	Currently no specific tests (additional specific monograph requirements may apply)

RECTAL ROUTE

The rectal route is into the rectum. Rectally administered products may produce local effect(s) or delivery to the systemic circulation.

Softening time determination

■ ■2S (USP39)

of lipophilic suppositories: The test is intended to determine, under defined conditions, the time that elapses until a suppository maintained in water at $37 \pm 0.5^\circ$ softens to the extent that it no longer offers resistance when a defined weight is applied (see *Table 5*).

Relative foam density: Determine relative foam density by weighing a mass of foam (*m*) and a mass of the same volume of water (*e*) in a flat-bottom dish. Relative foam density = m/e .

Volume of foam expansion: Estimate the volume of foam expansion at 25° using a graduated buret and a foam-generating container equipped with a dose-actuating device and fitted to the buret.

■ ■2S (USP39)

Table 5. Drug Products Administered by the Rectal Route, with Product-Specific Tests

Rectal Route	
Dosage Form	Product-Specific Tests
Foams	<i>Minimum Fill (755)</i>
	Physical appearance (of the foam and of the collapsed foam)
	<i>Relative foam density</i>
	<i>Volume of foam expansion</i>
Ointments	<i>Minimum Fill (755)</i>
	<i>Topical and Transdermal Drug Products—Product Quality Tests (3)</i>
Suppositories	Softening time of lipophilic suppositories
Solutions	Currently no specific tests (additional specific monograph requirements may apply)
Suspensions	Currently no specific tests (additional specific monograph requirements may apply)

▲USP38

¹ ICH Q3B (R2) Impurities in New Drug Products, 2006, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3B_R2/Step4/Q3B_R2_Guideline.pdf Accessed 28 May 2015.

⟨5⟩ Inhalation and Nasal Drug Products—General Information and Product Quality Tests, *USP* 38 page 80 and *PF* 39(1) [Jan.–Feb. 2013]. There are five USP general chapters that link with USP’s taxonomy for pharmaceutical dosage forms: *Injections and Implanted Drug Products* ⟨1⟩, *Oral Drug Products—Product Quality Tests* ⟨2⟩, *Topical and Transdermal Drug Products—Product Quality Tests* ⟨3⟩, *Mucosal Drug Products—Product Quality Tests* ⟨4⟩, and *Inhalation and Nasal Drug Products—General Information and Product Quality Tests* ⟨5⟩. This taxonomy describes five routes of administration: injection or parenteral; gastrointestinal or oral; topical (dermal and transdermal); mucosal; and lungs or inhalation (for more information, see the *Stimuli* article titled *Product Quality and Product Performance General Chapters* in *PF* 39(3) [May–June 2013]). The five chapters provide critical product quality attributes for the various dosage forms by the various routes of administration. In addition to these five chapters, *Ophthalmic Products—Quality Tests* ⟨771⟩ outlines the critical product quality attributes for the various dosage forms that can be applied to the eye. Product quality tests assess physical, chemical, and microbial attributes. Taken together, the tests help ensure identity, strength, quality, and purity of a dosage form. The General Chapters—Dosage Forms Expert Committee is proposing the following revision to ⟨1⟩, ⟨2⟩, ⟨3⟩, ⟨4⟩, ⟨5⟩, and ⟨771⟩, in this issue of *PF*, to clarify the applicability of these chapters.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCDF: K. Zaidi.)

Correspondence Number—C131378

Comment deadline: November 30, 2015

⟨5⟩ INHALATION AND NASAL DRUG PRODUCTS—GENERAL INFORMATION AND PRODUCT QUALITY TESTS

Change to read:

1. INTRODUCTION

Inhalation drug products deliver a drug substance into the lungs by oral inhalation and include inhalation aerosols, inhalation powders, inhalation sprays, inhalation solution, inhalation suspension, solution for inhalation, and drug for inhalation solution dosage forms. Nasal drug products deliver drugs into the nasal cavity and include nasal aerosols, nasal sprays, nasal solutions, and nasal powder dosage forms. This chapter does not address nasal products in the gels and ointments form. See *Table 1* for established names and definitions of these dosage forms. Definitions of these drug product dosage forms, brief information about their manufacture, and a glossary of dosage form names can be found in *Pharmaceutical Dosage Forms* ⟨1151⟩.

■ [Note—All references to chapters above 1000 are for informational purposes only, for use as a helpful resource. These chapters are not mandatory unless explicitly called out for application.] ■_{2S} (*USP39*)

Table 1. Established Names and Definitions

Established Name	Definition
Inhalation Aerosol	A drug product for oral inhalation that is packaged under pressure and delivers a specified amount of therapeutically active ingredient(s) upon activation of an accurately metered valve system. Inhalation aerosols are more commonly known as metered-dose inhalers or MDIs.
Inhalation Powder	Drug powder for oral inhalation with the use of a device that aerosolizes and delivers an accurately metered amount of the therapeutically active ingredient(s). Inhalation Powders are more commonly known as dry powder inhalers or DPIs.
Inhalation Spray	A nonpressurized, accurately metered, liquid drug dosage form for oral inhalation that is packaged in a container that upon activation delivers fine droplets of the formulation
Inhalation Solution	A drug solution for oral inhalation with the use of a nebulization system
Inhalation Suspension	A drug suspension for oral inhalation with the use of a nebulization system
Solution for Inhalation	A drug solution for oral inhalation that must be diluted before it is administered with the use of a nebulization system
[Drug] for Inhalation Solution	A drug powder that, upon the addition of a suitable vehicle, yields a solution conforming in all respects to an Inhalation Solution
Nasal Aerosol	A drug product for local application into the nasal passages that is packaged under pressure and delivers a specified amount of therapeutically active ingredient(s) upon activation of an accurately metered valve system
Nasal Spray	A nonpressurized, accurately metered, liquid drug dosage form for local application into the nasal passages that is packaged in a container that upon activation delivers droplets of the formulation
Nasal Solution	A nonpressurized, liquid drug dosage form for local application into the nasal passages
Nasal Powder	Drug powder for local application into the nasal passages with the use of a device that delivers and aerosolizes an accurately metered amount of the therapeutically active ingredient(s)

General chapter (5) provides a framework to support new individual monographs. These are “moving forward” documents and are not intended to replace the need for individual monographs. Chapter (5) provides pick lists of consolidated common product quality test requirements in a concise and coherent fashion. If a monograph exists, it should contain all the tests required for the dosage form. If a specific drug product monograph is missing (not in existence), the general chapter provides the quality tests that can be used by manufacturers until the monograph needed for the dosage form appears in *USP-NF*. If a validated performance test procedure is available for the specific drug product, it is identified in a general chapter below (1000). Additional information, or information on promising technologies that have not yet been fully validated, may be presented in informational chapters above (1000).¹

- General chapter (5) provides lists of consolidated common product quality test requirements in a concise and coherent fashion. This general chapter applies, in part or in its entirety, when

referenced in a drug product monograph, and includes the quality tests for the specific route of administration. The quality tests listed can be used, as appropriate, by manufacturers toward the development of new drug product monographs for submission to the USP. If a validated performance test procedure is available for the specific drug product, it is identified in a general chapter below 1000. Additional information, or information on promising technologies that have not yet been fully validated, may be presented in informational chapters above 1000. ■2S (USP39)

Drug Product General Quality Tests and Performance Quality Tests

A USP drug product monograph contains tests, analytical procedures, and acceptance criteria. Drug products tests are divided into two categories: 1) those that assess general quality attributes, and 2) those that assess product performance quality, e.g., delivered dose uniformity and its physical characteristics, such as aerodynamic particle size distribution. General quality tests assess the integrity of the dosage form, whereas performance quality tests assess delivery of the drug and other attributes that may relate to in vivo drug performance.

Taken together, quality and performance tests ensure the identity, strength, quality, and purity of inhalation and nasal drug products.

The next two sections of this chapter list product quality attributes for inhalation drug products and for nasal drug products, respectively. The final section describes in greater detail the quality tests for inhalation and nasal drug products. *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* (601) contains product performance tests for inhalation and nasal drug products and should be used in conjunction with (5).

2. GENERAL QUALITY TESTS FOR INHALATION DRUG PRODUCTS

Inhalation Aerosol

DESCRIPTION

Aerosol in this context is a dosage form consisting of a liquid or solid preparation packaged under pressure and intended for administration as a fine mist. The descriptive term aerosol also refers to the fine mist of small droplets or solid particles that are emitted from the product. Inhalation aerosols, also known as MDIs, are preparations characterized by dispersion of the active pharmaceutical ingredient into the airways during oral inspiration for either local or systemic effect, and nasal aerosols, also known as nasal MDIs, are characterized by deposition in the nasal cavity for either local or systemic effect.

An aerosol formulation typically contains drug substance(s) dissolved or suspended in a propellant(s) or a mixture of propellant(s) and co-solvent(s) and possibly other suitable excipients. An inhalation aerosol drug product, commonly known as an MDI, delivers a specified amount and quality of therapeutically active ingredient(s) upon activation of an accurately metered valve system. General quality tests for inhalation aerosol drug products should include the following (see section IV later in this chapter for more detailed discussions of each test):

- Identification
- Assay
- Impurities and Degradation Products
- Water Content
- Foreign Particulate Matter

- Leachables
- Spray Pattern
- Microbial Limit
- Alcohol Content (if present)
- Net Fill Weight
- Leak Rate
- For performance quality tests, refer to <601>.

Inhalation Solution

DESCRIPTION

Inhalation solution drug products typically are water-based and are sterile preparations. They are intended for delivery to the lungs by nebulization with a specified external nebulizer. Such drug preparations typically are packaged in single-dose semipermeable containers and also include protective packaging to minimize ingress of volatile foreign contaminants, loss of solvent, and exposure to oxygen and light. Nebulization involves continuous generation and delivery to the patient of a fine mist of aqueous droplets containing a drug solution by means of ultrasonic energy, Venturi effect, or other appropriate mechanical/electrical means. General quality tests for inhalation solutions should include the following (see section IV later in this chapter for more detailed discussions of each test):

- Identification
- Assay
- Impurities and Degradation Products
- Content Uniformity (premetered)
- Assay for Antimicrobial Preservative and Stabilizing Excipients (if present)
- Sterility
- Foreign Particulate Matter
- pH
- Osmolality
- Leachables
- Net Fill Weight
- Weight Loss
- For performance quality tests, refer to *Products for Nebulization—Characterization Tests* <1601>.

Inhalation Suspension

DESCRIPTION

Inhalation suspension drug products typically are water-based and are sterile preparations. They are intended for delivery to the lungs by nebulization with a specified external nebulizer. Such drug preparations typically are packaged in single-dose semipermeable containers and also include protective packaging to minimize ingress of volatile foreign contaminants, loss of solvent, and exposure to oxygen and light. Nebulization involves continuous generation and delivery to the patient of a fine mist of aqueous droplets containing the formulation components by means of ultrasonic energy, Venturi effect, or other appropriate mechanical means. General quality tests for inhalation suspensions should include the following (see section IV later in this chapter for more detailed discussions of each test):

- Particle size distribution of the formulation in the immediate container

- For all other general quality attributes, refer to the previous *Inhalation Solution* attributes.

Solution for Inhalation

DESCRIPTION

Solution for inhalation drug products typically are water-based and are sterile preparations. Upon dilution, in accordance with labeling, including identity and amount of dilution vehicle, they are intended for delivery to the lungs by nebulization using an external nebulizer. Such drug preparations typically are packaged in single-dose semipermeable containers and also include a protective packaging to minimize ingress of volatile foreign contaminants, loss of solvent, and exposure to oxygen and light. Nebulization involves continuous generation and delivery to the patient of a fine mist of aqueous droplets containing the formulation components by means of ultrasonic energy, Venturi effect, or other appropriate mechanical/electrical means. General quality tests for solutions for inhalation should include the following (see section *IV* later in this chapter for more detailed discussions of each test):

- Clarity and color of solution upon dilution in accordance with the labeling
- For all other general quality attributes, refer to the previous *Inhalation Solution* attributes.

Drug for Inhalation Solution

DESCRIPTION (POWDER)

Drug for Inhalation Solution is a (specified color) drug powder formulation that upon the addition of a suitable vehicle, in accordance with labeling, including identity and amount of dilution vehicle, yields a solution conforming in all respects to the *Inhalation Solution* requirements. General quality tests for drug for inhalation solutions should include the following (see section *IV* later in this chapter for more detailed discussions of each test):

- Water content
- Clarity, color, and completeness of solution within specified time, upon reconstitution
- For all other general quality attributes, refer to the previous *Inhalation Solution* attributes upon (re)constitution of the drug product.

Inhalation Spray

DESCRIPTION

Inhalation spray drug products typically are water-based liquid formulations packaged in a compact container-closure system containing an integral spray pump unit that, upon activation, delivers an accurately metered amount of fine mist of droplets of the formulation. The droplets can be generated by various means such as mechanical action, power assistance, or energy from the patient's inspiration. The mechanisms by which they generate droplets distinguish the various types of inhalation sprays. These drug products may be unit-dose or multidose presentations. Inhalation spray drug products may be designed as premeasured or device-metered presentations. A premeasured unit contains a previously measured amount of liquid formulation in an individual container (e.g., a blister) that is inserted in the device by the patient before use. A device-metered product contains a sufficient amount of liquid formulation for a prescribed number of doses in a reservoir, and each dose is delivered as an accurately

metered spray by the device throughout the unit's life. General quality tests for inhalation sprays should include the following (see section *IV* later in this chapter for more detailed discussions of each test):

- Plume geometry
- For all other general quality attributes, refer to the previous *Inhalation Solution* attributes.
- For performance quality tests, refer to (601).

Inhalation Powder

DESCRIPTION

Inhalation powder drug products, commonly known as dry powder inhalers (DPIs), dispense powders for inhalation with the use of a device that aerosolizes and delivers an accurately metered amount of active ingredient(s) with consistent physical characteristics alone or with a suitable excipient(s). Current designs include premetered and device-metered DPIs, all of which rely on various energy sources to create and disperse the aerosol during patient inspiration. Premetered DPIs contain previously measured amounts of formulation in individual containers (e.g., capsules or blisters) that are inserted into the device before use. Premetered DPIs also may contain premetered dose units as ordered multidose assemblies in the delivery system. Device-metered DPIs have an internal reservoir that contains a sufficient quantity of formulation for multiple doses that are metered by the device itself during actuation by the patient. General quality tests for inhalation powders should include the following (see section *IV* later in this chapter for more detailed discussions of each test):

- Identification
- Assay
- Impurities and Degradation Products
- Content Uniformity (premetered)
- Water Content
- Foreign Particulate Matter
- Microbial Limit
- Net Content (device-metered)
- Residual Solvents
- Volatile and Semivolatile Leachables
- For performance quality tests, refer to (601).

3. GENERAL QUALITY TESTS FOR NASAL DRUG PRODUCTS

Nasal Aerosol

Refer to the previous *Inhalation Aerosol* attributes.

Nasal Spray

DESCRIPTION

Nasal spray drug products typically are water-based liquid formulations applied to the nasal cavity for local and/or systemic effects. They contain therapeutically active ingredient(s) dissolved or suspended in solution or mixtures of excipients in a nonpressurized compact

container–closure system. The container–closure system includes an integral spray pump unit that upon activation delivers a spray containing an accurately metered amount of fine mist of droplets of the formulation. Dispersion of the formulation as a spray typically is accomplished by forcing the formulation through the nasal actuator and its orifice. Often, such drug products are multidose device-metered (see *Inhalation Spray*) presentations in which the dose is metered by the spray pump. Nasal spray drug products also may be designed as premetered presentations. General quality tests for nasal sprays should include the following (see section IV later in this chapter for more detailed discussions of each test):

- Identification
- Assay
- Impurities and Degradation Products
- Assay for Preservative and Stabilizing Excipients (if present)
- Content Uniformity (premetered)
- Particle Size Distribution (for suspensions)
- Foreign Particulate Matter
- Spray Pattern
- Microbial Limit
- Leachables
- Net Fill Weight
- pH
- Osmolality
- Viscosity
- Sterility (premetered)
- For performance quality tests, refer to <601>.

Nasal Powder

Refer to the previous *Inhalation Powder* quality attributes.

Nasal Solution

DESCRIPTION

Nasal solutions are drug products that typically are water-based liquid formulations applied to the nasal cavity for local effect. They may contain drug substance(s) dissolved in solution or mixtures of excipients in a nonpressurized compact container–closure system. The container–closure system includes a delivery system that administers nonmetered amounts of drops or a fine mist of droplets of the formulation. Typically, such drug products are multidose presentations. General quality tests for nasal solution drug products should include the following (see section IV later in this chapter for more detailed discussions of each test):

- Identification
- Assay
- Impurities and Degradation Products
- Assay for Preservative and Stabilizing Excipients (if present)
- Foreign Particulate Matter
- Microbial Limit
- Leachables
- Net Fill Weight
- pH

- Osmolality
- Viscosity

Change to read:**4. DESCRIPTION OF PRODUCT QUALITY TESTS**

Product quality tests are listed as follows, and should be applied to inhalation and nasal drug products and to products for nebulization. Product-specific quality tests are addressed in product monographs.

Description

See the previous corresponding dosage forms and the respective labels for the monograph of a drug product.

Alcohol Content (if present)

If alcohol is used in a drug product formulation, a specific assay with appropriate acceptance criteria should be included.

Assay (strength and content uniformity)

The USP Assay test of a drug substance in the drug product container is determined by means of a validated stability-indicating procedure following *Validation of Compendial Procedures* (1225). The Assay test should measure available drug substance and its stability, including adherence of the drug substance to the container and closure components. Appropriate acceptance criteria can provide added assurance of manufacturing reproducibility and may ensure better conformance in other performance attributes (e.g., delivered dose uniformity). If a drug product is labeled to contain a single enantiomer of a chiral drug substance, analysts can use a chiral assay or a combination of an achiral assay and a validated procedure to control the presence of the undesired enantiomer as an impurity.

Assay for Preservative and Stabilizing Excipients (if present)

The assay of any preservative (e.g., an antimicrobial) or stabilizing excipient (e.g., an antioxidant, an agent specifically added to minimize or prevent degradation) in a multidose container should be determined analytically, typically with a validated stability-indicating procedure following current ICH Q2 guidance. The corresponding acceptance criteria normally are based on appropriate preservative effectiveness demonstrated by a microbial challenge test.

Content Uniformity for Premetered Dosage Forms

See *Uniformity of Dosage Units* (905).

Clarity and Color of Solution upon Dilution

Solution for inhalation and drug for inhalation solution dosage forms must be diluted and reconstituted in accordance with labeling before administration by nebulization. The type and amount of the vehicle used for dilution and reconstitution must be specified on the labeling. Appropriate studies must be undertaken to fully assess the clarity and color of the solution upon dilution and reconstitution. The studies also should include appropriate physical and chemical stability studies, as well as studies of performance characteristics.

Foreign Particulate Matter

Foreign particulate matter in these drug products should be adequately controlled. Particulate matter in inhalation and nasal drug products may originate during manufacturing and from formulation and container-closure components. For toxicological assessment, the type, origin, amount, and size of foreign particulates, including fine particulates (e.g., less than 10 μm), should be determined throughout the stability storage period.

Identification

A specific identification test or tests are used to verify the identity of the drug substance in the drug product. If a nonspecific method is used for identification, then it should be combined with a second independent and complementary method. A specific identification test for polymorphic forms should be carried out. Moreover, if the drug substance is a salt, an appropriate identification test also should be included for the counterion.

Impurities and Degradation Products

Validated stability-indicating analytical procedure(s) following current (1225) should be used to determine the levels of impurities and degradation products in a drug product. Typically, the acceptance criteria are set for individual, total unspecified, and total impurities and degradation products following current ICH Q3B. For reporting, identification, and qualification thresholds and other relevant information, follow current ICH Q3B guidance.

Leachables

Inhalation and nasal drug products should be evaluated for compounds that may leach from elastomeric and plastic components and from coatings of components of the container-closure system in direct contact with the formulation. Additionally, the drug product inadvertently may contain other residual contaminants from manufacturing and processing. Leachables may include polynuclear aromatics, nitrosamines, monomers, plasticizers, accelerators, antioxidants, and vulcanizing agents. Processing contaminants may include surface-treatment or processing agents that may dissolve, chemically associate, or become suspended in the formulation. Thus, throughout the expiration-dating period, the drug product should be evaluated for compounds that can migrate into the formulation from a variety of sources. The type of testing that should be performed depends on whether the formulation is a powder or a liquid and the composition of the container-closure system, e.g., a drug product packaged in a semipermeable container should be evaluated for ingress of volatile leachables. Appropriate specifications using validated analytical procedures should be applied to identify, monitor, and quantify the compounds in the drug product with appropriate minimum levels of quantification. Corresponding acceptance criteria should be established and justified from toxicological and safety

perspectives.

Leak Rate

Leak rate studies of inhalation and nasal aerosol drug products can be used during drug product development and characterization to support the selection of appropriate container–closure components (e.g., valve and canister) and drug product manufacturing parameters, including the crimping process. The specifications for the USP Leak Rate testing studies can include multiple units from each batch based on weight difference determination with time at a specified temperature. See *Leak Rate* (604) for additional information.

Microbial Limits

The microbial quality of dosage forms where indicated in *General Quality Tests for Inhalation Drug Products* and *General Quality Tests for Nasal Drug Products* normally is controlled by appropriate validated test(s) and acceptance criteria for total aerobic count, total yeasts and molds count, and freedom from designated indicator pathogens. Acceptance criteria can be expressed on a per-container basis. Refer to *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* (61) and *Alternative Microbiological Sampling Methods for Nonsterile Inhaled and Nasal Products* (610) for additional information.

Net Fill Weight

The total net weight of the formulation in the container should be assessed and controlled with a test and acceptance criteria.

Osmolality

To control the tonicity of the formulation of dosage forms where indicated in *General Quality Tests for Inhalation Drug Products*, the product should be tested for osmolality with appropriate specifications as described in *Osmolality and Osmolarity* (785).

pH

Appropriate specification for the pH of the formulation of dosage forms where indicated in *General Quality Tests for Inhalation Drug Products* and *General Quality Tests for Nasal Drug Products* should be established as described in *pH* (791).

Particle Size Distribution

For inhalation suspension and suspension nasal spray drug products, appropriate method(s) and corresponding acceptance criteria can be used for the determination of the particle size distribution of the drug substance particles in the formulation within the container.

Plume Geometry

Because various factors can affect the plume characteristics of the spray of an inhalation aerosol, inhalation spray, nasal aerosol, or nasal spray drug product, its full characterization is important for assessing the performance of the delivery system. Plume geometry can be

determined by a variety of procedures using appropriately validated methods. Plume geometry also can be controlled by appropriate acceptance criteria that measure spray pattern characteristics, including shape and size of the evolving spray plume under defined experimental and instrumental test conditions.

Reconstitution and Time (powder)

Drug for inhalation solution dosage forms must be (re)constituted before administration with the use of a specified nebulization system. Hence, appropriate compatibility studies should be undertaken to fully assess the type and amount of the solvent(s), as well as (re)constitution time for preparation of the final solution for patient administration. The compatibility studies also should include appropriate physical and chemical stability studies on the reconstituted solution, including its performance characterization.

Residual Solvent

Suitable and validated tests should be used to determine the levels of any solvent(s) in the drug product. Refer to *Residual Solvents* (467) for additional information.

Spray Pattern

Because various factors can affect the spray pattern of an inhalation aerosol, nasal aerosol, or nasal spray drug product, full spray pattern characterization is important for assessing the performance of the specific valve and the actuator or the pump. The spray pattern can be determined using appropriately validated methods and corresponding acceptance criteria that measure the shape, density, and size of the pattern. The test procedure for spray patterns normally is specific to the drug product and may include, among others, the distance between the mouthpiece and the measurement plane or collection surface, minimum number of actuations per spray pattern to enable discrimination, orientation of the collection surface relative to the mouthpiece, and visualization procedure(s).

Sterility

All inhalation water-based dosage forms are sterile preparations and should meet the requirements of *Sterility Tests* (71).

Viscosity

A test for viscosity with appropriate acceptance criteria should be included for dosage forms where indicated in *General Quality Tests for Inhalation Drug Products* and *General Quality Tests for Nasal Drug Products* as appropriate (see **•Viscosity—Capillary Methods (911)•**(CN 1-May-2015)).

Water Content

Appropriate specification for water content of dosage forms where indicated in *General Quality Tests for Inhalation Drug Products* and *General Quality Tests for Nasal Drug Products* should be established to ensure the drug product's continued stability and acceptable performance of the drug product. Validated analytical procedures should be used as described in *Water*

Determination (921). Proceed as directed in (921) with the following modification: provide the closed-system titrating vessel with an opening through which passes a coarse-porosity gas dispersion tube connected to a sampling cylinder.

Weight Loss

Drug products should be evaluated for weight loss, e.g., drug products packaged in semipermeable containers, to assess the moisture-loss protective properties of the overall container–closure system.

± All references to chapters above 1000 are for information only, for use as a helpful resource. These chapters are not mandatory unless explicitly called out for application.

■ 2S (USP39)

BRIEFING

(89.1) **Collagenase I**. This new proposed general chapter is to provide analytical procedures to aid in the assessment of quality for collagenase I isolated from *Clostridium histolyticum*. This chapter, *Collagenase I* (89.1), is part of a series of general chapters that are under development to address the qualities of the enzymes used as ancillary material in pharmaceutical manufacturing. Another proposed chapter in the series, *Collagenase II* (89.2), also appears in this issue of *PF*. Recombinant trypsin was the first enzyme to be introduced in this manner, and is published in *Enzymes Used As Ancillary Material in Pharmaceutical Manufacturing* (89).

This chapter does not discuss the applications of collagenase I but rather focuses on tests to assess the qualities as a process material. Furthermore, this chapter does not provide ways to limit the application of collagenase I in the final medicinal product. The activity of collagenase I is determined by using the chromogenic peptide substrate 4-phenylazobenzoyloxycarbonyl-Pro-Leu-Gly-Pro-d-Arg. The liquid chromatographic procedure in the test for *Purity* is based on analyses performed with the GE Healthcare Mono Q 5/50 GL brand of L91 column. New Reference Standards, USP Collagenase I RS and USP Collagenase II RS, are proposed for use during assessment of the system suitability for the proposed *Assay* and test for *Purity*.

(BIO2: E. Chang.)

Correspondence Number—C153137

Comment deadline: November 30, 2015

Add the following:

■ (89.1) **COLLAGENASE I**

IANTNSEKYD FEYLNGLSYT ELTNLIKNIK WNQINGLFNY STGSQKFFGD
 KNRVQAIINA LQESGRITYA NDMKGIEFTT EVLRAGFYLG YYNDGLSYLN
 DRNFQDKCIP AMIAIQKNPN FKLGTAVQDE VITSLGKLG NASANAEEVN
 NCVPLKQFR ENLNQYAPDY VKGTAVNELI KGIEFDGSA AYEKDVKTMP
 WYGLDPPFIN ELKALGLYGN ITSATEWASD VGIYYLSKFG LYSTNRNDIV
 QSLEKAVDMY KYGKIAFVAM ERITWDYDGI GSNKKVDHD KFLDDAEKHY
 LPKTYTFDNG TFIIRAGDKV SEEKIKRLYW ASREVKSQFH RVVGNDAKALE
 VGNADDVLTN KIFNSPEEYK FNTNINGVST DNGGLYIEPR GTFYTYERTP
 QQSIFSLLEL FRHEYTHYLQ ARYLVLDLWG QGPFYKRNRL TWDEGTAEF
 FAGSTRSGV LPRKSILGYL AKDKVDHRYL LKKTLSNGVD DSDWFMFNYG
 FAVAHYLYEK DMPTFIKMNK AILNTDVKSY DEIIRKLSDD ANKNTYEQNH
 IQELADKYQG AGIPLVSDDY LKDHGKAS EVYSEISKAA SLTNTSVTAE
 KSQYFNTFTL RGTYTGETSK GEFKWDDEMS KKLDTLESL AKNSWSGYKT
 LTAYFTNYRV TSDNKVQYDV VFHGLVDNA DISNNKAPIA KVTGPSTGAV
 GRNIEFSGKD SKDEGKIVS YDWDGFGAT SRGNKSVHAY KKAGTYNVTL
 KVTDDKGATA TESFTIEIKN EDTTTPITKE MEPNDIIEA NGPIVEGVTV
 KGDNLGSDA DTFYFDVKED GDVTIELPYS GSNFTWLVY KEGDDQNHIA
 SGIDKNNSKV GTFKSTKGRH YVFIYKHDSA SNISYSLNIK GLGNEKLEKE
 ENNDSSDKAT VIPNFNTTMQ GSSLGDSRD YSFEVKEEG EVNIELDKKD
 EFGVTWTLHP ESNINDRITY GQVDGNKVSN KVKLKRPKYV LLVYKYSGG
 NYELRVNK

C₅₀₉₉H₇₇₇₁N₁₃₂₉O₁₆₁₀S₁₄ 113,897 daltons (for β subtype)
 [[9001-12-1]].

DEFINITION

Collagenase I (EC 3.4.24.3), isolated from *Clostridium histolyticum* and encoded by *colG* gene (GenBank accession number BAA77453.1), is a key raw material used in the dissociation or destruction of a broad range of tissue types. Collagenase I is a metallo protease that acts as an endoprotease and also exhibits a tripeptidylcarboxypeptidase activity. It shows endopeptidic activity with the main cleavage site found in front of the human collagen duplex amino acids glycine–proline. Hydrolysis takes place near the ends of the triple helical domains of collagen. Collagenase I is also known as class I collagenase and consists of three subtypes: α , β , and Γ . Collagenase I β is the full-length enzyme while collagenase I α (68,000 Da) and collagenase I Γ (79,000 Da) are thought to be proteolytic degradation products of collagenase I β caused by other proteases present in *C. histolyticum* (mainly a trypsin-like enzyme and clostripain/endoproteinase Arg C).

Collagenase I can be provided in a liquid formulation consisting of 5 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and 1 mM calcium chloride pH 7.5, and stored as a frozen liquid. The specific activity of collagenase I is 0.10–0.60 Units/mg of protein using 4-phenylazobenzoyloxycarbonyl-Pro-Leu-Gly-Pro-d-Arg as the substrate described in the *Assay*. The peak area for collagenase I is NLT 90% as determined by HPLC described in the test for *Purity*. The test for *Clostripain activity* is used to assess the activity of the clostripain impurity and the acceptance criterion is NMT 0.5 Units/mg of protein. The test for *Trypsin Activity* is used to assess the activity of the trypsin-like enzyme impurity and the acceptance criterion is NMT 0.5 Units/mg of protein.

IDENTIFICATION

- **A.** It meets the requirements in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Purity*.

ASSAY

• Procedure

Tris buffer: 0.1 M Tris pH 7.1, prepared as follows. Dissolve 6.05 g of tris(hydroxymethyl)aminomethane (Tris) in 400 mL of water, and adjust with 2 N hydrochloric acid to a pH of 7.1 (at 25 ± 1°). Dilute with water to a final volume of 500

mL.

Substrate solution: Dissolve 10 mg of 4-phenylazobenzoyloxycarbonyl (PZ)-Pro-Leu-Gly-Pro-d-Arg in 0.2 mL of methanol, and dilute this solution with *Tris buffer* to a final volume of 10 mL. [Note—Use a freshly prepared solution only.]

Calcium chloride solution: 0.1 M, prepared as follows. Weigh 1.47 g of calcium chloride dihydrate in a volumetric flask, and dilute with water to a final volume of 100 mL.

Citric acid solution: 0.025 M, prepared as follows. Weigh 525 mg of citric acid monohydrate in a volumetric flask, and dilute with water to a final volume of 100 mL.

Extraction mixture: To one test tube per sample to be assayed, pipet 5.0 mL of ethyl acetate, and 1.0 mL of *Citric acid solution*. [Note—Use a freshly prepared mixture only.]

Drying tube: Into one test tube per sample to be assayed, add 0.35–0.40 g of sodium sulfate anhydrous. Seal the test tube with parafilm.

Standard solution: Dilute USP Collagenase I RS with *Tris buffer* in the range of 1:50 to 1:100 (v/v). [Note—Avoid freezing and thawing USP Collagenase I RS. After withdrawing USP Collagenase I RS, wipe off the outside of the plastic pipette tips to remove any residual solution.]

Sample solution: Dilute Collagenase I with *Tris buffer* to an appropriate dilution to achieve the absorbance range of 0.3–0.9 from the *Analysis*. Prepare in triplicate. [Note—Avoid freezing and thawing the collagenase I sample. After withdrawing the collagenase I sample, wipe off the outside of the tip to remove any residual solution.]

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 320 nm

Pathlength: 1 cm

Temperature: 25°

Analysis

Samples: *Standard solution* and *Sample solution*

Transfer 1.0 mL of *Substrate solution* and 0.2 mL of *Calcium chloride solution* into a test tube, and equilibrate the test tube to 25°. Start the reaction by adding 0.05 mL of *Standard solution* or *Sample solution*. Prepare a blank by replacing the *Standard solution* or *Sample solution* with 0.05 mL of *Tris buffer*. Mix and incubate for exactly 15 min at 25°. Transfer 0.5 mL of the reaction to the test tube containing 6.0 mL of *Extraction mixture*. Vortex immediately for 20 s. Transfer 3 mL of the ethyl acetate phase (upper layer) into a *Drying tube* using a glass pipette, and vortex thoroughly. Transfer the supernatant to a disposable, semi-micro cuvette suitable for UV absorbance with a Pasteur pipette. Record the absorbance.

Calculate the activity of collagenase I in Units/mL:

$$\text{Result} = (A - A_B) \times [V_T \times V_E / (\epsilon \times V \times V_R \times B \times T)] \times D$$

A = absorbance of the *Standard solution* or *Sample solution*

A_B = absorbance of the blank

V_T = volume of the reaction mixture, 1.25 mL

V_E = volume of ethyl acetate in the *Extraction mixture*, 5.0 mL

ϵ = extinction coefficient for 320 nm, 21 ($\mu\text{mol}^{-1}\cdot\text{cm}^{-1}$)

V = volume of the *Standard solution* or *Sample solution*, 0.05 mL

V_R = volume of the reaction transferred to *Extraction mixture*, 0.5 mL

B = absorption pathlength, 1 cm

T = incubation time, 15 min

D = dilution factor

[Note—One Unit will release the equivalent of 1 μmol of PZ-Pro-Leu from PZ-Pro-Leu-Gly-Pro-d-Arg per min under the conditions of the Assay.]

Calculate the specific activity of collagenase I in Units/mg of protein:

$$\text{Result} = \text{Activity}/C$$

Activity = activity of collagenase I (Units/mL)

C = protein concentration (mg/mL)

System suitability

Samples: *Standard solution* and *Sample solution*

Suitability requirements

Average calculated activity: 90%–110% of the value on the label, *Standard solution*

Absorbance: 0.3–0.9, *Standard solution* and *Sample solution*

Acceptance criteria: 0.10–0.60 Units/mg of protein

PURITY

• Procedure

Solution A: 20 mM Tris and 1 mM calcium chloride pH 7.5, prepared as follows. Dissolve 2.42 g of Tris and 147 mg of calcium chloride dihydrate in 900 mL of water. Adjust with 2 N hydrochloric acid to a pH of 7.5. Dilute with water to a final volume of 1000 mL.

Solution B: 20 mM Tris, 1 mM calcium chloride, and 1 M sodium chloride pH 7.5, prepared as follows. Dissolve 2.42 g of Tris, 147 mg of calcium chloride dihydrate, and 58.44 g of sodium chloride in 900 mL of water. Adjust with 2 N hydrochloric acid to a pH of 7.5. Dilute with water to a final volume of 1000 mL.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
22	85	15
32	0	100
34	100	0
40	100	0

Storage buffer: 5 mM HEPES and 1 mM calcium chloride pH 7.5, prepared as follows. Dissolve 1.19 g of HEPES and 147 mg of calcium chloride dihydrate in 900 mL of water. Adjust with 4 N sodium hydroxide solution to a pH of 7.5. Dilute with water to a final volume of 1000 mL.

Standard solution: Thaw USP Collagenase I RS at room temperature shortly before use and mix. Store on ice or at 5°. Dilute with *Storage buffer* to achieve a protein concentration of 5.5 mg/mL. Transfer to an HPLC vial and keep at 5°. Prepare in duplicate and inject each duplicate once.

Collagenase II solution: Thaw USP Collagenase II RS at room temperature shortly before use and mix. Store on ice or at 5°. Dilute with *Storage buffer* to achieve a protein concentration of 5.5 mg/mL. Transfer to an HPLC vial and keep at 5°. Prepare in duplicate, and inject each duplicate once.

Sample solution: Dilute Collagenase I with *Storage buffer* to achieve a protein concentration of 5.5 mg/mL and keep at 5°.

Blank: *Storage buffer*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 5-mm × 5-cm; 10-µm packing L91

Temperatures

Column: 25°

Autosampler: 5°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirement: The chromatogram from the *Standard solution* corresponds to the typical chromatogram provided with USP Collagenase I RS.

Analysis

Sample: *Sample solution*

The *Blank* should be considered for integration. Identify the peak corresponding to collagenase II by comparing its retention time to the main peak from the *Collagenase II solution*. Evaluate the purity of Collagenase I using the area-% method but excluding peaks associated with the *Blank*. All shoulders in the fronting and tailing are separated by dropping a perpendicular line at the main peak. The time of integration is approximately 25 min.

Acceptance criteria: NLT 90% for the main peak of collagenase I and NMT 3% of collagenase II

IMPURITIES

- **Clostripain Activity**

Potassium phosphate buffer: 0.1 M pH 7.6, prepared as follows. Dissolve 1.36 g of monobasic potassium phosphate in water, and dilute to 100 mL. Dissolve 2.28 g of dibasic potassium phosphate trihydrate in water, and dilute to 100 mL. Adjust the pH of the second solution to 7.6 with the first solution.

Dithiothreitol solution: 0.194 M, prepared as follows. Dissolve 60 mg of dithiothreitol (DTT) in 2 mL of *Potassium phosphate buffer*.

Calcium chloride solution: 0.01 M, prepared as follows. Dissolve 147 mg of calcium chloride dihydrate in 100 mL of water.

Substrate stock solution: 38 mM, prepared as follows. Dissolve 13 mg of benzoyl-L-arginine ethyl ester hydrochloride (BAEE·HCl) in 1 mL of *Potassium phosphate buffer*.

Substrate solution: 0.73 mM BAEE·HCl, 7.8 mM DTT, and 0.4 mM calcium chloride, prepared as follows. Transfer 0.5 mL of *Substrate stock solution*, 1.0 mL of *Dithiothreitol solution*, and 1.0 mL of *Calcium chloride solution* to a 25-mL volumetric flask, and dilute with *Potassium phosphate buffer* to volume.

Sample solution: Prepare in such a way that $\Delta A/\text{min}$ lies in the 0.02–0.06 range. Dilute with ice-cold *Potassium phosphate buffer* if necessary.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Pathlength: 1 cm

Analytical wavelength: 255 nm

Temperature: 25°

Analysis

Sample: *Sample solution*

Transfer 3.0 mL of *Substrate solution* into a cuvette, and equilibrate the cuvette to 25°. Start the reaction by adding 0.05 mL of *Sample solution*. Prepare a blank by replacing the *Sample solution* with 0.05 mL of *Potassium phosphate buffer*. Mix well. Determine the change in absorbance ($\Delta A/\text{min}$) from the linear range of the reaction. Assay the *Sample solution* in triplicate.

System suitability

Sample: *Sample solution*

Suitability requirement: 0.02–0.06 for $\Delta A/\text{min}$

Calculate the activity of clostripain in Units/mL in the portion of collagenase I taken:

$$\text{Result} = [V_T / (\epsilon \times V_U \times B)] \times \Delta A/\text{min} \times D$$

V_T = volume of the reaction mixture, 3.05 mL

ϵ = extinction coefficient for 255 nm, $0.81(\text{L} \cdot \text{mmol}^{-1} \cdot \text{cm}^{-1})$

V_U = volume of the *Sample solution*, 0.05 mL

B = absorption pathlength, 1 cm

$\Delta A/\text{min}$ = change in absorbance from the linear range of the reaction

D = dilution factor

Calculate the specific activity in Units/mg of protein:

$$\text{Result} = \text{Activity}/C$$

Activity = activity of clostripain (Units/mL)

C = protein concentration (mg/mL)

Acceptance criteria: NMT 0.5 Units of clostripain activity per mg of protein

• Trypsin Activity

Buffer: 0.1 M Tris and 0.02 M calcium chloride pH 8.0, prepared as follows. Dissolve 6.05 g of Tris and 1.45 g of calcium chloride dihydrate in 400 mL of water. Adjust with 2 N hydrochloric acid to a pH of 8.0 (at $25 \pm 1^\circ$). Dilute with water to a final volume of 500 mL.

Substrate stock solution: Dissolve 10 mg of carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate,¹ accurately weighed, in 1.5 mL of water. Store on ice. [Note—Use freshly prepared solution only.]

Substrate solution: Prepare a solution by mixing 9.2 mL of *Buffer* and 1.0 mL of *Substrate stock solution*. Store on ice. [Note—Use freshly prepared solution only.]

Sample solution: Undiluted Collagenase I solution

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: Visible

Analytical wavelength: 405 nm

Pathlength: 1 cm

Temperature: 25°

Analysis

Sample: *Sample solution*

Transfer 1.02 mL of *Substrate solution* into a polystyrene, semi-micro cuvette, allow the temperature to stabilize, equilibrate the cuvette to 25° , and wait for 10 min. Start the reaction by adding 0.10 mL of *Sample solution*. Start recording the absorbance and continue for at least 5 min after the addition of the *Sample solution*. Determine the change in absorbance ($\Delta A/\text{min}$) from the linear range of the reaction. Assay the *Sample solution* in triplicate. [Note—Use polyethylene pipette tips to transfer the *Sample solution*. The pipette tip should not be wet before transfer, and each pipette tip should be used only for transferring one sample. After withdrawing the *Sample solution*, wipe off the outside of the tip to remove any residual solution. After adding the *Sample solution* to the *Substrate solution*, rinse the tip by pipetting the solution up and down 2–3 times, discard the tip, and mix.]

System suitability

Sample: *Sample solution*

Suitability requirement: >0.01 for $\Delta A/\text{min}$

Calculate the activity of trypsin in Units/mL in the portion of Collagenase I taken:

$$\text{Result} = [V_T / (\epsilon \times V_U \times B)] \times \Delta A/\text{min} \times D$$

V_T = volume of the reaction mixture, 1.12 mL

ϵ = extinction coefficient for 405 nm, $10.4 \text{ (mmol}^{-1} \cdot \text{l cm}^{-1}\text{)}$

V_U = volume of the *Sample solution*, 0.10 mL

B = absorption pathlength, 1 cm

$\Delta A/\text{min}$ = change in absorbance from the linear range of the reaction

D = dilution factor

Calculate the specific activity in Units/mg of protein

$$\text{Result} = \text{Activity}/C$$

Activity = activity of trypsin (Units/mL)

C = protein concentration (mg/mL)

Acceptance criteria: NMT 0.5 Units of trypsin activity per mg of protein

SPECIFIC TESTS

• Protein Content

Sample solutions: Dilute Collagenase I in water. Prepare at least in triplicate.

[Note—Prepare the dilution using plastic pipette tips and not glass pipettes. Carefully wipe off the outside of the tip to remove any residual solution.]

Blank solution: Water

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 280 nm

Pathlength: 1 cm

System suitability

Samples: *Sample solutions*

Suitability requirement: Absorbance is in the range of 0.10–1.00.

Analysis

Samples: *Sample solutions* and *Blank solution*

Determine the net absorbance of *Sample solutions* by subtracting the absorbance of the *Blank solution* from the absorbance of the *Sample solution*. Determine the average net absorbance of *Sample solutions*.

Calculate the protein concentration in mg/mL:

$$\text{Result} = A_U \times D / \epsilon$$

A_U = average net absorbance of the *Sample solutions*
 D = dilution factor
 ϵ = extinction coefficient ($A_{280}1\%/cm$) for collagenase, 1.4

- **Bacterial Endotoxins Test** (85): NMT 50 USP Endotoxin Units/mg of protein
- **Microbial Enumeration Tests** (61): The total bacterial count is NMT 100 cfu/mL.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store in closed containers at -15° to -25° .
 - **Labeling:** The labeling states that the material is derived from *Clostridium histolyticum* along with the lot number, product or catalog number, and storage conditions.
 - **USP Reference Standards** (11)
 - USP Collagenase I RS
 - USP Collagenase II RS
 - USP Endotoxin RS
- 2S (USP39)

¹ A suitable carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate is Chromozym TRY from Roche Applied Science (catalog number 10378496103) or equivalent.

BRIEFING

(89.2) **Collagenase II.** This new proposed general chapter is to provide analytical procedures to aid in the assessment of quality for collagenase II isolated from *Clostridium histolyticum*. This chapter, *Collagenase II* (89.2), is part of a series of general chapters that are under development to address the qualities of the enzymes used as ancillary material in pharmaceutical manufacturing. Another proposed chapter in the series, *Collagenase I* (89.1), also appears in this issue of *PF*. Recombinant trypsin is the first enzyme to be introduced in this manner, and was published in *Enzymes Used As Ancillary Material in Pharmaceutical Manufacturing* (89).

This chapter does not discuss the applications of collagenase II but rather focuses on tests to assess the qualities as a process material. Furthermore, this chapter does not provide ways to limit the application of collagenase II in the final medicinal product. The activity of collagenase II is determined by using the chromogenic peptide substrate 4-phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-d-Arg. The liquid chromatographic procedure in the test for *Purity* is based on analyses performed with the GE Healthcare Mono Q 5/50 GL brand of L91 column. A new Reference Standard, USP Collagenase II RS, is proposed for use during assessment of the system suitability for the proposed *Assay* and test for *Purity*.

(BIO2: E. Chang.)

Correspondence Number—C153138

Comment deadline: November 30, 2015

Add the following:

■ (89.2) COLLAGENASE II

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VQNESKRYTV SYLKLNYVD LVDLLVKEI ENLPDLFQYS SDAKEFYGNK
TRMSFIMDEI GRRAPQYTEI DHKGIPTLVE VVRAGFYLGF HNKELNEINK
RSFKERVIPS ILAIQKNPNF KLGTEVQDKI VSATGLLAGN ETAPPEVNN
FTPIIQDCIK NIDRYALDDL KSKALFNLA APTYDITEYL RATKEKPENT
PWYKIDGFI NELKLLALYG KINNNNSWII DNGIYHIAPL GKLSNNKIG
IETLTEMKV YPYLSMHLQ SADQIKRHVD SKDAEGNKIP LDKFKEGKE
KYCPTYTFD DGKVIKAGA RVEEEKVRL YWASKEVNSQ FFRVYGDIP
LEEGNPDZIL TMVIYNPEE YKLSNVLGY DTNNGMYIE PEGTFFTYER
EAQESTYLE ELFRHEYTHY LQGRYAVPGQ WGRTKLYDND RLWYEEGGA
ELFAGSTRTS GILPRKSLVS NIHNTTRNRR YKLSDTVHSK YGASFIFYNY
ACMFMDMYN KDMGILNKLN DLAKNNDVDG YDNYIRLSS NYALNDKYQD
HMQERIDNYE NLTVPFVADD YLVRHAYKNP NEIYSEISEV AKLKDAKSEV
KKSQYFSTFT LRGSYTGAS KGKLEDQKAM NKFIIDSLKK LDTYSWSGYK
TLTAYFTNYK VDSSNRVTYD VVFHGYL PNE GDSKNSLPYG KINGTYKGT
KEKIKFSSEG SFDPDGKIVS YEWDFGDGN SNEENPEHSY DKVGTYYVKL
KVTDDKGESS VSTTTAEIKD LSENKLPVYI MHVPKSGALN QKVVFYKGT
YDPDGSIAGY QWDFDGSDF SSEQNPSHY TKKGEYTVL RVMDSSGQMS
EKTMKIKITD PVPYIGTEKE PNNKETSAG PIVPGIPVSG TIENSDQDY
FYFDVITPGE VKIDINKLGY GGATWVYDE NNAVSVYATD DGQNLSGFKF
ADKPGRYIYH LYMFNGSYMP YRINIEGVSV R

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C₅₀₂₈H₇₆₆₆N₁₃₀₀O₁₅₆₄S₂₁ 112,023 (for ζ subtype)
[[9001-12-1]].

DEFINITION

Collagenase II (EC 3.4.24.3), isolated from *Clostridium histolyticum* and encoded by colH gene (GenBank accession number BAA06251.1), is a key raw material used in the dissociation or destruction of a broad range of tissue types. Collagenase II is a metallo protease that acts as an endoprotease and also exhibits a tripeptidylcarboxypeptidase activity. It shows endopeptidic activity with the main cleavage site found in front of the human collagen duplex amino acids glycine–proline. Hydrolysis takes place in the interior of the triple helical domains of collagen.

Collagenase II is also known as class II collagenase and consists of three subtypes: Δ, ε, and ζ. Collagenase II ζ is the full-length enzyme while collagenase II Δ (100,000 Da) and II ε (110,000 Da) are thought to be proteolytic degradation products of collagenase II ζ caused by other proteases present in *C. histolyticum* (mainly a trypsin-like enzyme and clostripain/endoproteinase Arg C).

Collagenase II can be provided in a liquid formulation consisting of 5 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and 1 mM calcium chloride pH 7.5, and stored as a frozen liquid. The specific activity of collagenase II is 10–16 Units/mg of protein using 4-phenylazobenzoyloxycarbonyl-Pro-Leu-Gly-Pro-d-Arg as the substrate described in the Assay. The peak area for collagenase II is NLT 90% as determined by HPLC described in the test for Purity. The test for Clostripain Activity is used to assess the activity of the clostripain impurity and the acceptance criterion is NMT 0.5 Units/mg of protein. The test for Trypsin Activity is used to assess the activity of the trypsin-like enzyme impurity and the acceptance criterion is NMT 0.5 Units/mg of protein.

IDENTIFICATION

- **A.** It meets the requirements in the Assay.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for Purity.

ASSAY

• Procedure

Tris buffer: 0.1 M Tris pH 7.1, prepared as follows. Dissolve 6.05 g of tris(hydroxymethyl)aminomethane (Tris) in 400 mL of water, and adjust with 2 N hydrochloric acid to a pH of 7.1 (at 25 ± 1°). Dilute with water to a final volume of 500

mL.

Substrate solution: Dissolve 10 mg of 4-phenylazobenzoyloxycarbonyl (PZ)-Pro-Leu-Gly-Pro-d-Arg in 0.2 mL of methanol, and dilute this solution with *Tris buffer* to a final volume of 10 mL. [Note—Use a freshly prepared solution only.]

Calcium chloride solution: 0.1 M, prepared as follows. Weigh 1.47 g of calcium chloride dihydrate in a volumetric flask, and dilute with water to a final volume of 100 mL.

Citric acid solution: 0.025 M, prepared as follows. Weigh 525 mg of citric acid monohydrate in a volumetric flask, and dilute with water to a final volume of 100 mL.

Extraction mixture: To one test tube per sample to be assayed, pipet 5.0 mL of ethyl acetate, and 1.0 mL of *Citric acid solution*. [Note—Use a freshly prepared mixture only.]

Drying tube: Into one test tube per sample to be assayed, add 0.35–0.40 g of sodium sulfate anhydrous. Seal the test tube with parafilm.

Standard solution: Dilute USP Collagenase II RS with *Tris buffer* in the range of 1:2000 to 1:4000 (v/v). [Note—Avoid freezing and thawing USP Collagenase II RS. After withdrawing USP Collagenase II RS, wipe off the outside of the plastic pipette tips to remove any residual solution.]

Sample solution: Dilute Collagenase II with *Tris buffer* to an appropriate dilution to achieve the absorbance range of 0.3–0.9 from the *Analysis*. Prepare in triplicate. [Note—Avoid freezing and thawing the collagenase II sample. After withdrawing the collagenase II sample, wipe off the outside of the tip to remove any residual solution.]

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 320 nm

Pathlength: 1 cm

Temperature: 25°

Analysis

Samples: *Standard solution* and *Sample solution*

Transfer 1.0 mL of *Substrate solution* and 0.2 mL of *Calcium chloride solution* into a test tube, and equilibrate the test tube to 25°. Start the reaction by adding 0.05 mL of *Standard solution* or *Sample solution*. Prepare a blank by replacing the *Standard solution* or *Sample solution* with 0.05 mL of *Tris buffer*. Mix and incubate for exactly 15 min at 25°. Transfer 0.5 mL of the reaction to the test tube containing 6.0 mL of *Extraction mixture*. Vortex immediately for 20 s. Transfer 3 mL of the ethyl acetate phase (upper layer) into a *Drying tube* using a glass pipette, and vortex thoroughly. Transfer the supernatant to a disposable, semi-micro cuvette suitable for UV absorbance with a Pasteur pipette. Record the absorbance.

Calculate the activity of collagenase II in Units/mL:

$$\text{Result} = (A - A_B) \times [V_T \times V_E / (\epsilon \times V \times V_R \times B \times T)] \times D$$

A = absorbance of the *Standard solution* or *Sample solution*

A_B = absorbance of the blank

V_T = volume of the reaction mixture, 1.25 mL

V_E = volume of ethyl acetate in the *Extraction mixture*, 5.0 mL

ϵ = extinction coefficient for 320 nm, 21 ($\mu\text{mol}^{-1}\cdot\text{cm}^{-1}$)

V = volume of the *Standard solution* or *Sample solution*, 0.05 mL

V_R = volume of the reaction transferred to *Extraction mixture*, 0.5 mL

B = absorption pathlength, 1 cm

T = incubation time, 15 min

D = dilution factor

[Note—One Unit will release the equivalent of 1 μmol of PZ-Pro-Leu from PZ-Pro-Leu-Gly-Pro-d-Arg per min under the conditions of the Assay.]

Calculate the specific activity of collagenase II in Units/mg of protein:

$$\text{Result} = \text{Activity}/C$$

Activity = activity of collagenase II (Units/mL)

C = protein concentration (mg/mL)

System suitability

Samples: *Standard solution* and *Sample solution*

Suitability requirements

Average calculated activity: 90%–110% of the value on the label, *Standard solution*

Absorbance: 0.3–0.9, *Standard solution* and *Sample solution*

Acceptance criteria: 10–16 Units/mg of protein

PURITY

• Procedure

Solution A: 20 mM Tris and 1 mM calcium chloride pH 7.5, prepared as follows. Dissolve 2.42 g of Tris and 147 mg of calcium chloride dihydrate in 900 mL of water. Adjust with 2 N hydrochloric acid to a pH of 7.5. Dilute with water to a final volume of 1000 mL.

Solution B: 20 mM Tris, 1 mM calcium chloride, and 1 M sodium chloride pH 7.5, prepared as follows. Dissolve 2.42 g of Tris, 147 mg of calcium chloride dihydrate, and 58.44 g of sodium chloride in 900 mL of water. Adjust with 2 N hydrochloric acid to a pH of 7.5. Dilute with water to a final volume of 1000 mL.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
22	85	15
32	0	100
34	100	0
40	100	0

Storage buffer: 5 mM HEPES and 1 mM calcium chloride pH 7.5, prepared as follows. Dissolve 1.19 g of HEPES and 147 mg of calcium chloride dihydrate in 900 mL of water. Adjust with 4 N sodium hydroxide solution to a pH of 7.5. Dilute with water to a final volume of 1000 mL.

Standard solution: Thaw USP Collagenase II RS at room temperature shortly before use and mix. Store on ice or at 5°. Dilute with *Storage buffer* to achieve a protein concentration of 5.5 mg/mL. Transfer to an HPLC vial and keep at 5°. Prepare in duplicate and inject each duplicate once.

Sample solution: Dilute Collagenase II with *Storage buffer* to achieve a protein concentration of 5.5 mg/mL and keep at 5°.

Blank: *Storage buffer*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 5-mm × 5-cm; 10-µm packing L91

Temperatures

Column: 25°

Autosampler: 5°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirement: The chromatogram from the *Standard solution* corresponds to the typical chromatogram provided with USP Collagenase II RS.

Analysis

Sample: *Sample solution*

The *Blank* should be considered for integration. Evaluate the purity of Collagenase II using the area-% method but excluding peaks associated with the *Blank*. All shoulders in the fronting and tailing are separated by dropping a perpendicular line at the main peak. The time of integration is approximately 25 min.

Acceptance criteria: NLT 90% for the main peak of collagenase II

IMPURITIES

• Clostripain Activity

Potassium phosphate buffer: 0.1 M pH 7.6, prepared as follows. Dissolve 1.36 g of monobasic potassium phosphate in water, and dilute to 100 mL. Dissolve 2.28 g of dibasic potassium phosphate trihydrate in water, and dilute to 100 mL. Adjust the pH of the second solution to 7.6 with the first solution.

Dithiothreitol solution: 0.194 M, prepared as follows. Dissolve 60 mg of dithiothreitol (DTT) in 2 mL of *Potassium phosphate* buffer.

Calcium chloride solution: 0.01 M, prepared as follows. Dissolve 147 mg of calcium chloride dihydrate in 100 mL of water.

Substrate stock solution: 38 mM, prepared as follows. Dissolve 13 mg of benzoyl-L-arginine ethyl ester hydrochloride (BAEE·HCl) in 1 mL of *Potassium phosphate buffer*.

Substrate solution: 0.73 mM BAEE·HCl, 7.8 mM DTT, and 0.4 mM calcium chloride, prepared as follows. Transfer 0.5 mL of *Substrate stock solution*, 1.0 mL of *Dithiothreitol solution*, and 1.0 mL of *Calcium chloride solution* to a 25-mL volumetric flask, and dilute with *Potassium phosphate buffer* to volume.

Sample solution: Prepare in such a way that $\Delta A/\text{min}$ lies in the 0.02–0.06 range. Dilute with ice-cold *Potassium phosphate buffer* if necessary.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Pathlength: 1 cm

Analytical wavelength: 255 nm

Temperature: 25°

Analysis

Sample: *Sample solution*

Transfer 3.0 mL of *Substrate solution* into a cuvette, and equilibrate the cuvette to 25°. Start the reaction by adding 0.05 mL of *Sample solution*. Prepare a blank by replacing the *Sample solution* with 0.05 mL of *Potassium phosphate buffer*. Mix well. Determine the change in absorbance ($\Delta A/\text{min}$) from the linear range of the reaction. Assay the *Sample solution* in triplicate.

System suitability

Sample: *Sample solution*

Suitability requirement: 0.02–0.06 for $\Delta A/\text{min}$

Calculate the activity of clostripain in Units/mL in the portion of collagenase II taken:

$$\text{Result} = [V_T / (\epsilon \times V_U \times B)] \times \Delta A/\text{min} \times D$$

V_T = volume of the reaction mixture, 3.05 mL

ϵ = extinction coefficient for 255 nm, $0.81(\text{L} \cdot \text{mmol}^{-1} \cdot \text{cm}^{-1})$

V_U = volume of the *Sample solution*, 0.05 mL

B = absorption pathlength, 1 cm

$\Delta A/\text{min}$ = change in absorbance from the linear range of the reaction

D = dilution factor

Calculate the specific activity in Units/mg of protein:

$$\text{Result} = \text{Activity}/C$$

Activity = activity of clostripain (Units/mL)

C = protein concentration (mg/mL)

Acceptance criteria: NMT 0.5 Units of clostripain activity per mg of protein

● **Trypsin Activity**

Buffer: 0.1 M Tris and 0.02 M calcium chloride pH 8.0, prepared as follows. Dissolve 6.05 g of Tris and 1.45 g of calcium chloride dihydrate in 400 mL of water. Adjust with 2 N hydrochloric acid to a pH of 8.0 (at $25 \pm 1^\circ$). Dilute with water to a final volume of 500 mL.

Substrate stock solution: Dissolve 10 mg of carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate,¹ accurately weighed, in 1.5 mL of water. Store on ice. [Note—Use freshly prepared solution only.]

Substrate solution: Prepare a solution by mixing 9.2 mL of *Buffer* and 1.0 mL of *Substrate stock solution*. Store on ice. [Note—Use freshly prepared solution only.]

Sample solution: Undiluted Collagenase II solution

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: Visible

Analytical wavelength: 405 nm

Pathlength: 1 cm

Temperature: 25°

Analysis

Sample: *Sample solution*

Transfer 1.02 mL of *Substrate solution* into a polystyrene semi-micro cuvette, allow the temperature to stabilize, equilibrate the cuvette to 25°, and wait for 10 min. Start the reaction by adding 0.10 mL of *Sample solution*. Start recording the absorbance and continue for at least 5 min after the addition of the *Sample solution*. Determine the change in absorbance ($\Delta A/\text{min}$) from the linear range of the reaction. Assay the *Sample solution* in triplicate. [Note—Use polyethylene pipette tips to transfer the *Sample solution*. The pipette tip should not be wet before transfer, and each pipette tip should be used only for transferring one sample. After withdrawing the *Sample solution*, wipe off the outside of the tip to remove any residual solution. After adding the *Sample solution* to the *Substrate solution*, rinse the tip by pipetting the solution up and down 2–3 times, discard the tip, and mix.]

System suitability

Sample: *Sample solution*

Suitability requirement: >0.01 for $\Delta A/\text{min}$

Calculate the activity of trypsin in Units/mL in the portion of Collagenase II taken:

$$\text{Result} = [V_T / (\epsilon \times V_U \times B)] \times \Delta A/\text{min} \times D$$

V_T = volume of the reaction mixture, 1.12 mL

ϵ = extinction coefficient for 405 nm, $10.4 \text{ (mmol}^{-1} \cdot \text{l cm}^{-1}\text{)}$

V_U = volume of the *Sample solution*, 0.10 mL

B = absorption pathlength, 1 cm
 $\Delta A/\text{min}$ = change in absorbance from the linear range of the reaction
 D = dilution factor

Calculate the specific activity in Units/mg of protein:

$$\text{Result} = \text{Activity}/C$$

Activity = activity of trypsin (Units/mL)
 C = protein concentration (mg/mL)

Acceptance criteria: NMT 0.5 Units of trypsin activity per mg of protein

SPECIFIC TESTS

• Protein Content

Sample solutions: Dilute Collagenase II in water. Prepare at least in triplicate.

[Note—Prepare the dilution using plastic pipette tips and not glass pipettes. Carefully wipe off the outside of the tip to remove any residual solution.]

Blank solution: Water

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 280 nm

Pathlength: 1 cm

System suitability

Samples: *Sample solutions*

Suitability requirement: Absorbance is in the range of 0.10–1.00.

Analysis

Samples: *Sample solutions* and *Blank solution*

Determine the net absorbance of *Sample solutions* by subtracting the absorbance of the *Blank solution* from the absorbance of the *Sample solution*. Determine the average net absorbance of *Sample solutions*.

Calculate the protein concentration in mg/mL:

$$\text{Result} = A_U \times D / \epsilon$$

A_U = average net absorbance of the *Sample solutions*

D = dilution factor

ϵ = extinction coefficient ($A_{280}1\%/cm$) for collagenase, 1.4

- **Bacterial Endotoxins Test** (85): NMT 50 USP Endotoxin Units/mg of protein
- **Microbial Enumeration Tests** (61): The total bacterial count is NMT 100 cfu/mL.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store in closed containers at -60° to -90° .

- **Labeling:** The labeling states that the material is derived from *Clostridium histolyticum* along with the lot number, product or catalog number, and storage conditions.
- **USP Reference Standards** (11)
 - USP Collagenase II RS
 - USP Endotoxin RS
- 2S (USP39)

¹ A suitable carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate is Chromozym TRY from Roche Applied Science (catalog number 10378496103) or equivalent.

BRIEFING

(121.1) **Physicochemical Analytical Procedures for Insulins**, *USP 38* page 195. On the basis of comments received, it is proposed to revise the chapter as follows:

1. Incorporate animal derived insulins prepared from bovine and porcine pancreas into the chapter.
2. In *Peptide Mapping*, clarify the *Tailing factor* requirements, and better define the activity units of *Staphylococcus aureus* strain V8 (*S. aureus* V8) protease.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(BIO1: M. Crivellone.)

Correspondence Number—C157594

Comment deadline: November 30, 2015

(121.1) PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS

INTRODUCTION

Change to read:

Several physicochemical procedures are used for the assessment of the quality attributes of Insulin Human, and

■ ■ 2S (USP39)

various insulin analogues,

■ and animal derived insulins, ■ 2S (USP39)

hereafter called insulins. Of these procedures, peptide mapping and determination of high molecular weight proteins are similar for the various insulin drug substances and drug products. This chapter describes the tests for peptide mapping and for quantitative analysis of high molecular weight proteins that can be used for the various insulins.

Specific instructions that deviate from the general procedures outlined here are given in the respective drug substance or drug product monographs for the different insulins.

Change to read:

PEPTIDE MAPPING

Insulin digestion is performed using *Staphylococcus aureus* strain V8 (*S. aureus* V8) protease

■ (also referred to as Endoproteinase Glu-C), ■ 2S (USP39)

a serine endoproteinase that cleaves at the C-terminal side of glutamyl and aspartyl residues

in phosphate buffer at pH 7.8. This protease is specific for Glu-C digestion in ammonium bicarbonate or other non-phosphate-containing buffers at pH 7.8. The presence of proline on the carboxy side of the peptide bond inhibits cleavage. The buffer system used should cleave all glutamyl bonds in insulin without cleavage of the aspartyl bonds. In general, insulin is digested into four peptides in the buffer system described in the *Sample solution* below. The following show the amino acid differences of the insulin analogues compared to Insulin Human and the *in silico* differences in the fragments obtained upon digestion with *S. aureus* V8 protease.

Figure 1 shows the four fragments of Insulin Human following digestion with *S. aureus* V8 protease.

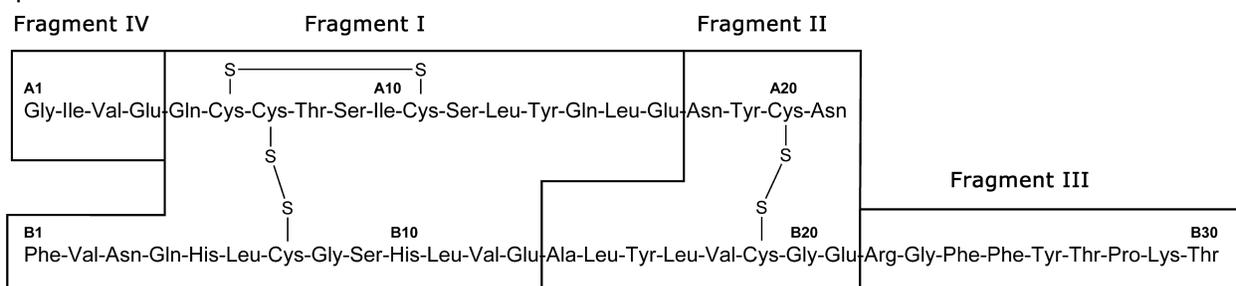


Figure 1. Insulin Human: *S. aureus* V8 protease digest fragments.

Table 1 shows the amino acid differences between insulin analogues,

■ animal derived insulins, ■ 2S (USP39) and Insulin Human.

Table 1. Amino Acid Differences between Insulin Analogues, ■ Animal Derived Insulins, ■ 2S (USP39) and Insulin Human

Insulin Analogue ■ 2S (USP39)	Amino Acid Differences from Insulin Human
Insulin aspart	B28 Asp
Insulin glargine	A21 Gly, two Arg added to C-terminus of B chain
Insulin glulisine	B3 Lys, B29 Glu
Insulin lispro	B28 Lys, B29 Pro
■ Insulin porcine	B30 Ala
Insulin bovine	A8 Ala, A10 Val, B30 Ala ■ 2S (USP39)

Table 2 shows specific *S. aureus* V8 protease digest fragments of insulin analogues

■ and animal derived insulins. ■ 2S (USP39)

Amino acid differences compared to Insulin Human are highlighted.

Table 2. Amino Acid Differences: Insulin Human, and ■ Insulin ■ 2S (USP39) Analogues, ■ and Animal Derived Insulins ■ 2S (USP39) (Differences with Insulin Human Are Shown in Boldface)

Insulin Analogue ■ ■ 2S (USP39)	Amino Acid Differences Compared to Insulin Human^a	
Insulin aspart	Fragment III	(B22) Arg-Gly-Phe-Phe-Tyr-Thr- Asp -Lys-Thr (B30)
Insulin glargine	Fragment II	(A18) Asn-Tyr-Cys- Gly (A21)
	Fragment III	(B22) Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Thr- Arg-Arg (B32)
Insulin glulisine	Fragment I	(B1) Phe-Val- Lys -Gln-His-Leu-Cys-Gly-Ser-His-Leu (B11)
	Fragment III	(B22) Arg-Gly-Phe-Phe-Tyr-Thr-Pro- Glu -Thr (B30)
Insulin lispro	Fragment III	(B22) Arg-Gly-Phe-Phe-Tyr-Thr- Lys-Pro -Thr (B30)
■ Insulin porcine	Fragment III	(B22) Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys- Ala (B30)
Insulin bovine	Fragment I	(A5) Gln-Cys-Cys- Ala -Ser- Val -Cys-Ser-Leu-Tyr-Gln-Leu-Glu (A17)
	Fragment III	(B22) Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys- Ala (B30) ■ 2S (USP39)
^a A and B denote the A and B chains of insulin, respectively; numbers denote amino acid position in the chain.		

● Peptide Mapping Procedure

The following procedure is applicable for preparing peptide maps of Insulin Human, and

■ ■ 2S (USP39)

insulin analogues,

■ and animal derived insulins. ■ 2S (USP39)

HEPES buffer: Dissolve 2.38 g of HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) in about 90 mL of water in a 100-mL volumetric flask. Adjust with 5 M sodium hydroxide to a pH of 7.5, dilute with water to volume, and mix.

Sulfate buffer: 2.0 M ammonium sulfate and 0.5 M sulfuric acid (1:1). Mix, and filter.

● Insulin Digestion

The following procedure provides efficient cleavage of the glutamyl bonds of insulin. [Note—Volumes up to 20-fold higher can be used as long as the ratio of the solutions remains the same. If interfering autolysis byproducts are observed in the chromatogram when a digest of the enzyme alone is run, the enzyme-to-insulin ratio must be decreased and digestion time must be increased.]

Enzyme solution: Prepare a 1-mg/mL solution of *S. aureus* V8 protease in water (approximately 500 units/mg

■ using casein as the substrate). ■ 2S (USP39)

Sample solution: Prepare a 2.0-mg/mL solution of the insulin to be examined in 0.01 N hydrochloric acid. To a clean vial add 25 μ L of the 2.0-mg/mL insulin solution, 100 μ L of HEPES buffer, and 20 μ L of Enzyme solution (final ratio is 25:100:20). Cap the vial, and

incubate at 25° for 6 h. Stop the reaction by adding an equal volume of *Sulfate buffer*. Longer incubation times may be needed for analogues with poor solubility at pH 7.5.

Standard solution: Prepare at the same time and in the same manner a solution of the appropriate USP Insulin Reference Standard as directed in the *Sample solution*.

- **Peptide Fragment Determination**

Determine the peptide fragments using the following peptide mapping procedure (see *Biotechnology-Derived Articles—Peptide Mapping* (1055)).

Solution A: Acetonitrile, water, and *Sulfate buffer* (100:700:200). Filter, and degas.

Solution B: Acetonitrile, water, and *Sulfate buffer* (400:400:200). Filter, and degas.

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	95	5
3	95	5
30	41	59
35	20	80
40	95	5
50	95	5

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 10-cm; 5-µm packing *L1*

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 50–100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Chromatogram comparability: In the chromatogram obtained from the *Standard solution*, identify the peaks due to digest fragments I, II, III, and IV. The chromatogram of the *Standard solution* corresponds to that of the typical chromatogram provided with the appropriate USP Insulin Reference Standard.

Resolution: There should be complete separation of the peaks due to fragments II and III. The resolution is defined in the applicable insulin monograph.

Tailing factor: NMT 1.5

■ for digest fragments II and III ■_{2S} (USP39)

Analysis

Samples: *Sample solution* and *Standard solution*

Condition the *Chromatographic system* by running at initial conditions, $t = 0$ min, for at least 15 min. Carry out a blank gradient program before injecting the digests. Separately inject equal volumes of the *Standard solution* and the *Sample solution*, and record the responses of each peak.

Acceptance criteria: The chromatographic profile of the *Sample solution* corresponds to that of the *Standard solution*.

LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS

- **Procedure**

Solution A: 1 mg/mL of L-arginine in water

Mobile phase: *Solution A*, acetonitrile, and glacial acetic acid (65:20:15). Filter, and degas.

Resolution solution: Store a suitable amount of insulin drug substance at room temperature for a sufficient period of time (5–10 days, or as needed) to obtain insulin with more than 0.4% high molecular weight proteins. Prepare a 4-mg/mL solution in 0.01 N hydrochloric acid. Store the solution in a refrigerator, and use within 7 days. Alternatively, dissolve about 4 mg of USP High Molecular Weight Insulin Human RS in 1 mL of 0.01 N hydrochloric acid.

Sample solution: In a small vial, prepare a 4-mg/mL solution of insulin in 0.01 N hydrochloric acid, and mix to dissolve. Store in a refrigerator, and use within 7 days.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 276 nm

Column: 7.8-mm × 30-cm; 5- to 10- μ m packing L20

Temperatures

Autosampler: It is advisable to use a refrigerated autosampler.

Column: Ambient

Flow rate: 0.5 mL/min

Injection volume: 100 μ L

System suitability

Sample: *Resolution solution*

Suitability requirements

Retention times: Between 13 and 17 min for the polymeric insulin complexes, about 17.5 min for the covalent insulin dimer, and between 18 and 22 min for the insulin monomer, with salts eluting after the insulin monomer

Peak-to-valley ratio: The ratio of the height of the covalent insulin dimer peak to the height of the valley between the covalent insulin dimer peak and the insulin monomer peak is NLT 2.0.

Analysis

Sample: *Sample solution*

Disregard any peaks with retention times greater than that of the insulin monomer.

Calculate the percentage of high molecular weight proteins in the portion of Insulin taken:

$$\text{Result} = \frac{\sum r_H}{(\sum r_H + r_M)} \times 100$$

$\sum r_H$ = sum of the responses of all peaks with retention times less than that of the insulin monomer

r_M = peak response of the insulin monomer

Acceptance criteria: As stated in the applicable insulin monograph

ADDITIONAL REQUIREMENTS

- **USP Reference Standards** <11>
USP High Molecular Weight Insulin Human RS (alternative, optional)

BRIEFING

<127> **Flow Cytometric Enumeration of CD34+ Cells.** The CD34+ Cells Expert Panel recommends and the Biologics and Biotechnology Monographs 3 Expert Committee proposes this new general chapter to provide a test method to enumerate CD34+ cells in samples of peripheral blood, leukapheresis products, bone marrow, and cord blood. CD34+ cell enumeration by flow cytometry is a rare event analysis; therefore, specific gating instructions are provided in the single-platform, flow cytometric method for CD34+ cell enumeration described in this chapter. A new Reference Standard (RS), USP CD34+ Cells RS, is proposed for use during assessment of the *System suitability requirements* for the method.

(BIO3: R. Potts.)

Correspondence Number—C158590

Comment deadline: November 30, 2015

Add the following:

▪ <127> **FLOW CYTOMETRIC ENUMERATION OF CD34+ CELLS**

INTRODUCTION

The CD34 antigen is expressed on the surface of almost all human hematopoietic stem and progenitor cells. The absolute number of CD34+ hematopoietic stem cells (HSCs) has been shown to correlate with in vitro colony-forming unit (CFU) assay activity and with clinical engraftment in hematopoietic grafts prepared from peripheral blood, bone marrow, and cord blood sources. The single-platform, flow cytometric CD34+ cell enumeration method described here is based on a clinical laboratory protocol,¹ established by the International Society of Hematotherapy and Graft Engineering (ISHAGE), now known as the International Society for Cellular Therapy (ISCT). This protocol can be used to enumerate CD34+ cells in samples of peripheral blood, leukapheresis products, bone marrow, and cord blood. CD34+ cell enumeration by flow cytometry is a rare event analysis, which requires specific gating instructions that are provided in this chapter. Furthermore, the USP CD34+ Cells RS has been developed to assess the reagents and ensure the correct gating during data acquisition and analysis.

IDENTIFICATION OF CD34+ HEMATOPOIETIC STEM CELLS

CD34+ cell enumeration by flow cytometry is a rare event analysis. For the analysis of CD34+ HSCs, cell samples are stained with fluorescently labeled antibodies against both the HSC antigen CD34 and the pan-leukocyte antigen CD45. Five parameters—forward light scatter (FSC), side light scatter (SSC), CD34 staining, CD45 staining, and viability dye staining are combined in a sequential, or Boolean, gating strategy to identify viable CD34+ cells. CD34+ HSCs have FSC and SSC characteristics similar to lymphocytes; expressing both CD45 and CD34, and exhibiting dim CD45 expression and low SSC characteristics. Viability dye does not stain live cells, allowing the exclusion of dead cells from the analysis of viable cell preparations. For analysis of nonviable, fixed (preserved) cell preparations such as the USP CD34+ Cells Reference Standard (RS), either the flow cytometer viable cell analysis gate is fully opened to include all cells, or the *Viability dye* is omitted from the analysis.

ENUMERATION CONSIDERATIONS

The CD34+ cell enumeration method described here relies on the use of synthetic fluorescent microspheres (counting beads) as internal enumeration controls. Homogeneous counting beads are added to the cell sample at a known concentration and volume, or the counting beads may be procured, pre-aliquoted, and lyophilized in special sample tubes, in which all subsequent cell staining and processing steps are conducted. To avoid the loss of counting beads, the wash steps are omitted, and protein-containing sample buffers are used. An ammonium chloride-based, red blood cell (RBC)-lysing protocol is used for fresh cell preparations; no lysing is necessary for frozen, thawed, or fixed cell preparations.

After the cells are stained and processed, the counting beads and cells are simultaneously analyzed on a flow cytometer. The number of CD34+ cells/ μL in the cell sample can be directly calculated by comparing the absolute number of target CD34+ cells and the number of counting beads detected in the same data file. The USP CD34+ Cells RS is used to verify that the correct reagents and flow cytometer gating parameters were used.

EQUIPMENT SPECIFICATIONS

The following equipment is needed:

- A pipettor capable of accurately reverse-pipetting microliter volumes

A flow cytometer with the following minimum specifications (see *Flow Cytometry* (1027)):

- Detection capabilities for FSC; SSC; "green" fluorescence emission (range, 510–550 nm); "yellow" fluorescence emission (range, 560–590 nm); and "red" fluorescence emission (>600 nm)
- Light-scatter and fluorescence measurements that are correlated to time
- Light-scatter resolution allowing for the identification of lymphocytes, monocytes, granulocytes, and fluorescent counting beads
- Detection and data acquisition rates of at least 5000 cells/s
- A 488-nm laser excitation source

- Fluorescence intensity sensitivity allowing for the detection of cellular autofluorescence
- Analysis software, including a data file structure in the Flow Cytometry Standard (FCS) or equivalent, with spectral overlap correction capability

FLOW CYTOMETER INSTRUMENT SETUP AND CONSIDERATIONS

Note—For general information, see <1027> and the flow cytometer manufacture's recommendations.

Ensure that the sheath fluid receptacle is filled, the waste fluid receptacle is empty, and all caps are tightly closed. Verify that the laser power, system vacuum, and pressure are properly set.

Using fluorochrome-conjugated calibration microspheres, verify proper system alignment by measuring the average detection rate, mean fluorescence, mean FSC, mean SSC, and the respective calculated coefficient of variance (CV) values as measured by each detector. All values should fall within the ranges recommended by the manufacturer of the flow cytometer instrument. Adjust the detector threshold to ensure that all counting bead events are included. Use either antibody-binding microspheres or preserved cells, such as the USP CD34+ Cells RS, to establish a matrix of detector values while adjusting for spectral overlap (compensation). Note that although either microspheres or cells can be stained with anti-CD34 phycoerythrin (PE) and anti-CD45 fluorescein isothiocyanate (FITC) antibodies, only fresh cells can be stained with *Viability dye*.

REAGENTS

Phosphate-buffered saline (PBS): 138 mM sodium chloride, 2.7 mM potassium chloride, 8 mM dibasic sodium phosphate, and 1.47 mM monobasic potassium phosphate. [Note—pH 7.0–7.4. If needed, adjust pH with hydrochloric acid.]

Dilution buffer: 1% (w/v) Bovine serum albumin or human serum albumin in *PBS*

10X Stock ammonium chloride RBC lysis buffer: 1.5 M ammonium chloride, 0.01 M sodium bicarbonate, and 0.01 M ethylenediaminetetraacetic acid (EDTA)²

Diluted (1X) RBC lysis buffer: 10X Stock ammonium chloride RBC lysis buffer diluted 1:10 with water

Anti-CD34 PE: Phycoerythrin (PE)-conjugated mouse IgG1 anti-human CD34 antibody (clones QBEnd10, 8G12, 581, Birma K3, or equivalent class II or class III PE-conjugated anti-CD34 antibodies), appropriately titrated

Anti-CD45 FITC: Fluorescein isothiocyanate (FITC) conjugated mouse IgG1 anti-human CD45 antibody (clones J33, T29/33, or HLE-1 or equivalent FITC-conjugated anti-CD45 antibodies), appropriately titrated

Viability dye: 1 µg/mL 7-Aminoactinomycin D (7-AAD) or equivalent, freshly diluted from a stock solution of 100 µg/mL³

Instrument calibration beads: Fluorochrome-conjugated instrument calibration microspheres⁴

Suspended counting beads: Fluorochrome-conjugated counting beads, formulated in liquid

suspension⁵

Lyophilized counting bead tube: Fluorochrome-conjugated counting beads, formulated and lyophilized in a counting tube⁶

System suitability standard USP CD34+ Cells RS: Reconstitute the entire vial of USP CD34+ Cells RS with 0.5 mL of distilled water.

Cell samples: Fresh, fixed, or cryopreserved and thawed cell suspensions containing CD34+ cells, with a minimum of 100,000 nucleated cells/sample. Cell samples may include peripheral blood, leukapheresis product, cord blood, or bone marrow.

SAMPLE PREPARATION

Use reverse-pipetting techniques for all sample dilutions and transfers. Dilute *Cell samples* with *Dilution buffer* to obtain a total nucleated cell concentration of $10\text{--}30 \times 10^6$ cells/mL. Add *Anti-CD45 FITC* and *Anti-CD34 PE* to a 12-mm \times 75-mm polystyrene tube (or a *Lyophilized counting bead tube*), followed by *Viability dye* to a final concentration of 1 $\mu\text{g/mL}$. Add 100 μL of well-mixed *Cell samples* to the bottom of the tube, and mix. Incubate for 20 min protected from light. Add 2 mL of *Diluted (1X) RBC lysis buffer* to fresh samples or add 2 mL of *Dilution buffer* to a fixed or frozen and thawed sample; vortex to mix. For fresh *Cell samples*, incubate for 10 min protected from light; for fixed or frozen then thawed *Cell samples*, no incubation time is needed. Place samples on ice. Samples should be analyzed within 1 h of lysis.

Immediately before acquiring data on the flow cytometer, pipet 100 μL of well-mixed *Suspended counting beads* (if not using *Lyophilized counting bead tubes*) to the prepared *Cell samples* described above. Cap the tube, and gently mix by inversion. Do not add counting beads to *Cell samples* intended to be used for adjustment of the instrumentation compensation matrix.

Immediately proceed to flow acquisition to collect a minimum of 75,000 CD45+ events and a minimum of 100 CD34+ cells. Acquire and analyze data by creating regions and logical gates (manually, or by using software appropriate for the flow cytometer), as described in *Table 1*.

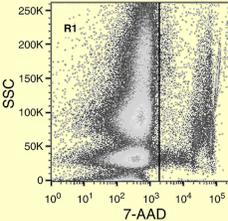
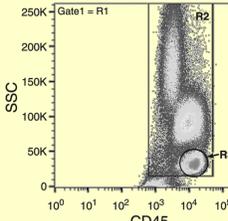
System suitability requirements: Use the USP CD34+ Cells RS to verify that the gating strategy described below allows the detection and quantitation of CD34+ HSCs. Note that for fixed *Cell samples*, the viability gate (*R1* in *Table 1*) must be opened to include all events; alternatively, the *Viability dye* can be omitted. Calculate the number of CD34+ HSCs/ μL in the USP CD34+ Cells RS.

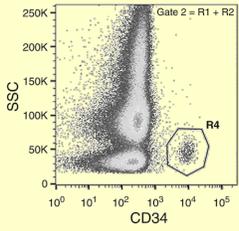
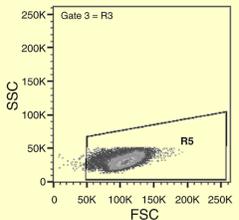
Acceptance criteria: The results should fall within the range provided in the USP CD34+ Cells RS certificate.

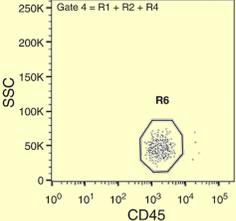
DATA ACQUISITION AND ANALYSIS

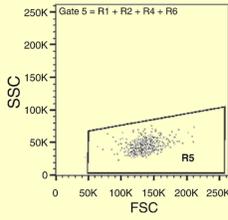
For flow acquisition, collect a minimum of 75,000 CD45+ events and a minimum of 100 CD34+ cells. Acquire and analyze data by creating regions and logical gates (manually, or using software appropriate for the flow cytometer) as described in *Table 1*. The best results are obtained when cell and bead data (events) are displayed as bivariate dot plots. *Table 1* contains representative dot plots and gating strategies for fresh cell samples. Dot plot displays may vary slightly, depending on the cell sample, flow cytometer, and software used.

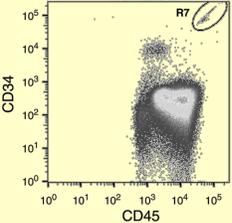
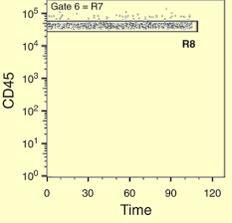
Table 1. Dot Plot Descriptions and Gating Instructions

Step	Format	Purpose	Gating Instructions	Representative Dot Plots and Gating Strategies
1	SSC (linear) vs. Viability dye fluorescence (log)	Define viable cells	<ul style="list-style-type: none"> • Display all unselected events. Create region <i>R1</i> around events with little-to-no fluorescence. • Included in the region: <i>R1</i>, viable cells, debris • Excluded from the region: Dead and dying cells, counting beads 	 <p>A dot plot showing SSC (y-axis, 0 to 250K) versus 7-AAD (x-axis, log scale from 10⁰ to 10⁵). A vertical gate labeled 'R1' is positioned at approximately 10¹ on the x-axis, selecting the population with low 7-AAD fluorescence.</p>
2	SSC (linear) vs. CD45 fluorescence (log)	Define viable CD45+ leukocytes and lymphocytes	<ul style="list-style-type: none"> • Display <i>R1</i>-selected events. Create region <i>R2</i> around events with CD45+ fluorescence, excluding debris (i.e., events with very low SSC). Within <i>R1</i>, create region <i>R3</i> around events with high CD45+ fluorescence and low SSC. • Included in the region: <i>R2</i> is viable CD45+ leukocytes; <i>R3</i> is viable lymphocytes. • Excluded from the region: <i>R2</i> is cell debris, platelets, and unlysed RBCs; <i>R3</i> is granulocytes, monocytes, and other non-lymphocytes. 	 <p>A dot plot showing SSC (y-axis, 0 to 250K) versus CD45 (x-axis, log scale from 10⁰ to 10⁵). A vertical gate labeled 'Gate1 = R1' is shown at the far left. Within this gate, two regions are defined: 'R2' is a large population of CD45+ cells, and 'R3' is a small population of CD45+ cells with low SSC.</p>

Step	Format	Purpose	Gating Instructions	Representative Dot Plots and Gating Strategies
3	SSC (linear) vs. CD34 fluorescence (log)	Define viable CD34+ cells	<ul style="list-style-type: none"> • Display <i>R2</i>-selected events. Create region <i>R4</i> around events with CD34+ fluorescence and low SSC. • Included in the region: <i>R4</i> is viable CD34+ cells. • Excluded from the region: CD34- cells 	
4	SSC (linear) vs. FSC (linear)	Define lymphocytes and blasts	<ul style="list-style-type: none"> • Display <i>R3</i>-selected events. Create region <i>R5</i> to exclude debris (low SSC and low FSC). • Included in the region: <i>R5</i> is viable lymphocytes and blasts. • Excluded from the region: Small debris and events with high SSC values 	

Step	Format	Purpose	Gating Instructions	Representative Dot Plots and Gating Strategies
5	SSC (linear) vs. CD45 fluorescence (log)	Define CD45 dim CD34+ cells	<ul style="list-style-type: none"> • Display <i>R4</i>-selected events. Create region <i>R6</i> around events with low-to-intermediate SSC and intermediate CD45 expression. • Included in the region: <i>R6</i> is viable CD34+ HSCs. • Excluded from the region: Cells that express high levels of CD45 	

Step	Format	Purpose	Gating Instructions	Representative Dot Plots and Gating Strategies
6	SSC (linear) vs. FSC (linear)	Confirm CD34+ HSCs	<ul style="list-style-type: none"> • Display R6-selected events, and paste a copy of the R5 gate parameters into the plot. Events in R5 have light scatter characteristics similar to lymphocytes and blasts, confirming the identity of the events as CD34+ HSCs. • Included in the region: R5 is confirmed viable CD34+ HSCs. • Excluded from the region: Debris and other irrelevant events 	

Step	Format	Purpose	Gating Instructions	Representative Dot Plots and Gating Strategies
7	CD34 fluorescence (log) vs. CD45 fluorescence (log)	Quantify total counting beads	<ul style="list-style-type: none"> • Display all ungated events. Create region <i>R7</i> around events with both high CD34+ and high CD45+ fluorescence. Included in the region: <i>R7</i> is total counting beads. <i>R5</i> have light scatter characteristics similar to lymphocytes and blasts, confirming the identity of the events as CD34+ HSCs. • Included in the region: <i>R7</i> is total counting beads. • Excluded from the region: Cells and debris 	 <p>A dot plot showing CD34 fluorescence (log scale, 10⁰ to 10⁵) on the y-axis versus CD45 fluorescence (log scale, 10⁰ to 10⁵) on the x-axis. A gate labeled 'R7' is drawn around a cluster of events in the upper right quadrant, representing high CD34+ and high CD45+ events.</p>
8	CD45 fluorescence (log) vs. time (linear)	Quantify "singlet" (e.g., unaggregated) counting beads	<ul style="list-style-type: none"> • When recommended by the counting bead manufacturer, display <i>R7</i>-selected events. Create <i>R8</i> around events with lower fluorescence intensity. <i>R8</i> is singlet counting beads. • Included in the region: <i>R8</i> is singlet counting beads. • Excluded from the region: Counting bead aggregates 	 <p>A dot plot showing CD45 fluorescence (log scale, 10⁰ to 10⁵) on the y-axis versus Time (linear scale, 0 to 120) on the x-axis. A gate labeled 'R8' is drawn around a horizontal band of events at a lower CD45 intensity, representing singlet counting beads.</p>

CALCULATION OF ABSOLUTE CD34 NUMBER

The absolute number of viable CD34+ cells/ μ L is calculated as follows:

$$\text{Viable CD34+ cells}/\mu\text{L} = (\text{number of CD34+ cells})/(\text{number of counting beads}) \times (\text{bead concentration} \times DF)$$

DF = sample dilution factor

The number of CD34+ cells is determined from the viable CD34+ cells (region *R5* from *Table 1*, Step 6). Depending on the recommendations of the counting bead manufacturer, the number of counting beads is determined from either the total counting beads (region *R7* from *Table 1*, graph 7) or the singlet counting beads (region *R8* from *Table 1*, graph 8). The bead concentration is specified by the counting bead manufacturer.

Table 2. Troubleshooting Guidelines (see also (1027))

Problems	Possible Reasons	Solutions	Comments
Unable to clearly define the bead gate (<i>R7</i>) in <i>Table 1</i> , graph 7 due to fluorescent debris	Degraded fresh cell sample; fixed sample; too many platelets; incomplete RBC lysis; nucleated RBCs (e.g., in cord blood)	Use a far-red fluorescence channel (>600 nm), instead of FITC, to gate counting beads.	Counting beads are highly fluorescent in all channels. Autofluorescence interference decreases dramatically at longer wavelengths (>600 nm).
Unable to find CD34+ population in, graph 3	Incorrect compensation settings for PE and <i>Viability dye</i>	Repeat the spectral compensation setup procedure, and adjust settings accordingly.	Make sure that counting beads are not in the compensation matrix sample, because they can interfere with compensation settings.
Unsure of gating for singlet or total beads	High levels of counting bead aggregates	Follow the counting bead manufacturer's recommendation for including or excluding aggregates.	Counting bead concentration calculations will vary by manufacturer.
Want to use a different CD34 antibody clone	Multi-parametric flow cytometry considerations	Alternative antibody clones must be carefully validated against the specified CD34 antibodies. Choose a class II or class III antibody that detects all CD34 isoforms and glycoforms.	Class I antibodies do not detect all CD34 glycoforms.
Want to use a different CD45 antibody clone	Multi-parametric flow cytometry considerations	Alternative antibody clones must be carefully validated against the specified CD45 antibodies. Choose an antibody that detects all isoforms and all glycoforms of CD45.	

ADDITIONAL REQUIREMENTS

USP Reference Standards (11)USP CD34+ Cells RS \blacksquare 2S (USP39)

¹ Sutherland DR., Keeney M, Gratama JW. "Enumeration of CD34+ Hematopoietic Stem and Progenitor Cells", *Current Protocols in Cytometry*. Ed. Tuan Hoang. John Wiley & Sons, Inc., Hoboken, NJ 2003, 6.4.1–6.4.24.

² A suitable fixative-free RBC lysis reagent can be obtained from BD Biosciences: BD Pharm Lyse™ Lysing Buffer Catalog No. 555899, BD Ammonium Chloride Lysing Solution (10X) Catalog No 344563, or equivalent.

³ Suitable reagents can be obtained from BD Biosciences as part of a kit, Catalog No. 344563, or BD Biosciences Catalog No. 555899, or equivalent.

⁴ A suitable reagent can be obtained from BD Biosciences: Catalog No. 641319, or equivalent.

⁵ Suitable reagents can be obtained from Dako: Catalog No. K2370 (in a kit) or Dako Catalog No. S2366, or equivalent.

⁶ A suitable reagent can be obtained from BD Biosciences: Catalog No. 340334.

BRIEFING

(212) **Oligosaccharide Analysis**, page 7582 of the *Second Supplement to USP 38*. On the basis of requests for a revision, it is proposed to add a procedure of *High-Performance Anion Exchange Chromatography with Fluorometric Detection* for analysis of *N*-linked oligosaccharides containing multi-sialylated, multi-antennary complex type chains. To support this proposed analytical procedure, two USP Reference Standards (RS) have been added to this chapter: USP Oligosaccharide System Suitability Mixture C RS and USP Oligosaccharide System Suitability Mixture D RS:

- USP Oligosaccharide System Suitability Mixture C RS is a mixture of bi-, tri-, and tetra-antennary *N*-linked oligosaccharides with variable sialylation with or without fucose [also containing sialyl Lewis x (Le^x) type structures]; the mixture was released from human α 1 acid-glycoprotein by peptide *N*-glycosidase F (PNGase F).
- USP Oligosaccharide System Suitability Mixture D RS is a mixture of sialylated bi- and tri-antennary complex type structures with an additional sialic acid on one of the tri-antennary structure; the mixture was released from bovine fetuin by PNGase F.

This new liquid chromatographic procedure for the *High-Performance Anion Exchange Liquid Chromatography with Fluorometric Detection* test was based on analyses performed with the Dionex CarboPac PA1 4.0-mm \times 5-cm, 10- μ m packing brand of guard column and the 4.0-mm \times 25-cm, 10- μ m packing brand of L46 column.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCBA: E. Chang.)

Correspondence Number—C157899

Comment deadline: November 30, 2015

Add the following:**■ (212) OLIGOSACCHARIDE ANALYSIS****INTRODUCTION**

Change to read:

Analysis of the asparagine (Asn-) linked oligosaccharide (also known as the *N*-linked oligosaccharide or *N*-glycan) composition of selected recombinant therapeutic proteins has become necessary for product characterization or release specifications of these proteins. For information on the characterization and assessment of protein glycosylation, see *Glycoprotein and Glycan Analysis—General Considerations* (1084), which may be a helpful, but not mandatory, resource. Chapter (1084) covers general analytical strategies and also highlights criteria for selecting appropriate methods for specific analytical challenges, thereby providing the context for this chapter. This chapter focuses on the analysis of *N*-linked oligosaccharides that are released from glycosylated recombinant therapeutic proteins. ~~that contain relatively simple biantennary and high mannose chains, with no or low levels of sialylated, phosphorylated, or sulfated structures~~

- ~~2S~~ (USP39)

Therefore, the chapter provides 1) a generic procedure for enzymatic release of *N*-glycans using peptide *N*-glycosidase F (PNGase F), 2) two different approaches for fluorophore labeling of the released *N*-linked oligosaccharides, and 3) ~~four~~

- ~~five~~ ~~2S~~ (USP39)

analytical procedures based on chromatographic and electrophoretic separation, as well as performance criteria for the separation. Alternative analytical strategies are discussed in (1084). Although the procedures in this chapter are validated, the procedures must be verified with the individual specific product (see *Verification of Compendial Procedures* (1226), which may be a helpful but not mandatory resource). Furthermore, validation is required when the procedure is optimized for a specific product (see *Validation of Compendial Procedures* (1225), which may be a helpful but not mandatory resource). Data analysis, quantitation, and lot release specifications, which are expected to be product specific, will be found in individual product monographs; therefore, these aspects are not covered in this chapter.

Labeling oligosaccharides with a fluorophore before analysis provides an important advantage of enhanced detection of oligosaccharides during separation, because oligosaccharides have no or low absorptivity of either ultraviolet (UV) or visible light and no significant fluorophore groups. This chapter describes two different methods for fluorophore labeling of oligosaccharides: derivatization of oligosaccharides using 2-aminobenzamide (2-AB) for liquid chromatographic separation, and labeling of oligosaccharides with 8-aminopyrene-1,3,6-trisulfonic acid (APTS) for capillary electrophoresis (CE) separation. Although electrochemical detection (pulsed amperometric detection or pulsed electrochemical detection) is also used in oligosaccharide analysis by using a strong base elution, the electrochemical detection method is less sensitive and has a higher noise to signal ratio. Therefore, this detection method is not addressed in this chapter.

Many different chromatography and electrophoresis methods have been developed to analyze oligosaccharides, and the procedures described in this chapter have been used successfully for this purpose. The choice of procedures discussed is meant to reflect the most commonly and broadly used approaches for supporting current recombinant glycosylated biological medicines in commerce. These analytical procedures include normal phase or hydrophilic interaction liquid chromatography (HILIC), high-performance anion exchange liquid chromatography (HPAEC), and CE. Several other chromatographic modes that separate oligosaccharides by size, shape, and polarity are also commonly used for oligosaccharide separation, but those other chromatographic modes are not discussed in this chapter. The general principles of

chromatography are covered in *Chromatography* (621), and therefore (621) should be considered within that context.

USP Reference Standards (RS) have been developed to assess system suitability for these analytical procedures:

- USP Oligosaccharide System Suitability Mixture A RS consists of a mixture of partially sialylated, partially galactosylated, and partially fucosylated biantennary *N*-linked oligosaccharides that were released from human polyclonal immunoglobulin G (IgG) by PNGase F.
- USP Oligosaccharide System Suitability Mixture B RS consists of a mixture of high-mannose *N*-linked oligosaccharides with traces of hybrid chains that were released from bovine ribonuclease B (RNase B) by PNGase F.
-
- USP Oligosaccharide System Suitability Mixture C RS is a mixture of bi-, tri-, and tetra-antennary *N*-linked oligosaccharides with variable sialylation with or without fucose (Fuc; also containing Le^x type structures); the mixture was released from human α1 acid-glycoprotein by PNGase F.
- USP Oligosaccharide System Suitability Mixture D RS is a mixture of sialylated bi- and tri-antennary complex type structures with an additional sialic acid on one of the tri-antennary structure; the mixture was released from bovine fetuin by PNGase F.
- 2S (USP39)

Change to read:

ANALYTICAL PROCEDURES OVERVIEW

Table 1 outlines the applications and separation principles of the analytical procedures included in this chapter. The choice of the appropriate RS will depend on the expected glycan content of the product being analyzed.

Table 1

Analytical Procedures	Type of <i>N</i>-linked Oligosaccharides to Be Separated	Applicable USP Reference Standards
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<p><i>Normal phase liquid chromatography/HILIC procedure 1:</i></p> <ul style="list-style-type: none"> • Column: 2.0-mm × 15-cm; 3-µm packing L68 • More oligosaccharide peaks compared to <i>HILIC procedure 2</i> • Longer mobile phase with shallow gradient compared to <i>HILIC procedure 2</i> • Enhanced sensitivity for smaller sample size 	<p>Relatively simple biantennary chains with no or low levels of sialylated structures, after 2-AB labeling</p>	<p>USP Oligosaccharide System Suitability Mixture A RS</p>
	<p>High-mannose chains, after 2-AB labeling</p>	<p>USP Oligosaccharide System Suitability Mixture B RS</p>
<p><i>Normal phase liquid chromatography/HILIC procedure 2:</i></p> <ul style="list-style-type: none"> • Column: 4.6-mm × 25-cm; 5-µm packing L68 • Fewer oligosaccharide peaks observed compared to <i>HILIC procedure 1</i> • Shorter mobile phase with less shallow gradient compared to <i>HILIC procedure 1</i> 	<p>Relatively simple biantennary chains with no or low levels of sialylated structures, after 2-AB labeling</p>	<p>USP Oligosaccharide System Suitability Mixture A RS</p>
	<p>High-mannose chains, after 2-AB labeling</p>	<p>USP Oligosaccharide System Suitability Mixture B RS</p>

<p><i>HPAEC</i></p> <ul style="list-style-type: none"> ■ <i>procedure 1</i> ■ 2S (USP39) <ul style="list-style-type: none"> • Guard column: 4.0-mm × 5-cm; 10-µm packing L46 • Column: 4.0-mm × 25-cm; 10-µm packing L46 • Mobile phase: 0–75 mM sodium acetate in isocratic 0.15 N sodium hydroxide • Selectivity is different from <i>HILIC procedures 1</i> and <i>2</i> with respect to the size and composition of oligosaccharide 	<p>Relatively simple biantennary chains with no or low levels of sialylated structures, after 2-AB labeling</p>	<p>USP Oligosaccharide System Suitability Mixture A RS</p>
<ul style="list-style-type: none"> ■ <p><i>HPAEC procedure 2</i></p> <ul style="list-style-type: none"> • Guard column: 4.0-mm × 5-cm; 10-µm packing L46 • Column: 4.0-mm × 25-cm; 10-µm packing L46 • Mobile phase: 150–450 mM sodium acetate in isocratic 0.15 N sodium hydroxide • Suitable for separation of charged <i>N</i>-linked oligosaccharides 	<p>High-mannose chains, after 2-AB labeling</p>	<p>USP Oligosaccharide System Suitability Mixture B RS</p>
	<p>Bi-, tri-, and tetra-antennary <i>N</i>-linked oligosaccharides with variable sialylation, after 2-AB labeling</p>	<p>USP Oligosaccharide System Suitability Mixture C RS</p>
	<p>Sialylated bi- and tri-antennary complex type structures with an additional sialic acid on one of the tri-antennary structure, after 2-AB labeling</p>	<p>USP Oligosaccharide System Suitability Mixture D RS ■ 2S (USP39)</p>

Capillary electrophoresis <ul style="list-style-type: none"> • Appropriate for APTS-oligosaccharides • Capillary: 50-μm inner diameter with 50 cm of total length and 40 cm of separation length 	Relatively simple biantennary chains with no or low levels of sialylated structures, after APTS labeling	USP Oligosaccharide System Suitability Mixture A RS
	High-mannose chains, after APTS labeling	USP Oligosaccharide System Suitability Mixture B RS

Change to read:**SAMPLE PREPARATION**

See *Appendix (Table 17)* for abbreviations and structures of glycans.

Ideally, the samples should be free of salts, excipients, and other carrier materials that may interfere with the analysis. This can be achieved by either 1) dialysis against water, a suitable buffer, or a volatile buffer using an appropriate membrane, 2) sample trapping on a solid-phase extraction (SPE) cartridge followed by washing away of salts and excipients and elution of the required sample, or 3) ultrafiltration using an appropriate membrane.

[Note—A control sample with a known glycan profile should be included in the overall procedure to confirm correct performance of the analysis. A reaction blank control only containing the buffer matrix of the glycoprotein sample can also be included in the overall procedure.]

- **Enzymatic Release of *N*-Glycans**

[Note—The following is a generic method that should be optimized for individual products depending on the amount of glycoprotein to be digested and the glycan structures, especially on the basis of the protein to glycan ratio in the molecule and the accessibility of the sugars.]

Procedure 1 for chromatographic separation

Enzyme reaction buffer: 50 mM sodium phosphate pH 7.5 or 15 mM tris(hydroxymethyl)-aminomethane, and adjust with hydrochloric acid to a pH of 7.0.

Digestion with PNGase F: Transfer 0.1–2.25 mg of glycoprotein to a vial, and adjust with *Enzyme reaction buffer* to a final volume of 30–100 μL . Add PNGase F to the glycoprotein sample in a ratio of 0.5–15 units of PNGase F to 0.1 mg of glycoprotein. Incubate at 37° for 12–24 h. Cool at room temperature for about 5 min. Vortex gently and centrifuge briefly. [Note—One unit of PNGase F is defined as the amount of enzyme required to catalyze the release of *N*-linked oligosaccharides from 1.0 nmol of denatured ribonuclease B per min at a pH of 7.5 at 37°, and is equal to 1 IUB milliunit. Complete digestion by PNGase F can be assessed by observing a mobility shift of the deglycosylated proteins on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by Coomassie Blue staining or on SDS-capillary gel electrophoresis (SDS-CGE), as shown by reduction in mass of approximately 2 kDa per glycan chain cleaved.]

Two methods are described below for performing the separation of released glycans from glycoprotein.

Method 1, using an ultrafiltration membrane with a molecular weight cutoff of 30

kDa:¹ [Note—Ultrafiltration membranes with a lower molecular weight cutoff may be used

for proteins < 150 kDa.] Remove trace amounts of glycerin by centrifuging at 11,000 $\times g$ and rinsing the membrane with 0.5 mL of water. Discard the permeate. Add 0.1 mL of water into the sample reservoir and then add the digested glycoprotein sample to the reservoir. Rinse the reaction vial with 0.1 mL of water and add to the sample reservoir. Centrifuge at 11,000 $\times g$ and collect the permeate. Rinse the sample reservoir twice using 0.1 mL of water for each wash and collect the permeate. Combine the permeates (approximately 0.5 mL) and bring the samples to complete dryness using a centrifugal evaporator without heat.

Method 2, using a reversed-phase SPE cartridge:² Add 2.0 mL of methanol into a syringe, attach the syringe to the cartridge, and use the syringe plunger to pass methanol through to waste. Add into a syringe 6.0 mL of 5% (v/v) acetic acid prepared in water. Attach the syringe to the cartridge and use the syringe plunger to pass the acetic acid solution through to waste. Gently apply the digested sample onto an individual cartridge. Into a 1-mL syringe add 0.5 mL of 5% (v/v) acetic acid, attach the syringe to the cartridge, and use the syringe plunger to pass the acetic acid through to waste. To a 2- to 3-mL syringe add 1.5 mL of 5% (v/v) acetic acid. Attach the syringe to the cartridge, and use the syringe plunger to pass the acetic acid solution through, collecting the eluting solution into a 1.5-mL tube. Bring the samples to complete dryness using a centrifugal evaporator without heat.

Procedure 2 for capillary electrophoresis separation

Enzyme reaction buffer: 50 mM sodium phosphate, pH 7.5

Digestion with PNGase F: Add 2 μL of PNGase F (5 units/ μL) to 50 μg of glycoprotein sample, and adjust with *Enzyme reaction buffer* to a final volume of 50 μL . Incubate at 37° for 18 h. Separate released oligosaccharides by centrifugation using a centrifugal filter with a 10,000 molecular weight cut-off.³ In a centrifugal vacuum evaporator, dry the released oligosaccharides to dryness.

[Note—Follow the guidelines listed in *Table 1* to select the appropriate USP RS and label.]

• **2-AB Labeling⁴ for Liquid Chromatographic Separation**

Solution A: Mix glacial acetic acid and dimethyl sulfoxide (3:7, v/v).

Solution B: Add 1.5 mL of *Solution A* to 75 mg of 2-AB. Mix well by gentle vortexing to completely dissolve 2-AB.

Labeling solution: Add 1 mL of *Solution B* to 63 mg of sodium cyanoborohydride. Mix well by gentle vortexing. Cap the mixture tightly and incubate at 70° for 1–2 min for complete dissolution. Cool at room temperature for 10 min. Use this *Labeling solution* within 1 h of its preparation. Use this solution within 1 h after its preparation, and protect the solution from light.

2-AB labeled standard solution: Add 5–15 μL of *Labeling solution* to 1 vial of USP Oligosaccharide System Suitability Mixture A RS, USP Oligosaccharide System Suitability Mixture B RS,

■ USP Oligosaccharide System Suitability Mixture C RS, or USP Oligosaccharide System Suitability Mixture D RS, ■_{2S} (USP39) and mix well.

2-AB labeled sample solution: Add 5–15 μL of *Labeling solution* to the dried glycan sample after enzymatic release and separation steps, and mix well.

Procedure: Immediately incubate the *2-AB labeled standard solution* and the *2-AB labeled*

sample solution at 37° for 16–18 h or at 65° for 2 h. Allow to cool at room temperature for 10 min. Centrifuge briefly.

■ **Dried 2-AB labeled standard or Dried 2-AB labeled sample:** ■_{2S} (USP39)

Two methods for the removal of free 2-AB are described below.

Method 1, using a gel filtration spin column: Prepare suitable G-10 microcentrifuge spin columns⁵ by tapping the column gently to ensure that the dry resin is settled at the bottom. Remove the caps, and place the column in a 2-mL collection tube. Add 0.5 mL of water to the resin, and let it swell for at least 15 min. A longer swelling time (up to 24 h) is acceptable if maintained at 2°–8°. Centrifuge the column at maximum speed for 5–10 s. Remove the water from the collection tube. Wash the resin by adding 0.5 mL of water, and centrifuge the column. Repeat the washing step one more time. After removing the water from the final wash step, spin at maximum speed for 10 s to remove residual water from the resin. Residual water is sufficiently removed if the resin appears white. Place the column in a new, labeled collection tube. Add 100 µL of water to the *2-AB labeled standard solution* and the *2-AB labeled sample solution*, and then apply the entire solution to the center of each washed G-10 column, respectively. Place the G-10 columns in the microcentrifuge and spin at approximately 200 ×g for 1 min. Apply the flow-through (approximately 90–120 µL) to the center of the second unused but previously washed G-10 column and spin at approximately 200 ×g for 1 min. Transfer the repeat flow-through (approximately 60–100 µL) to a 0.5-mL microcentrifuge tube. Dry the eluates by centrifugal evaporation with no heat.

Method 2, using an SPE cartridge: Prepare suitable SPE cartridges⁶ by washing with 1.0 mL of water, followed by 5 × 1.0 mL of 30% (v/v) acetic acid, and then 1.0 mL of acetonitrile. Apply the *2-AB labeled standard solution* and the *2-AB labeled sample solution* to the center surface of the cartridge discs, and allow the solution to incubate on the disc for 15–20 min. Wash each disc with 1 mL of acetonitrile, followed by 6 × 1.0 mL of 96% (v/v) acetonitrile, allowing each aliquot to drain before the next is applied. Elute the *2-AB labeled standard solution* and the *2-AB labeled sample solution* with 3 × 0.5 mL of water, allowing each aliquot to drain before the next is applied. Dry the eluates by centrifugal evaporation with no heat. [Note—Very small hydrophobic glycans may be lost during this procedure. During method verification, it should be confirmed that no oligosaccharide species are lost during the 2-AB removal step by comparing the chromatographic profiles of an unextracted reaction blank, an un-extracted sample, and an extracted sample.]

● **APTS Labeling for Capillary Electrophoresis Separation**

APTS labeling reagent: Dissolve 5 mg of trisodium APTS in 48 µL of 15% (v/v) acetic acid.

1 M sodium cyanoborohydride: 1 M sodium cyanoborohydride in tetrahydrofuran

Run buffer: Dissolve 1.492 g of triethanolamine (TEA) and 10 g of glycerol, accurately weighed, in 80 mL of water. Adjust with 1 N hydrochloric acid to a pH of 7.5, and dilute with water to a final volume of 100 mL.

Sample buffer: Dilute 1.0 mL of *Run buffer* with 9.0 mL of water.

APTS-labeled standard solution: Add 2 µL of the *APTS labeling reagent* and 2 µL of 1 M *sodium cyanoborohydride* to a vial of USP Oligosaccharide System Suitability Mixture A RS or USP Oligosaccharide System Suitability Mixture B RS. Incubate at 55° for 90 min. Add 46 µL of water to quench the reaction and mix. Transfer 5 µL of the APTS-labeled USP RS

to 1.995 mL of *Sample buffer* before separation. [Note—The volume of *Sample buffer* may need to be adjusted so that the fluorescence signals of the oligosaccharides are similar to those from the *APTS-labeled sample solution*.]

APTS-labeled sample solution: Add 2 μL of *APTS labeling reagent* and 2 μL of 1 M sodium cyanoborohydride to the dried glycan sample after enzymatic release and separation steps. Incubate at 55° for 90 min. Add 46 μL of water to quench the reaction and mix. Transfer 5 μL of the APTS-labeled glycan solution to 95 μL of *Sample buffer* before separation.

[Note—Follow the guidelines listed in *Table 1* to select the appropriate USP RS and label.]

Change to read:

SEPARATION AND IDENTIFICATION OF OLIGOSACCHARIDES

• **Normal Phase Chromatography/Normal Phase Chromatography/HILIC**

Procedure 1

1.4 M formic acid solution: Mix 273 mL of water with 15 mL of 98%–100% formic acid.

1.4 M ammonia solution: Mix 155 mL of water with 40 mL of 26% ammonia solution.

Ammonium formate buffer: Add 1.4 M ammonia solution to 1.4 M formic acid solution.

Solution A: Acetonitrile, Ammonium formate buffer, and water (75: 4.3: 20.7)

Solution B: Acetonitrile, Ammonium formate buffer, and water (54: 8.3: 37.7)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	79	21
80.0	47	53
81.0	0	100
92.0	0	100
93.0	79	21
113.0	79	21

Standard solution: Reconstitute the *Dried 2-AB labeled standard* with NMT 500 μL of water. [Note—The volume of water may need to be adjusted so that the fluorescence signals from the *Standard solution* are similar to those from the *Sample solution*.]

Sample solution: Reconstitute the *Dried 2-AB labeled sample* with an appropriate volume of water. [Note—Use a ratio of 30 μL of water per 100 μg of glycoprotein used in the *Enzymatic Release of N-Glycans* test as a starting point.]

Blank solution: Buffer matrix of glycoprotein sample carried through the *Sample Preparation* procedure

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Fluorescence (250-nm excitation wavelength and 428-nm emission wavelength)

Column: 2.0-mm × 15-cm; 3- μ m packing L68

Temperatures

Column: 45°

Autosampler: 2°–8°

Flow rate: 0.2 mL/min

Injection volume: 2 μ L

System suitability

Samples: *Standard solution* and *Blank solution*

Suitability requirements

Blank: No peak in the chromatogram of the *Blank solution* within the retention time window at 5–113 min

Chromatogram similarity: ~~If USP Oligosaccharide System Suitability Mixture A RS is used, the chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture A RS. If USP Oligosaccharide System Suitability Mixture B RS is used, the chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture B RS.~~

- The chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the Certificate for either USP Oligosaccharide System Suitability Mixture A RS or USP Oligosaccharide System Suitability Mixture B RS. ■2S (USP39)

Presence of *N*-glycan species: If USP Oligosaccharide System Suitability Mixture A RS is used, identify peaks corresponding to G0, G0F, G1Fa, G1Fb, G2F, A1F, and A2F (see *Table 3*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture A RS. If USP Oligosaccharide System Suitability Mixture B RS is used, identify peaks corresponding to oligomannose (MAN) MAN-5, MAN-6, MAN-7, MAN-8, and MAN-9 (see *Table 4*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture B RS.

Relative retention times: Corresponding to the relative retention times of *N*-glycans listed in *Table 3* or *Table 4*

Table 3. Standard Solution using USP Oligosaccharide System Suitability Mixture A RS

<i>N</i> -Glycan	■ Approximate k_{2S} (USP39) Relative Retention Time
G0 (NGA2)	0.47
G0F (NGA2F)	0.57
G1Fa (NA1F, FA2G1, or NA2G1F)	0.75
G1Fb (NA1F, FA2G1, or NA2G1F)	0.78
G2F (NA2F)	1.00
A1F (G2fS1)	1.39
A2F (G2fS2)	1.76

Table 4. Standard Solution using USP Oligosaccharide System Suitability Mixture B RS

<i>N</i> -Glycan	■ Approximate k_{2S} (USP39) Relative Retention Time
MAN-5	0.74
MAN-6	1.00
MAN-7	1.29
MAN-8	1.60
MAN-9	1.85

Analysis: Equilibrate the *Chromatographic system* for at least 1 h with starting conditions described in *Table 2*. Inject the *Blank solution* until the baseline is stable (1–3 injections).

Sample: *Sample solution*

Integrate peaks in the resulting chromatogram, and report the relative peak areas of glycan structures relevant to the product. A base line is drawn from the first to the last peak. For general information on chromatographic peak integration, see <621>.

Procedure 2

Solution A: 250 mM ammonium formate, pH 4.0, prepared as follows. Mix 500 mL of water with 9.8 mL of 96% formic acid. Adjust with 30% ammonium hydroxide to a pH of 4.0 ± 0.1. Dilute with water to a final volume of 1000 mL. Pass the solution through a 0.22-µm pore size filter composed of cellulose acetate membrane.

Solution B: Acetonitrile

Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0.0	20	80
2.0	30	70
67.0	52	48
67.1	80	20
73.0	80	20
73.1	20	80
85.0	20	80

Standard solution: Reconstitute the *Dried 2-AB labeled standard* with NLT 500 μL of water. [Note—The volume of water may need to be adjusted so that the fluorescence signals from the *Standard solution* are similar to those from the *Sample solution*.]

Sample solution: Reconstitute the *Dried 2-AB labeled sample* with an appropriate volume of water. [Note—Use a ratio of 50 μL of water per 100 μg of glycoprotein used in the *Enzymatic Release of N-Glycans* test as a starting point.]

Blank solution: Buffer matrix of glycoprotein sample carried through the *Sample Preparation* procedure

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Fluorescence; set the excitation and emission wavelengths as listed in *Table 6*.

Table 6

Time (min)	Excitation (nm)	Emission (nm)
0.00	330	420
1.00	330	420
1.01	400	500
10.00	400	500
10.01	330	420
85.00	330	420

Column: 4.6-mm \times 25-cm; 5- μm packing L68

Temperatures

Column: 35°

Autosampler: 2°–8°

Flow rate: 0.4 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Chromatogram similarity: If USP Oligosaccharide System Suitability Mixture A RS is

~~used, the chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture A RS. If USP Oligosaccharide System Suitability Mixture B RS is used, the chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture B RS.~~

■ The chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the Certificate for either USP Oligosaccharide System Suitability Mixture A RS or USP Oligosaccharide System Suitability Mixture B RS. ■_{2S (USP39)}

Presence of N-glycan species: If USP Oligosaccharide System Suitability Mixture A RS is used, identify peaks corresponding to *G0, G0F, G1Fa, G1Fb, G2, G2F, A1F, and A2F* (see *Table 7*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture A RS. If USP Oligosaccharide System Suitability Mixture B RS is used, identify peaks corresponding to *MAN-5, MAN-6, MAN-7, MAN-8, and MAN-9* (see *Table 8*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture B RS.

Relative retention times: Corresponding to the relative retention times of *N*-glycans listed in *Table 7* or *Table 8*

Table 7. Standard Solution using USP Oligosaccharide System Suitability Mixture A RS

<i>N</i> -Glycan	■ Approximate ■ _{2S (USP39)} Relative Retention Time
G0 (NGA2)	0.78
G0F (NGA2F)	0.83
G1Fa (NA1F, FA2G1, or NA2G1F)	0.91
G1Fb (NA1F, FA2G1, or NA2G1F)	0.92
G2 (NA2)	0.96
G2F (NA2F)	1.00
A1F (G2fS1)	1.11
A2F (G2fS2)	1.20

Table 8. Standard Solution using USP Oligosaccharide System Suitability Mixture B RS

<i>N</i> -Glycan	■ Approximate _{2S (USP39)} Relative Retention Time
MAN-5	0.77
MAN-6	0.86
MAN-7	0.93
MAN-8	1.00
MAN-9	1.05

Analysis

Sample: *Sample solution*

Integrate peaks in the resulting chromatogram and report the relative peak areas of glycan structures relevant to the product. For general information on chromatographic peak integration, see (621).

- **High-Performance Anion Exchange Liquid Chromatography with Fluorometric Detection**

- **Procedure 1** _{2S (USP39)}

Solution A: Water; degas before use.

Solution B: 0.5 M sodium acetate, prepared as follows. Dissolve 20.5 g of anhydrous sodium acetate in 450 mL of water and mix well. Dilute with water to a final volume of 500 mL. Filter the solution through a membrane of NMT 0.45- μ m pore size, and degas before use.

Solution C: 0.5 M sodium acetate, prepared as follows. To 972 mL of water, add 26 mL of 50% (w/w) sodium hydroxide solution. Pass the solution through an alkaline-resistant filter membrane of NMT 0.45- μ m pore size, and degas before use.

Mobile phase: See *Table 9*.

Table 9

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)	Elution
0.0	70	0	30	Initial condition
15.0	70	0	30	0–75 mM sodium acetate, isocratic 0.15 N sodium hydroxide
65.0	55	15	30	
66.0	0	50	50	250 mM sodium acetate, 0.25 N sodium hydroxide wash
74.0	0	50	50	
75.0	70	0	30	Re-equilibrium
90.0	70	0	30	

Standard solution: Reconstitute the *Dried 2-AB labeled standard* with NMT 500 μ L of water. [Note—The volume of water may need to be adjusted so that the fluorescence signals from the *Standard solution* are similar to those from the *Sample solution*.]

Sample solution: Reconstitute the *Dried 2-AB labeled sample* with an appropriate volume

of water. [Note—Use a ratio of 20 μL of water per 100 μg of glycoprotein used in the *Enzymatic Release of N-Glycans* test as a starting point.]

Blank solution: Buffer matrix of glycoprotein sample carried through the *Sample Preparation* procedure

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Fluorescence (330-nm excitation wavelength and 420-nm emission wavelength)

Columns

Guard: 4.0-mm \times 5-cm; 10- μm packing L46

Analytical: 4.0-mm \times 25-cm; 10- μm packing L46

Temperatures

Column: 25°

Autosampler: 4°

Flow rate: 0.8 mL/min

Injection volume: 50 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Chromatogram similarity: ~~If USP Oligosaccharide System Suitability Mixture A RS is used, the chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture A RS. If USP Oligosaccharide System Suitability Mixture B RS is used, the chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture B RS.~~

- ~~The chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the Certificate for either USP Oligosaccharide System Suitability Mixture A RS or USP Oligosaccharide System Suitability Mixture B RS.~~ ■_{2S} (USP39)

Presence of N-glycan species: If USP Oligosaccharide System Suitability Mixture A RS is used, identify peaks corresponding to G0F, G1F, G0, G2F, A1F, and A2F (see *Table 10*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture A RS. If USP Oligosaccharide System Suitability Mixture B RS is used, identify peaks corresponding to MAN-5, MAN-6, MAN-7, MAN-8, and MAN-9 (see *Table 11*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture B RS.

Relative retention times: Corresponding to the relative retention times of N-glycans listed in *Table 10* or *Table 11*

Table 10. Standard Solution using USP Oligosaccharide System Suitability Mixture A RS

<i>N</i> -Glycan	■ Approximate k_{2S} (USP39) Relative Retention Time
G0F (NGA2F)	0.67
G1F (NA1F, FA2G1, or NA2G1F)	0.86
G0 (NGA2)	0.92
G2F (NA2F)	1.00
A1F (G2fS1)	1.75
A2F (G2fS2)	2.22

Table 11. Standard Solution using USP Oligosaccharide System Suitability Mixture B RS

<i>N</i> -Glycan	■ Approximate k_{2S} (USP39) Relative Retention Time
MAN-5	0.86
MAN-6	1.00
MAN-7	1.09
MAN-8	1.15
MAN-9	1.24

Analysis: Equilibrate the *Chromatographic system* with the initial *Mobile phase* conditions described in *Table 9* for a minimum of 15 min. Inject 50 μ L of water for the first injection.

Sample: *Sample solution*

Integrate peaks in the resulting chromatogram and report the relative peak areas of glycan structures relevant to the product. For general information on chromatographic peak integration, see (621).

■ Procedure 2

Solution A: Water; degas before use

Solution B: 0.5 M sodium acetate, prepared as follows. Dissolve 41.0 g of sodium acetate anhydrous in 900 mL of water. Transfer the solution to a 1-L volumetric flask, and bring with water to volume. Filter the solution through a nylon membrane with NMT 0.45- μ m pore size, and degas before use.

Solution C: 0.5 M sodium hydroxide, prepared as follows. To 900 mL of water, add 26 mL of 50% (w/w) sodium hydroxide solution, and mix well. Bring the solution with water to the final volume of 1000 mL. Filter the solution through an alkaline-resistant nylon membrane with NMT 0.45- μ m pore size, and degas before use.

Mobile phase: See *Table 12*.

Table 12

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)	Elution
0.0	80	10	10	Initial condition
15.0	80	10	10	50–150 mM sodium acetate, isocratic 0.05 N sodium hydroxide
70.0	60	30	10	150–450 mM sodium acetate, isocratic 0.05 N sodium hydroxide
94.0	0	90	10	Sodium acetate wash, no gradient
99.0	0	90	10	
105	0	10	90	
110	00	10	90	Sodium hydroxide wash, no gradient
111	80	10	10	Re-equilibrium
130	80	10	10	

Standard solution: Reconstitute the *Dried 2-AB labeled standard* with NMT 500 μL of water. [Note—The volume of water may need to be adjusted so that the fluorescence signals from the *Standard solution* are similar to those from the *Sample solution*.]

Sample solution: Reconstitute the *Dried 2-AB labeled sample* with an appropriate volume of water. [Note—Use a ratio of 20 μL of water per 100 μg of glycoprotein used in the *Enzymatic Release of N-Glycans* test as a starting point.]

Blank solution: Buffer matrix of sample carried through the *Sample Preparation* procedure.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Fluorescence (330-nm excitation wavelength and 420-nm emission wavelength)

Columns

Guard: 4.0-mm \times 5-cm; 10- μm packing L46

Analytical: 4.0-mm \times 25-cm; 10- μm packing L46

Temperatures

Column: 25°

Autosampler: 4°

Flow rate: 0.5 mL/min

Injection volume: 25 μL

System suitability

Samples: *Standard solution* and *Blank solution*

Suitability requirements

Blank: The chromatogram with *Blank solution* must not have any interfering components in the regions of *N-glycan* elution.

Chromatogram similarity: The chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the Certificate for either USP Oligosaccharide System Suitability Mixture C RS or USP Oligosaccharide System Suitability Mixture D RS.

Presence of *N*-glycan species: If USP Oligosaccharide System Suitability Mixture C RS is used, identify peaks corresponding to A1 (peak 1 and peak 2), A2, and A3 (see *Table 13*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the Certificate. If Oligosaccharide System Suitability Mixture D RS is used, identify peaks corresponding to A1 (peak 1), A2, A3, and A3G3S4 (see *Table 14*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the Certificate.

Relative retention times: Corresponding to the relative retention times of *N*-glycans listed in *Table 13* or *Table 14*

Table 13. Standard Solution using USP Oligosaccharide System Suitability Mixture C RS

<i>N</i> -Glycan	Approximate Relative Retention Time
A1 (G1S1), peak 1	0.31
A1 (G1S1), peak 2	0.32
A2 (G2S2)	0.73
A3 (G3S3)	1.00

Table 14. Standard Solution using USP Oligosaccharide System Suitability Mixture D RS

<i>N</i> -Glycan	Approximate Relative Retention Time
A1 (G1S1), peak 1	0.23
A2 (G2S2)	0.57
A3 (G3S3)	0.78
A3G3S4 (S4NA3, or A3 + Sa)	1.00

Analysis [Note—Equilibrate the column with initial mobile phase conditions for a minimum of 15 min.]

Sample: *Sample solution*

Inject 25 μ L of water, and run the gradient program at least once to equilibrate the column and system. Integrate peaks in the resulting chromatogram, and report the relative peak areas of glycan structures relevant to the product. For general information on chromatographic peak integration, see (621).

■ 2S (USP39)

● **Capillary Electrophoresis**

Run buffer: Dissolve 1.492 g of TEA and 10 g of glycerol in 80 mL of water. Adjust with 1 N hydrochloric acid to a pH of 7.5 and dilute with water to a final volume of 100 mL.

Standard solution: *APTS-labeled standard solution* prepared as directed in the test for 8-Aminopyrene-1,3,6-trisulfonic Acid (APTS) Labeling for CE Separation

Sample solution: *APTS-labeled sample solution* prepared as directed in the test for 8-

Aminopyrene-1,3,6-trisulfonic Acid (APTS) Labeling for CE Separation

Blank solution: Buffer matrix of glycoprotein sample carried through the *Sample Preparation* procedure

Instrumental conditions

Mode: CE

Detector: Laser-induced fluorescence (488-nm excitation wavelength and 520-nm emission wavelength)

Capillary: 50- μ m inner diameter bare fused-silica with 50 cm total length and 40 cm separation length

Preconditioning of the capillary: Between each run, rinse for 5 min at 40 psi with 0.5 N sodium hydroxide, followed by water for 1 min at 40 psi. [Note—Precondition a new capillary or as needed: Rinse for 5 min at 20 psi with methanol, followed by water for 1 min at 50 psi, 1 N hydrochloric acid for 5 min at 20 psi, water for 1 min at 50 psi, 0.5 N sodium hydroxide for 25 min at 20 psi, and water for 5 min at 50 psi.]

Prefilling of the capillary: Rinse for 5 min at 40 psi with *Run buffer*.

Sample injection: 10 s hydrodynamic (pressure) injection

Voltage: 22 kV for 60 min

Temperatures

Capillary cartridge: 18°

Sample storage: 20°

System suitability

Sample: *Standard solution*

Suitability requirements

Electropherogram similarity: If USP Oligosaccharide System Suitability Mixture A RS is used, the electropherogram obtained from the *Standard solution* corresponds to that of the reference electropherogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture A RS. If USP Oligosaccharide System Suitability Mixture B RS is used, the electropherogram obtained from the *Standard solution* corresponds to that of the reference electropherogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture B RS.

- The electropherogram obtained from the *Standard solution* corresponds to that of the reference electropherogram provided with the Certificate for either USP Oligosaccharide System Suitability Mixture A RS or USP Oligosaccharide System Suitability Mixture B RS. ■_{2S} (USP39)

Presence of N-glycan species: If USP Oligosaccharide System Suitability Mixture A RS is used, identify peaks corresponding to *G2F*, *G2*, *G1Fa*, *G1Fb*, *G0F*, *MAN-5*, *G0*, and *A1F* (see *Table 15*; see also *Appendix*) in the electropherogram obtained from the *Standard solution* by comparing the reference electropherogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture A RS. If USP Oligosaccharide System Suitability Mixture B RS is used, identify peaks corresponding to *MAN-5*, *MAN-6*, *MAN-7*, *MAN-8*, and *MAN-9* (see *Table 16*; see also *Appendix*) in the electropherogram obtained from the *Standard solution* by comparing the reference electropherogram provided with the USP Certificate for USP Oligosaccharide System

Suitability Mixture B RS.

Relative migration times: Corresponding to relative migration times listed in *Table 15* or *Table 16*

Table 15. Standard Solution using USP Oligosaccharide System Suitability Mixture A RS

<i>N</i> -Glycan	■ Approximate μ_{2S} (USP39) Relative Migration Time
G2F (NA2F)	0.79
G2 (NA2)	0.84
G1Fa (NA1F, FA2G1, or NA2G1F)	0.87
G1Fb (NA1F, FA2G1, or NA2G1F)	0.90
G0F (NGA2F)	1.00
MAN-5	1.03
G0 (NGA2)	1.12
A1F (G2fS1)	1.18

Table 16. Standard Solution using USP Oligosaccharide System Suitability Mixture B RS

<i>N</i> -Glycan	■ Approximate μ_{2S} (USP39) Relative Migration Time
MAN-9	0.82
MAN-8	0.85
MAN-7	0.91, 0.92, 0.94
MAN-6	1.00
MAN-5	1.11

Analysis

Sample: *Sample solution*

Integrate peaks in the resultant electropherogram and report the relative peak areas of glycan structures relevant to the product.

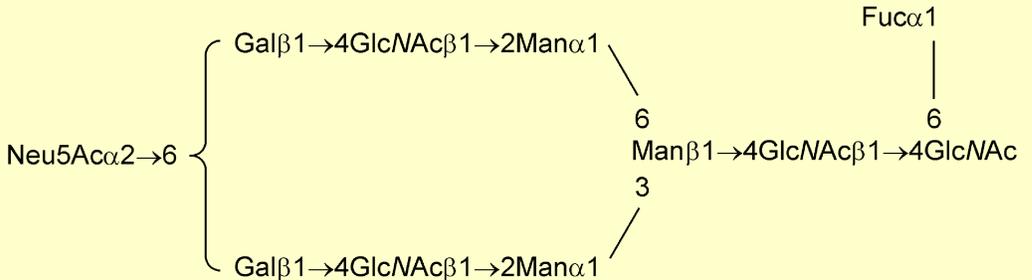
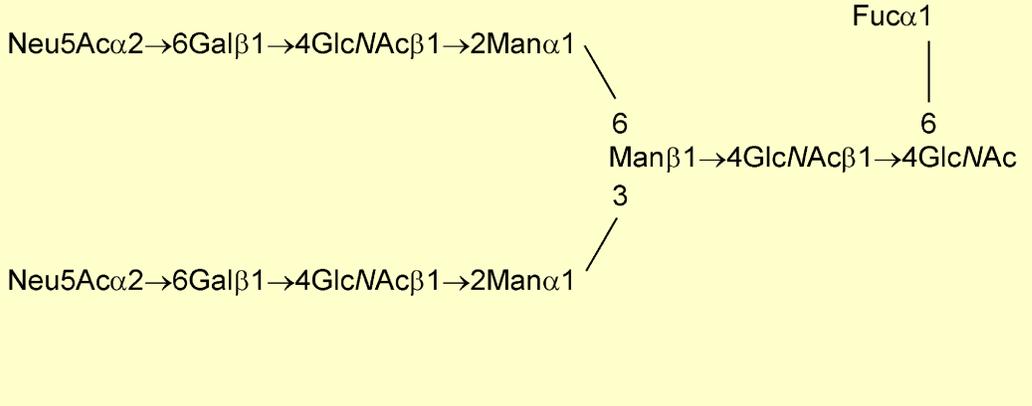
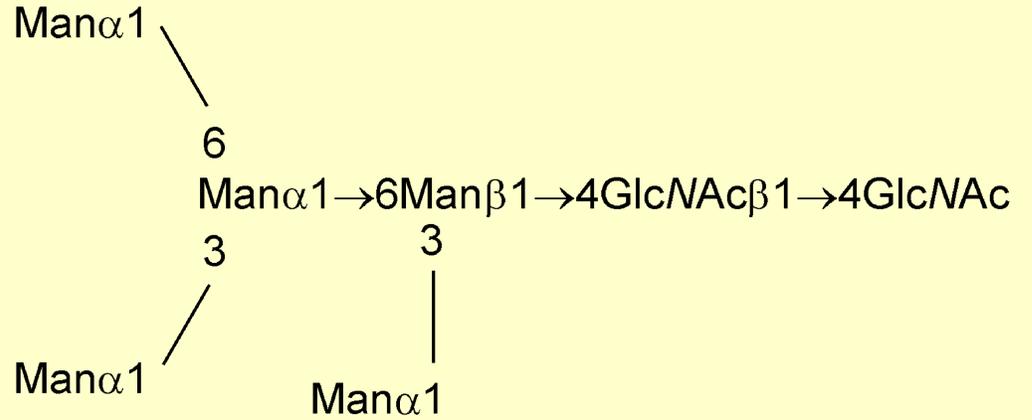
ADDITIONAL REQUIREMENTS

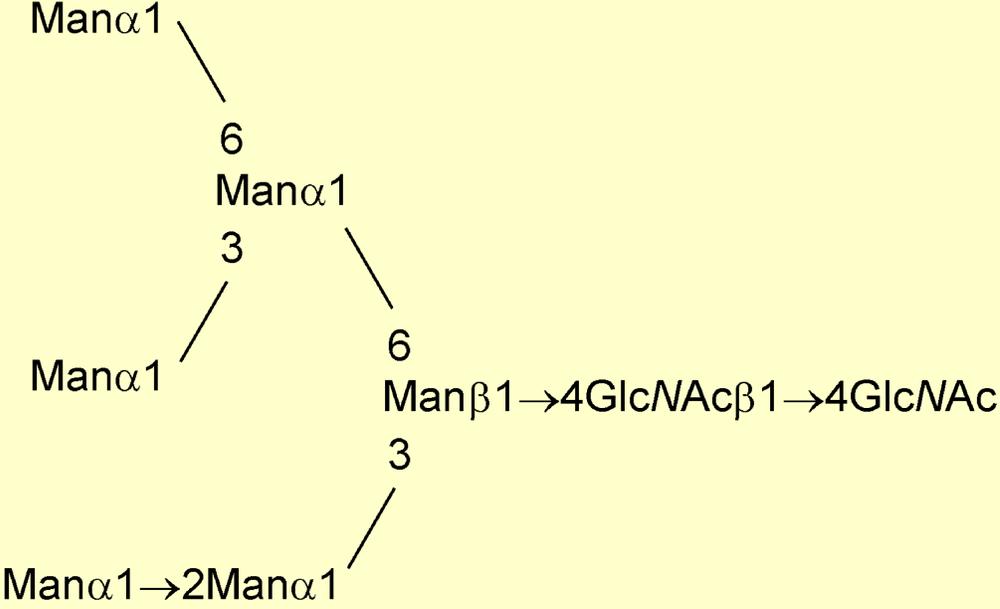
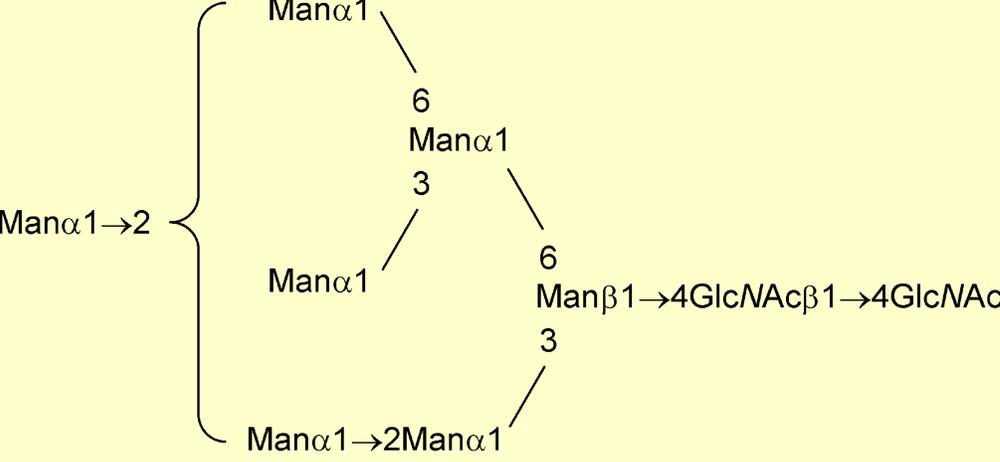
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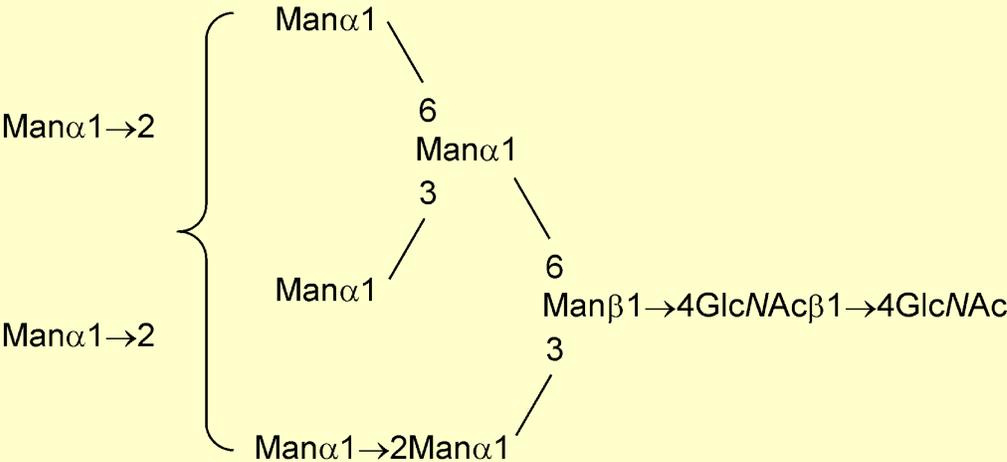
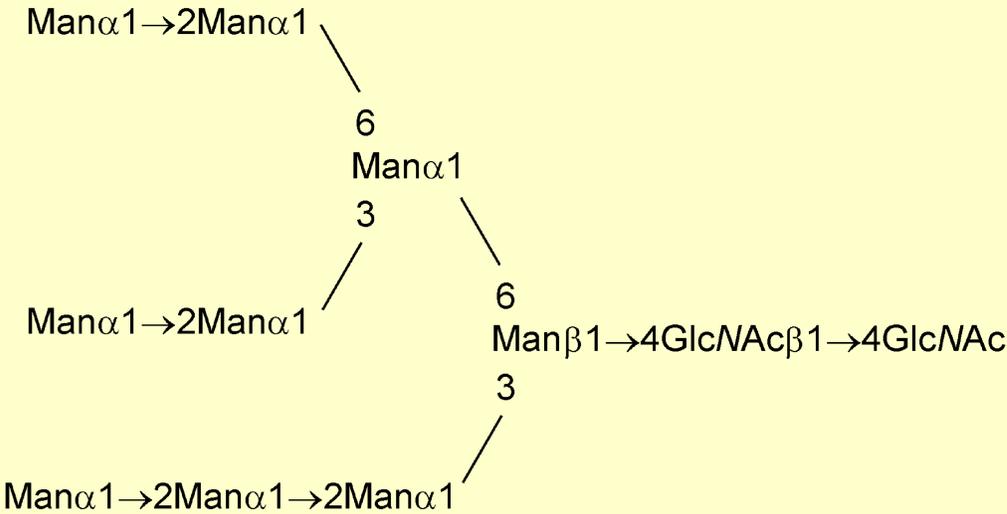
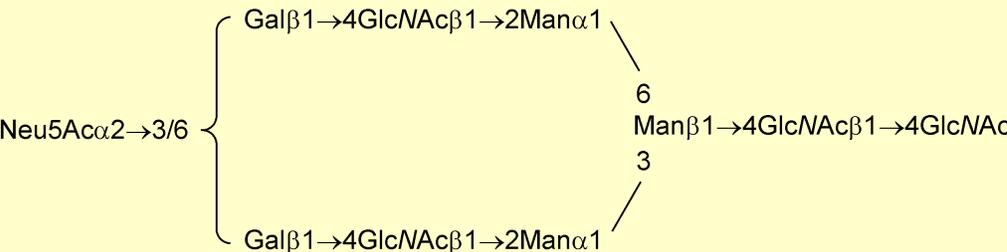
- **USP Reference Standards** (11)
 - USP Oligosaccharide System Suitability Mixture A RS
 - USP Oligosaccharide System Suitability Mixture B RS
 - USP Oligosaccharide System Suitability Mixture C RS
 - USP Oligosaccharide System Suitability Mixture D RS ■_{2S} (USP39)

Change to read:

APPENDIX

Glycan	Description
<p>A1F (G2fS1)</p>	<p>Monosialo-fucosylated biantennary oligosaccharide</p> 
<p>A2F (G2fS2)</p>	<p>Disialo-fucosylated biantennary oligosaccharide</p> 
<p>MAN-5</p>	<p>Oligomannose 5 Oligomannose <i>N</i>-linked oligosaccharide with 5 mannosyl residues</p> 

Glycan	Description
MAN-6	<p>Oligomannose 6 Oligomannose <i>N</i>-linked oligosaccharide with 6 mannosyl residues</p>  <p>The diagram shows a central mannose residue (Manα1) with a branch of 6 mannose residues (Manα1) attached via a 6 linkage. This central mannose is also linked to another mannose residue (Manα1) via a 3 linkage. This second mannose residue is further linked to a third mannose residue (Manα1) via a 3 linkage. Finally, the third mannose residue is linked to a fourth mannose residue (Manα1) via a 2 linkage. The fourth mannose residue is linked to a core fucose (GlcNAc) via a 6 linkage, which is further linked to another GlcNAc via a 4 linkage.</p>
MAN-7	<p>Oligomannose 7 Oligomannose <i>N</i>-linked oligosaccharide with 7 mannosyl residues</p>  <p>The diagram shows a central mannose residue (Manα1) with a branch of 6 mannose residues (Manα1) attached via a 6 linkage. This central mannose is also linked to another mannose residue (Manα1) via a 3 linkage. This second mannose residue is further linked to a third mannose residue (Manα1) via a 3 linkage. Finally, the third mannose residue is linked to a fourth mannose residue (Manα1) via a 2 linkage. The fourth mannose residue is linked to a core fucose (GlcNAc) via a 6 linkage, which is further linked to another GlcNAc via a 4 linkage. A bracket on the left side of the diagram groups the central mannose and the branch of 6 mannose residues, with the label Manα1\rightarrow2.</p>

Glycan	Description
<p>MAN-8</p>	<p>Oligomannose 8 Oligomannose <i>N</i>-linked oligosaccharide with 8 mannosyl residues</p>  <p>The diagram shows a central $\text{Man}\alpha 1$ residue. It is linked to another $\text{Man}\alpha 1$ residue above it via a 6-link. To the left, two $\text{Man}\alpha 1$ residues are attached to the central one via 3-links, with a bracket indicating a $\text{Man}\alpha 1 \rightarrow 2$ linkage between them. To the right, a $\text{Man}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 4\text{GlcNAc}$ chain is attached to the central $\text{Man}\alpha 1$ via a 6-link. A 3-link connects the central $\text{Man}\alpha 1$ to a $\text{Man}\alpha 1$ residue below it, which is then linked to another $\text{Man}\alpha 1$ residue via a 2-link.</p>
<p>MAN-9</p>	<p>Oligomannose 9 Oligomannose <i>N</i>-linked oligosaccharide with 9 mannosyl residues</p>  <p>The diagram shows a central $\text{Man}\alpha 1$ residue. It is linked to another $\text{Man}\alpha 1$ residue above it via a 6-link. To the left, two $\text{Man}\alpha 1$ residues are attached to the central one via 3-links, with a bracket indicating a $\text{Man}\alpha 1 \rightarrow 2\text{Man}\alpha 1$ linkage between them. To the right, a $\text{Man}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 4\text{GlcNAc}$ chain is attached to the central $\text{Man}\alpha 1$ via a 6-link. A 3-link connects the central $\text{Man}\alpha 1$ to a $\text{Man}\alpha 1$ residue below it, which is then linked to another $\text{Man}\alpha 1$ residue via a 2-link.</p>
<p>■ A1 (G1S1)</p>	<p>Mono-sialylated, galactosylated, biantennary oligosaccharide</p>  <p>The diagram shows a central $\text{Man}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 4\text{GlcNAc}$ chain. A $\text{Man}\alpha 1$ residue is attached to the first GlcNAc of this chain via a 6-link. This $\text{Man}\alpha 1$ is then linked to another $\text{Man}\alpha 1$ residue above it via a 2-link. A bracket indicates a $\text{Neu5Ac}\alpha 2 \rightarrow 3/6$ linkage between these two $\text{Man}\alpha 1$ residues. A 3-link connects the central $\text{Man}\alpha 1$ to another $\text{Man}\alpha 1$ residue below it, which is then linked to a third $\text{Man}\alpha 1$ residue via a 2-link.</p>

Glycan	Description
<p>A2 (G2S2)</p>	<p>Di-sialylated, galactosylated, biantennary oligosaccharide</p> <p>Neu5Acα2→3/6Galβ1→4GlcNAcβ1→2Manα1</p> <p style="text-align: right;">6 Manβ1→4GlcNAcβ1→4GlcNAc 3</p> <p>Neu5Acα2→3/6Galβ1→4GlcNAcβ1→2Manα1</p>
<p>A3 (G3S3)</p>	<p>Tri-sialylated, galactosylated, triantennary oligosaccharide</p> <p>Neu5Acα2→3/6Galβ1→4GlcNAcβ1→2Manα1</p> <p style="text-align: right;">6 Manβ1→4GlcNAcβ1→4GlcNAc 3</p> <p style="text-align: center;">4 Manα1 2</p> <p>Neu5Acα2→3/6Galβ1→3/4GlcNAcβ1</p> <p>Neu5Acα2→3/6Galβ1→4GlcNAcβ1</p>
<p>A3G3S4 (S4NA3, or A3 + Sa)</p>	<p>Tetra-sialylated, galactosylated, triantennary oligosaccharide</p> <p>Neu5Acα2→3/6Galβ1→4GlcNAcβ1→2Manα1</p> <p style="text-align: right;">6 Manβ1→4GlcNAcβ1→4GlcNAc 3</p> <p style="text-align: center;">2 Manα1 4</p> <p style="text-align: center;">3 GlcNAcβ1 6</p> <p>Neu5Acα2→3/6Galβ1</p> <p>Neu5Acα2→3/6Galβ1→4GlcNAcβ1</p> <p>Neu5Acα2</p> <p>■ 2S (USP39)</p>

■ 2S (USP38)

¹ A suitable ultrafiltration membrane is YM-30 microcon centrifugal filter device from Millipore (catalog numbers 42422, 42409, and 42410) or an equivalent.

² A suitable SPE cartridge is Glyko GlycoClean R Cartridge from Prozyme (catalog number GKI-4756) or an equivalent.

³ A suitable centrifugal filter is EMD Millipore Ultra-0.5 Centrifugal Filter Unit (catalog number UFC501096).

⁴ A suitable 2-AB labeling kit is Glyko Signal 2-AB Labeling Kit from Prozyme (catalog number GKK-404), LudgerTage 2-AB labeling kit from QA Bio (catalog number LT-KAB-A2), or an equivalent.

⁵ A suitable prepacked Sephadex G-10 column is Macro Spin G-10 mini SEC column from Harvard Apparatus (catalog number 743900) or The Nest Group (catalog number SMM S010), or an equivalent.

⁶ A suitable cartridge to remove free 2-AB is Glyko GlycoClean S Cartridge from Prozyme (catalog number GKI-4726) or an equivalent.

BRIEFING

⟨467⟩ **Residual Solvents**, *USP 38* page 309. This revised version of general chapter *Residual Solvents* ⟨467⟩ represents the result of deliberations of the Residual Solvents Expert Panel, created under the umbrella of the Chemical Analysis Expert Committee. The proposed revisions also reflect the results of a survey conducted in the pharmaceutical industry during 2013. The following main changes are proposed:

1. A section titled *Control Strategy* has been added to better explain the different approaches that can be used to control the quantities of residual solvents in pharmacopeial articles.
2. Under the *Identification, Control, and Quantification of Residual Solvents* section, a more detailed decision tree has been added.
3. The *Analytical Procedures* section has been modernized to align with current format.
4. Under *Analytical Procedures*, text is added to indicate that, when the solvents likely to be present are known, system suitability requirements are based only on the solvents under investigation.
5. A new section titled *Verification and Validation* has been added to provide guidance on the verification process, when the procedures are being implemented in the laboratory, or the validation of alternative procedures.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCCA: H. Pappa.)

Correspondence Number—C156655

Comment deadline: November 30, 2015

⟨467⟩ RESIDUAL SOLVENTS

Change to read:

INTRODUCTION

~~This general chapter applies to existing drug substances, excipients, and products. All substances and products are subject to relevant control of solvents likely to be present in a substance or product.~~

~~Where the limits to be applied comply with those given below, tests for residual solvents are not generally mentioned in specific monographs, because the solvents employed may vary from~~

one manufacturer to another.

The objective of this general chapter is to provide acceptable amounts of residual solvents in pharmaceuticals for the safety of the patient. The chapter recommends the use of less toxic solvents and describes levels considered to be toxicologically acceptable for some residual solvents.

For pharmacopeial purposes, residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. The residual solvents are not completely removed by practical manufacturing techniques. Appropriate selection of the solvent for the synthesis of a drug substance or an excipient may enhance the yield, or determine characteristics such as crystal form, purity, and solubility. Therefore, the solvent may sometimes be a critical element in the synthetic process. This general chapter does not address solvents deliberately used as excipients, nor does it address solvates. However, the content of solvents in such products should be evaluated and justified.

Because residual solvents do not provide therapeutic benefit, they should be removed, to the extent possible, to meet ingredient and product specifications, good manufacturing practices, or other quality-based requirements. Drug products should contain no higher levels of residual solvents than can be supported by safety data. Solvents that are known to cause unacceptable toxicities (Class 1, *Table 1*) should be avoided in the production of drug substances, excipients, or drug products unless their use can be strongly justified in a risk-benefit assessment. Solvents associated with less severe toxicity (Class 2, *Table 2*) should be limited in order to protect patients from potential adverse effects. Ideally, less toxic solvents (Class 3, *Table 3*) should be used where practical. The complete list of solvents included in this general chapter is given in *Appendix 1*. These tables and the list are not exhaustive. For the purposes of this Pharmacopeia, when a manufacturer has received approval from a competent regulatory authority for the use of a new solvent not currently listed in this general chapter, it is the responsibility of that manufacturer to notify the USP regarding the identity of this solvent, the approved residual solvent limit in the article, and the appropriate test procedure for this residual solvent in the article. The USP will then address this topic in the individual monograph. When a new solvent has been approved through the ICH process, it will be added to the appropriate list in this general chapter. At that time, consideration will be given for removal of the specific solvent test requirement in the individual monograph.

Testing of drug substances, excipients, and drug products for residual solvents should be performed when production or purification processes are known to result in the presence of such residual solvents. It is only necessary to test for residual solvents that are used or produced in the manufacture or purification of drug substances, excipients, or products.

Although manufacturers may choose to test the drug product, a cumulative procedure may be used to calculate the residual solvent levels in the drug product from the levels in the ingredients used to produce the drug product. If the calculation results in a level equal to or below that provided in this general chapter, no testing of the drug product for residual solvents need be considered. If, however, the calculated level is above the recommended level, the drug product should be tested to ascertain whether the formulation process has reduced the relevant solvent level to within the acceptable amount. A drug product should also be tested if a residual solvent is used during its manufacture.

For the purposes of this Pharmacopeia, when a manufacturer has received approval from a competent regulatory authority for a higher level of residual solvent, it is the responsibility of that manufacturer to notify the USP regarding the identity of this solvent and the approved residual solvent limit in the article. The USP will then address this topic in the individual monograph.

See *Appendix 2* for additional background information related to residual solvents.

CLASSIFICATION OF RESIDUAL SOLVENTS BY RISK ASSESSMENT

The term *tolerable daily intake* (TDI) is used by the International Program on Chemical Safety (IPCS) to describe exposure limits of toxic chemicals, and the term *acceptable daily intake* (ADI) is used by the World Health Organization (WHO) and other national and international health authorities and institutes. The term *permitted daily exposure* (PDE) is defined as a pharmaceutically acceptable intake of residual solvents to avoid confusion of differing values for ADIs of the same substance.

Residual solvents assessed in this general chapter are listed in *Appendix 1* by common names and structures. They were evaluated for their possible risk to human health and placed into one of three classes as follows:

Residual Solvent Class	Assessment
Class 1	Solvents to be avoided
	Known human carcinogens
	Strongly suspected human carcinogens
	Environmental hazards
Class 2	Solvents to be limited
	Nongenotoxic animal carcinogens or possible causative agents of other irreversible toxicity, such as neurotoxicity or teratogenicity
Class 3	Solvents suspected of other significant but reversible toxicities
	Solvents with low toxic potential
	Solvents with low toxic potential to humans; no health-based exposure limit is needed [Note—Class 3 residual solvents have PDEs of 50 mg or more per day.*]
* For residual solvents with PDEs of more than 50 mg per day, see the discussion in the section <i>Class 3</i> under <i>Limits of Residual Solvents</i> .	

METHODS FOR ESTABLISHING EXPOSURE LIMITS

The method used to establish PDEs for residual solvents is presented in *Appendix 3*.

For articles that are designated "for veterinary use only", higher levels for the PDE and concentration limit may be justified in exceptional cases based upon the actual daily dose, actual target species, and relevant toxicological data and considering consumer safety impact. For the purpose of this Pharmacopeia, when a manufacturer has received approval from a competent regulatory authority for a higher limit, it is the responsibility of that manufacturer to

notify the USP regarding the approved residual solvent limit in the article and the justification. The USP will then address this topic in the individual monograph.

OPTIONS FOR DESCRIBING LIMITS OF CLASS 2 RESIDUAL SOLVENTS

Two options are available when setting limits for Class 2 residual solvents.

Option 1

The concentration limits in ppm stated in *Table 2* are used. They were calculated using the equation below by assuming a product weight of 10 g administered daily.

$$\text{Concentration (ppm)} = (1000 \mu\text{g}/\text{mg} \times \text{PDE})/\text{dose}$$

Here, PDE is given in terms of mg per day, and dose is given in g per day.

These limits are considered acceptable for all drug substances, excipients, and drug products. Therefore, this option may be applied if the daily dose is not known or fixed. If all drug substances and excipients in a formulation meet the limits given in *Option 1*, these components may be used in any proportion. No further calculation is necessary, provided that the daily dose does not exceed 10 g. Products that are administered in doses greater than 10 g per day are to be considered under *Option 2*.

Option 2

It is not necessary for each component of the drug product to comply with the limits given in *Option 1*. The PDE in terms of mg per day as stated in *Table 2* can be used with the known maximum daily dose and the equation above to determine the concentration of residual solvent allowed in a drug product. Such limits are considered acceptable, provided that it has been demonstrated that the residual solvent has been reduced to the practical minimum. The limits should be realistic in relation to analytical precision, manufacturing capability, and reasonable variation in the manufacturing process. The limits should also reflect contemporary manufacturing standards.

Option 2 may be applied by adding the amounts of a residual solvent present in each of the components of the drug product. The sum of the amounts of solvent per day should be less than that given by the PDE.

Consider an example of the application of *Option 1* and *Option 2* to acetonitrile concentration in a drug product. The permitted daily exposure to acetonitrile is 4.1 mg per day; thus, the *Option 1* limit is 410 ppm. The maximum administered daily weight of a drug product is 5.0 g, and the drug product contains two excipients. The composition of the drug product and the calculated maximum content of residual acetonitrile are given in the following table.

Component	Amount in Formulation (g)	Acetonitrile Content (ppm)	Daily Exposure (mg)
Drug substance	0.3	800	0.24
Excipient 1	0.9	400	0.36
Excipient 2	3.8	800	3.04
Drug product	5.0	728	3.64

Excipient 1 meets the *Option 1* limit, but the drug substance, excipient 2, and drug product do

not meet the *Option 1* limit. Nevertheless, the drug product meets the *Option 2* limit of 4.1 mg per day and thus conforms to the acceptance criteria in this general chapter.

Consider another example, using acetonitrile as the residual solvent. The maximum administered daily weight of a drug product is 5.0 g, and the drug product contains two excipients. The composition of the drug product and the calculated maximum content of residual acetonitrile are given in the following table.

Component	Amount in Formulation (g)	Acetonitrile Content (ppm)	Daily Exposure (mg)
Drug substance	0.3	800	0.24
Excipient 1	0.9	2000	1.80
Excipient 2	3.8	800	3.04
Drug product	5.0	1016	5.08

In this example, the drug product meets neither the *Option 1* nor the *Option 2* limit according to this summation. The manufacturer could test the drug product to determine whether the formulation process reduced the level of acetonitrile. If the level of acetonitrile was not reduced to the allowed limit during formulation, the product fails to meet the solvent limits as described in this chapter, and the manufacturer of the drug product should take other steps to reduce the amount of acetonitrile in the drug product. In some instances, the manufacturer may have received approval from a competent regulatory authority for such a higher level of residual solvent. If this is the case, it is the responsibility of that manufacturer to notify the USP regarding the identity of this solvent and the approved residual solvent limit in the article. The USP will then address this topic in the individual monograph.

ANALYTICAL PROCEDURES

Residual solvents are typically determined using chromatographic techniques such as gas chromatography. Compendial methods for testing for residual solvent content are described under the section *Identification, Control, and Quantification of Residual Solvents* in this general chapter. The *General Notices* discuss the use of other methods in special circumstances (see 6.30. *Alternative and Harmonized Methods and Procedures*). If Class 3 solvents are present, a nonspecific method such as loss on drying may be used.

REPORTING LEVELS OF RESIDUAL SOLVENTS

Manufacturers of pharmaceutical products need certain information about the content of residual solvents in drug substances or excipients in order to meet the criteria of this general chapter. The following statements are given as acceptable examples of the information that could be provided from a supplier of drug substances or excipients to a pharmaceutical manufacturer. The supplier might choose one of the following as appropriate:

- Only Class 3 solvents are likely to be present. Loss on drying is less than 0.5%.
- Only Class 2 solvents X, Y,... are likely to be present. All are below the *Option 1* limit. (Here the supplier would name the Class 2 solvents represented by X, Y,...)
- Only Class 2 solvents X, Y,... and Class 3 solvents are likely to be present. Residual Class 2 solvents are below the *Option 1* limit, and residual Class 3 solvents are below 0.5%.

The phrase "likely to be present", as used in the above examples, refers to the solvent used or produced in the final manufacturing step and to solvents that are used or produced in earlier manufacturing steps and not removed consistently by a validated process.

If Class 1 solvents are likely to be present, they should be identified and quantified. If solvents of Class 2 or 3 are present at greater than their *Option 1* limits or 0.5%, respectively, they should be identified and quantified.

LIMITS OF RESIDUAL SOLVENTS

Class 1 (solvents to be avoided)

Class 1 residual solvents (*Table 1*) should not be employed in the manufacture of drug substances, excipients, and drug products because of the unacceptable toxicities or deleterious environmental effects of these residual solvents. However, if their use in order to produce a medicinal product with a significant therapeutic advance is unavoidable, their levels should be restricted as shown in *Table 1*, unless otherwise stated in the individual monograph. The solvent 1,1,1-trichloroethane is included in *Table 1* because it is an environmental hazard. The stated limit of 1500 ppm is based on a review of safety data.

When Class 1 residual solvents are used or produced in the manufacture or purification of a drug substance, excipient, or drug product and are not removed by the process, these solvents should be identified and quantified. The procedures described in the section *Identification, Control, and Quantification of Residual Solvents* in this general chapter are to be applied wherever possible. Otherwise, an appropriate validated procedure is to be employed.

Table 1. Class 1 Residual Solvents
(Solvents that should be avoided)

Solvent	Concentration Limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

Class 2

Class 2 residual solvents (*Table 2*) should be limited in drug substances, excipients, and drug products because of the inherent toxicities of the residual solvents. PDEs are given to the nearest 0.1 mg per day, and concentrations are given to the nearest 10 ppm. The stated values do not reflect the necessary analytical precision of the determination procedure. Precision should be determined as part of the procedure validation.

If Class 2 residual solvents are present at greater than their *Option 1* limits, they should be identified and quantified. The procedures described in the section *Identification, Control, and Quantification of Residual Solvents* in this general chapter are to be applied wherever possible. Otherwise an appropriate validated procedure is to be employed. [Note—The following Class 2 residual solvents are not readily detected by the headspace injection conditions described in the section *Identification, Control, and Quantification of Residual Solvents* in this general

chapter: formamide, 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, *N*-methylpyrrolidone, and sulfolane. Other appropriate validated procedures are to be employed for the quantification of these residual solvents. Such procedures shall be submitted to the USP for review and possible inclusion in the relevant individual monograph. In addition, USP Residual Solvent Class 2—Mixture C RS can be used to develop an alternative procedure.]

Table 2. Class 2 Residual Solvents

Solvent	PDE (mg/day)	Concentration Limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cumene	0.7	70
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
1,2-Dimethoxyethane	1.0	100
<i>N,N</i> -Dimethylacetamide	10.9	1090
<i>N,N</i> -Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methylbutylketone	0.5	50
Methylcyclohexane	11.8	1180
Methylene chloride	6.0	600
<i>N</i> -Methylpyrrolidone	5.3	530
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetrahydrofuran	7.2	720
Tetraalin	1.0	100
Toluene	8.9	890
Trichloroethylene	0.8	80
Xylene*	21.7	2170

* Usually 60% *m*-xylene, 14% *p*-xylene, 9% *o*-xylene with 17% ethyl benzene.

Class 3

Class 3 residual solvents (Table 3) may be regarded as less toxic and of lower risk to human health than Class 1 and Class 2 residual solvents. Class 3 includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals. However, there are no

long-term toxicity or carcinogenicity studies for many of the residual solvents in Class 3. Available data indicate that they are less toxic in acute or short-term studies and negative in genotoxicity studies.

It is considered that amounts of these residual solvents of 50 mg per day or less (corresponding to 5000 ppm or 0.5% under *Option 1*) would be acceptable without justification. Higher amounts may also be acceptable, provided that they are realistic in relation to manufacturing capability and good manufacturing practice. For the purposes of this Pharmacopeia, when a manufacturer has received approval from a competent regulatory authority for such a higher level of residual solvent, it is the responsibility of that manufacturer to notify the USP regarding the identity of this solvent and the approved residual solvent limit in the article. The USP will then address this topic in the individual monograph. If a Class 3 solvent limit in an individual monograph is greater than 50 mg per day, that residual solvent should be identified and quantified. The procedures described in the section *Identification, Control, and Quantification of Residual Solvents* in this general chapter, with appropriate modifications to the standard solutions, are to be applied wherever possible. Otherwise, an appropriate validated procedure is to be employed.

Table 3. Class 3 Residual Solvents

(Limited by GMP or other quality-based requirements in drug substances, excipients, and drug products)

Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethylketone
<i>tert</i> -Butylmethyl ether	Methylisobutylketone
-	2-Methyl-1-propanol
Dimethyl sulfoxide	Pentane
Ethanol	1-Pentanol
Ethyl acetate	1-Propanol
Ethyl ether	2-Propanol
Ethyl formate	Propyl acetate
Formic acid	-

Other Residual Solvents

The residual solvents listed in *Table 4* may also be of interest to manufacturers of drug substances, excipients, or drug products. However, no adequate toxicological data on which to base a PDE was found.

Table 4. Other Residual Solvents

(For which no adequate toxicological data was found)

1,1-Diethoxypropane	Methyl isopropyl ketone
1,1-Dimethoxymethane	Methyltetrahydrofuran
2,2-Dimethoxypropane	Solvent hexane
Isooctane	Trichloroacetic acid
Isopropyl ether	Trifluoroacetic acid

IDENTIFICATION, CONTROL, AND QUANTIFICATION OF RESIDUAL SOLVENTS

Whenever possible, the substance under test needs to be dissolved to release the residual solvent. Because the USP deals with drug products, as well as active ingredients and excipients, it may be acceptable that in some cases, some of the components of the formulation will not dissolve completely. In those cases, the drug product may first need to be pulverized into a fine powder so that any residual solvent that may be present can be released. This operation should be performed as fast as possible to prevent the loss of volatile solvents during the procedure.

[Note—The organic-free water specified in the following procedures produces no significantly interfering peaks when chromatographed.]

Class 1 and Class 2 Residual Solvents

The following procedures are useful to identify and quantify residual solvents when the information regarding which solvents are likely to be present in the material is not available. When the information about the presence of specific residual solvents is available, only *Procedure C* is needed to quantify the amount of residual solvents present. A flow diagram for the application of the residual solvent limit tests is shown in *Figure 1*.

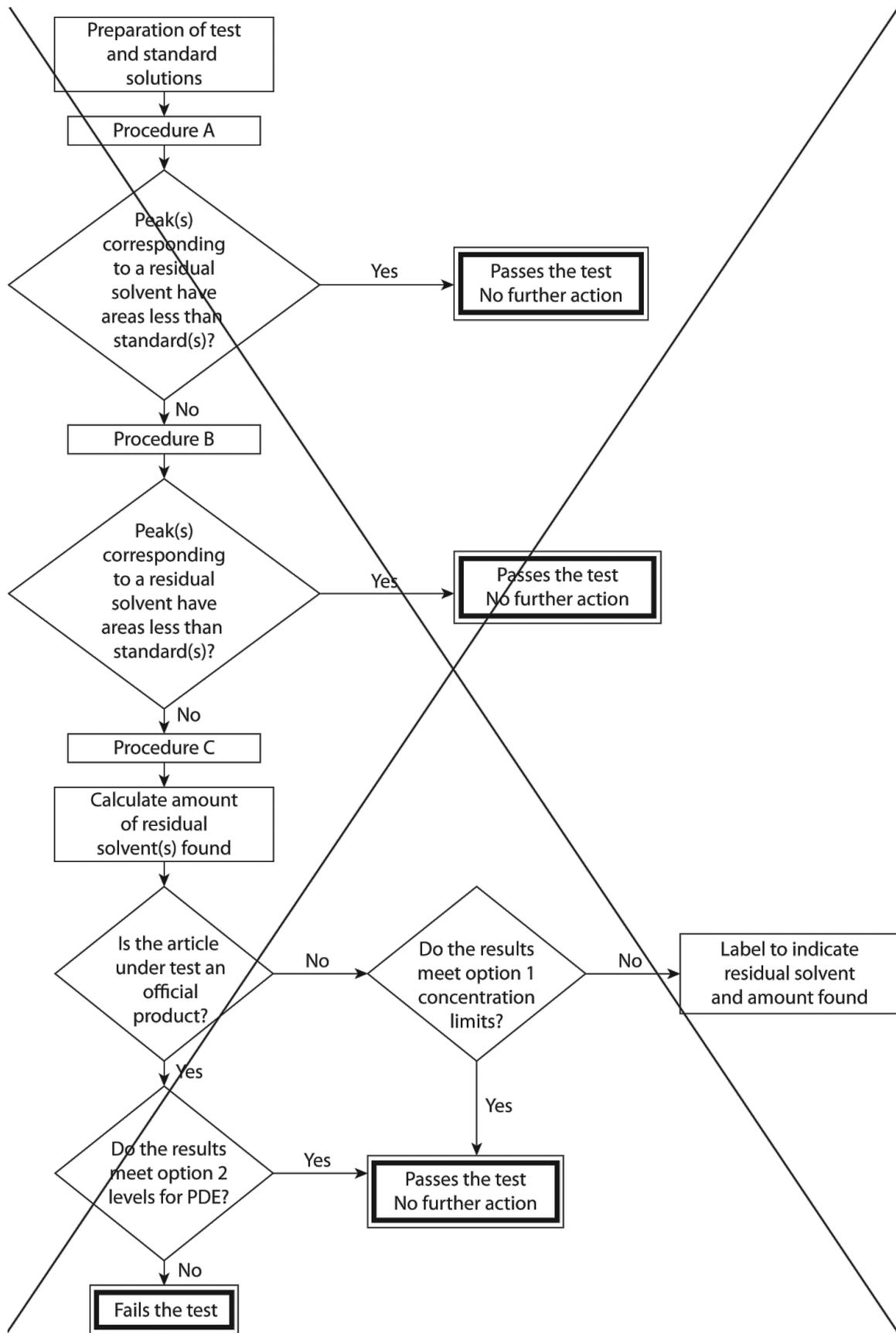


Figure 1. Diagram relating to the identification of residual solvents and the application of limit tests.

WATER-SOLUBLE ARTICLES

Procedure A—

~~*Class 1 Standard Stock Solution*—[Note—When transferring solutions, place the tip of the pipet just below the surface of the liquid, and mix.] Transfer 1.0 mL of USP Class 1 Residual Solvents Mixture RS to a 100-mL volumetric flask, previously filled with about 9 mL of dimethyl sulfoxide, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, previously filled with about 50 mL of water, dilute with water to volume, and mix. Transfer 10 mL of this solution to a 100-mL volumetric flask, previously filled with about 50 mL of water, dilute with water to volume, and mix.~~

~~*Class 1 Standard Solution*—Transfer 1.0 mL of *Class 1 Standard Stock Solution* to an appropriate headspace vial containing 5.0 mL of water (place the tip of the pipet just below the surface of the liquid for dispensing), apply the stopper, cap, and mix.~~

~~*Class 2 Standard Stock Solutions*—Transfer 1.0 mL of USP Residual Solvents Class 2 Mixture A RS to a 100-mL volumetric flask, dilute with water to volume, and mix. This is *Class 2 Standard Stock Solution A*. Transfer 1.0 mL of USP Residual Solvents Class 2 Mixture B RS to a 100-mL volumetric flask, dilute with water to volume, and mix. This is *Class 2 Standard Stock Solution B*.~~

~~*Class 2 Mixture A Standard Solution*—Transfer 1.0 mL of *Class 2 Standard Stock Solution A* to an appropriate headspace vial, add 5.0 mL of water, apply the stopper, cap, and mix.~~

~~*Class 2 Mixture B Standard Solution*—Transfer 5.0 mL of *Class 2 Standard Stock Solution B* to an appropriate headspace vial, add 1.0 mL of water, apply the stopper, cap, and mix.~~

~~*Test Stock Solution*—Transfer about 250 mg of the article under test, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with water to volume, and mix.~~

~~*Test Solution*—Transfer 5.0 mL of *Test Stock Solution* to an appropriate headspace vial, add 1.0 mL of water, apply the stopper, cap, and mix.~~

~~*Class 1 System Suitability Solution*—Transfer 1.0 mL of *Class 1 Standard Stock Solution* to an appropriate headspace vial, add 5.0 mL of *Test Stock Solution*, apply the stopper, cap, and mix.~~

~~*Chromatographic System* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame ionization detector and a 0.32-mm × 30-m fused silica column coated with a 1.8- μ m layer of phase G43 or a 0.53-mm × 30-m wide-bore column coated with a 3.0- μ m layer of phase G43. The carrier gas is nitrogen or helium with a linear velocity of about 35 cm/s, and a split ratio of 1:5. [Note—The split ratio can be modified in order to optimize sensitivity.] The column temperature is maintained at 40° for 20 min, then raised at a rate of 10° per min to 240°, and maintained at 240° for 20 min. The injection port and detector temperatures are maintained at 140° and 250°, respectively. Chromatograph the *Class 1 Standard Solution*, *Class 1 System Suitability Solution*, and *Class 2 Mixture A Standard Solution*, and record the peak responses as directed for *Procedure*: the signal-to-noise ratio of 1,1,1-trichloroethane in the *Class 1 Standard Solution* is NLT 5; the signal-to-noise ratio of each peak in the *Class 1 System Suitability Solution* is NLT 3; and the resolution, *R*, between acetonitrile and methylene chloride in the *Class 2 Mixture A Standard Solution* is NLT 1.0.~~

~~*Procedure*—[Note—It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.] Separately inject (following one of~~

the headspace operating parameter sets described in *Table 5*) equal volumes of headspace (about 1.0 mL) of the *Class 1 Standard Solution*, *Class 2 Mixture A Standard Solution*, *Class 2 Mixture B Standard Solution*, and *Test Solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If a peak response of any peak, other than a peak for 1,1,1-trichloroethane, in the *Test Solution* is greater than or equal to a corresponding peak in either the *Class 1 Standard Solution* or either of the two *Class 2 Mixture Standard Solutions*, or a peak response of 1,1,1-trichloroethane is greater than or equal to 150 times the peak response corresponding to 1,1,1-trichloroethane in the *Class 1 Standard Solution*, proceed to *Procedure B* to verify the identity of the peak; otherwise the article meets the requirements of this test.

Table 5. Headspace Operating Parameters

-	Headspace Operating Parameter Sets		
	1	2	3ntry>
Equilibration temperature (°)	80	105	80
Equilibration time (min)	60	45	45
Transfer line temperature (°) (if appropriate)	85	110	105
Syringe temperature (°) (if appropriate)	80–90	105–115	80–90
Carrier gas: nitrogen or helium at an appropriate pressure			
Pressurization time(s) (if appropriate)	≥60	≥60	≥60
Injection volume (mL)*	±	±	±
* Or follow the instrument manufacturer's recommendations, as long as the method criteria are met. Injecting less than this amount is allowed as long as adequate sensitivity is achieved.			

Procedure B—

Class 1 Standard Stock Solution, *Class 1 Standard Solution*, *Class 2 Standard Stock Solutions*, *Class 2 Mixture A Standard Solution*, *Class 2 Mixture B Standard Solution*, *Test Stock Solution*, *Test Solution*, and *Class 1 System Suitability Solution*—Prepare as directed for *Procedure A*.

Chromatographic System (see *Chromatography* (621))—The gas chromatograph is equipped with a flame ionization detector and a 0.32-mm × 30-m fused-silica column coated with a 0.25-µm layer of phase G16 or a 0.53-mm × 30-m wide-bore column coated with a 0.25-µm layer of phase G16. The carrier gas is nitrogen or helium with a linear velocity of about 35 cm/s and a split ratio of 1:5. [Note—The split ratio can be modified in order to optimize sensitivity.] The column temperature is maintained at 50° for 20 min, then raised at a rate of 6° per min to 165°, and maintained at 165° for 20 min. The injection port and detector temperatures are maintained at 140° and 250°, respectively. Chromatograph the *Class 1 Standard Solution* and the *Class 1 System Suitability Solution*, and record the peak responses as directed for *Procedure*: the signal-to-noise ratio of benzene in the *Class 1 Standard Solution* is NLT 5; the signal-to-noise ratio of each peak in the *Class 1 System Suitability Solution* is NLT 3; and the resolution, *R*, between acetonitrile and *cis*-dichloroethene in the *Class 2 Mixture A Standard Solution* is NLT 1.0.

Procedure—[Note—It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.] Separately inject (following one of

the headspace operating parameter sets described in *Table 5*) equal volumes of headspace (about 1.0 mL) of the *Class 1 Standard Solution*, *Class 2 Mixture A Standard Solution*, *Class 2 Mixture B Standard Solution*, and the *Test Solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If the peak response(s) in the *Test Solution* of the peak(s) identified in *Procedure A* is/are greater than or equal to a corresponding peak(s) in either the *Class 1 Standard Solution* or either of the two *Class 2 Mixture Standard Solutions*, proceed to *Procedure C* to quantify the peak(s); otherwise the article meets the requirements of this test.

Procedure C

Class 1 Standard Stock Solution, *Class 1 Standard Solution*, *Class 2 Standard Stock Solution A*, *Class 2 Mixture A Standard Solution*, *Test Stock Solution*, *Test Solution*, and *Class 1 System Suitability Solution*—Prepare as directed for *Procedure A*.

Standard Stock Solution—[Note—Prepare a separate *Standard Stock Solution* for each peak identified and verified by *Procedures A* and *B*. For the *Class 1* solvents other than 1,1,1-trichloroethane, prepare the first dilution as directed for the first dilution under *Class 1 Standard Stock Solution* in *Procedure A*.] Transfer an accurately measured volume of each individual USP Reference Standard corresponding to each residual solvent peak identified and verified by *Procedures A* and *B* to a suitable container, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a final concentration of 1/20 of the value stated in *Table 1* or *Table 2* (under *Concentration Limit*).

Standard Solution—Transfer 1.0 mL of this solution to an appropriate headspace vial, add 5.0 mL of water, apply the stopper, cap, and mix.

Spiked Test Solution—[Note—Prepare a separate *Spiked Test Solution* for each peak identified and verified by *Procedures A* and *B*.] Transfer 5.0 mL of *Test Stock Solution* to an appropriate headspace vial, add 1.0 mL of the *Standard Stock Solution*, apply the stopper, cap, and mix.

Chromatographic System (see *Chromatography* (621))—[Note—If the results of the chromatography from *Procedure A* are found to be inferior to those found with *Procedure B*, the *Chromatographic System* from *Procedure B* may be substituted.] The gas chromatograph is equipped with a flame ionization detector and a 0.32-mm × 30-m fused-silica column coated with a 1.8- μ m layer of phase G43 or a 0.53-mm × 30-m wide-bore column coated with a 3.0- μ m layer of phase G43. The carrier gas is nitrogen or helium with a linear velocity of about 35 cm/s, and a split ratio of 1:5. [Note—The split ratio can be modified in order to optimize sensitivity.] The column temperature is maintained at 40° for 20 min, then raised at a rate of 10° per min to 240°, and maintained at 240° for 20 min. The injection port and detector temperatures are maintained at 140° and 250°, respectively. Chromatograph the *Class 1 Standard Solution*, the *Class 1 System Suitability Solution*, and the *Class 2 Mixture A Standard Solution*, and record the peak responses as directed for *Procedure*: the signal to noise ratio of 1,1,1-trichloroethane in the *Class 1 Standard Solution* is NLT 5; the signal to noise ratio of each peak in the *Class 1 System Suitability Solution* is NLT 3; and the resolution, *R*, between acetonitrile and methylene chloride in the *Class 2 Mixture A Standard Solution* is NLT 1.0.

Procedure—[Note—It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.] Separately inject (following one of the headspace operating parameters described in *Table 5*) equal volumes of headspace (about 1.0 mL) of the *Standard Solution*, *Test Solution*, and the *Spiked Test Solution* into the

chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount, in ppm, of each residual solvent found in the article under test by the formula:

$$5(C/W)[r_U/(r_{ST}-r_U)]$$

in which C is the concentration, in μg per mL, of the appropriate USP Reference Standard in the *Standard Stock Solution*; W is the weight, in g, of the article under test taken to prepare the *Test Stock Solution*; and r_U and r_{ST} are the peak responses of each residual solvent obtained from the *Test Solution* and the *Spiked Test Solution*, respectively.

WATER-INSOLUBLE ARTICLES

Procedure A—[Note—Dimethyl sulfoxide may be substituted as an alternative solvent to dimethylformamide.]

Class 1 Standard Stock Solution—Transfer 1.0 mL of USP Class 1 Residual Solvents Mixture RS to a 100-mL volumetric flask previously filled with about 80 mL of dimethylformamide, dilute with dimethylformamide to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, previously filled with about 80 mL of dimethylformamide, dilute with dimethylformamide to volume, and mix (reserve a portion of this solution for the *Class 1 System Suitability Solution*). Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with dimethylformamide to volume, and mix.

Class 1 Standard Solution—Transfer 1.0 mL of *Class 1 Standard Stock Solution* to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.

Class 2 Standard Stock Solutions—Transfer 1.0 mL of USP Residual Solvents Class 2 Mixture A RS to a 100-mL volumetric flask, previously filled with about 80 mL of dimethylformamide, dilute with dimethylformamide to volume, and mix. This is *Class 2 Standard Stock Solution A*. Transfer 0.5 mL of USP Residual Solvents Class 2 Mixture B RS to a 10-mL volumetric flask, dilute with dimethylformamide to volume, and mix. This is *Class 2 Standard Stock Solution B*.

Class 2 Mixture A Standard Solution—Transfer 1.0 mL of *Class 2 Standard Stock Solution A* to an appropriate headspace vial containing 5.0 mL of water, apply the stopper, cap, and mix.

Class 2 Mixture B Standard Solution—Transfer 1.0 mL of *Class 2 Standard Stock Solution B* to an appropriate headspace vial containing 5.0 mL of water, apply the stopper, cap, and mix.

Test Stock Solution—Transfer about 500 mg of the article under test, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with dimethylformamide to volume, and mix.

Test Solution—Transfer 1.0 mL of *Test Stock Solution* to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.

Class 1 System Suitability Solution—Mix 5 mL of *Test Stock Solution* with 0.5 mL of the intermediate dilution reserved from *Class 1 Standard Stock Solution*. Transfer 1.0 mL of this solution to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.

Chromatographic System (see *Chromatography* (621))—The gas chromatograph is equipped with a flame ionization detector and a 0.53-mm \times 30-m wide-bore column coated with a 3.0- μm layer of phase G43. The carrier gas is helium with a linear velocity of about 35 cm/s and a split ratio of 1:3. [Note—The split ratio can be modified in order to optimize sensitivity.] The

column temperature is maintained at 40° for 20 min, then raised at a rate of 10° per min to 240°, and maintained at 240° for 20 min. The injection port and detector temperatures are maintained at 140° and 250°, respectively. Chromatograph the *Class 1 Standard Solution*, *Class 1 System Suitability Solution*, and *Class 2 Mixture A Standard Solution*, and record the peak responses as directed for *Procedure*: the signal-to-noise ratio of 1,1,1-trichloroethane in the *Class 1 Standard Solution* is NLT 5; the signal-to-noise ratio of each peak in the *Class 1 System Suitability Solution* is NLT 3; and the resolution, *R*, between acetonitrile and methylene chloride in the *Class 2 Mixture A Standard Solution* is NLT 1.0.

Procedure—[Note—It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.] Separately inject (use headspace operating parameters in column 3 of *Table 5* with a vial pressure of 10 psi) equal volumes of headspace (about 1.0 mL) of the *Class 1 Standard Solution*, *Class 2 Mixture A Standard Solution*, *Class 2 Mixture B Standard Solution*, and *Test Solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If a peak response of any peak, other than a peak for 1,1,1-trichloroethane, in the *Test Solution* is greater than or equal to a corresponding peak in either the *Class 1 Standard Solution* or either of the two *Class 2 Mixture Standard Solutions*, or a peak response of 1,1,1-trichloroethane is greater than or equal to 150 times the peak response corresponding to 1,1,1-trichloroethane in the *Class 1 Standard Solution*, proceed to *Procedure B* to verify the identity of the peak; otherwise, the article meets the requirements of this test.

Procedure B—

Class 1 Standard Stock Solution, *Class 1 Standard Solution*, *Class 1 System Suitability Solution*, *Class 2 Standard Stock Solutions*, *Class 2 Mixture A Standard Solution*, and *Class 2 Mixture B Standard Solution*, *Test Stock Solution*, and *Test Solution*—Proceed as directed for *Procedure A*.

Chromatographic System—Proceed as directed for *Procedure B* under *Water-Soluble Articles* with a split ratio of 1:3.[Note—The split ratio can be modified in order to optimize sensitivity.]

Procedure—[Note—It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.] Separately inject (use headspace operating parameters in column 3 of *Table 5* with a vial pressure of 10 psi) equal volumes of headspace (about 1.0 mL) of the *Class 1 Standard Solution*, *Class 2 Mixture A Standard Solution*, *Class 2 Mixture B Standard Solution*, and *Test Solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If the peak response(s) in *Test Solution* of the peak(s) identified in *Procedure A* is/are greater than or equal to a corresponding peak(s) in either the *Class 1 Standard Solution* or any of the two *Class 2 Mixture Standard Solutions*, proceed to *Procedure C* to quantify the peak(s); otherwise, the article meets the requirements of this test.

Procedure C—

Class 1 Standard Stock Solution, *Class 1 Standard Solution*, *Class 1 System Suitability Solution*, *Class 2 Standard Stock Solution A*, and *Class 2 Mixture A Standard Solution*—Proceed as directed for *Procedure A*.

Standard Stock Solution—[Note—Prepare a separate *Standard Stock Solution* for each peak identified and verified by *Procedures A* and *B*. For the *Class 1* solvents other than 1,1,1-

~~trichloroethane, prepare the first dilution as directed for the first dilution under *Class 1 Standard Stock Solution* in *Procedure A*.] Transfer an accurately measured volume of each individual USP Reference Standard corresponding to each residual solvent peak identified and verified by *Procedures A* and *B* to a suitable container, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a final concentration of 1/20 of the value stated in *Table 1* or *Table 2* (under *Concentration Limit*).~~

~~*Standard Solution*—Transfer 1.0 mL of the *Standard Stock Solution* to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.~~

~~*Test Stock Solution*—Proceed as directed for *Procedure A*.~~

~~*Test Solution*—Transfer 1.0 mL of the *Test Stock Solution* to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.~~

~~*Spiked Test Solution*—[Note—Prepare a separate *Spiked Test Solution* for each peak identified and verified by *Procedures A* and *B*.] Transfer 1.0 mL of *Test Stock Solution* to an appropriate headspace vial, add 1 mL of *Standard Stock Solution* and 4.0 mL of water, apply the stopper, cap, and mix.~~

~~*Chromatographic System*—Proceed as directed for *Procedure C* under *Water-Soluble Articles*.~~

~~*Procedure*—[Note—It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.] Separately inject (use headspace operating parameters in column 3 of *Table 5* with a vial pressure of 10 psi) equal volumes of headspace (about 1.0 mL) of the *Standard Solution*, *Test Solution*, and *Spiked Test Solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount, in ppm, of each residual solvent found in the article under test by the formula:~~

$$10 \times (C/W)[r_U/(r_{ST}-r_U)]$$

~~in which *C* is the concentration, in μg per mL, of the appropriate USP Reference Standard in the *Standard Stock Solution*; *W* is the weight, in g, of the article under test taken to prepare the *Test Stock Solution*; and r_U and r_{ST} are the peak responses of each residual solvent obtained from *Test Solution* and *Spiked Test Solution*, respectively.~~

Class 3 Residual Solvents

~~If Class 3 solvents are present, the level of residual solvents may be determined as directed under *Loss on Drying* (731) when the monograph for the article under test contains a loss on drying procedure specifying an upper limit of no more than 0.5% (per *Option 1* in this general chapter), or a specific determination of the solvent may be made. If there is no loss on drying procedure in the monograph for the article under test or if a Class 3 solvent limit in an individual monograph is greater than 50 mg per day (corresponding to 5000 ppm or 0.5% under *Option 1*), the individual Class 3 residual solvent or solvents present in the article under test should be identified and quantified, and the procedures as described above, with appropriate modifications to the standard solutions, are to be applied wherever possible. Otherwise, an appropriate validated procedure is to be employed. USP Reference Standards, where available, should be used in these procedures.~~

~~Acceptable daily intake (ADI): The maximum acceptable intake of toxic chemicals per day. This term is used by the World Health Organization (WHO).~~

~~Genotoxic carcinogens: Carcinogens that produce cancer by affecting genes or chromosomes.~~

~~Lowest observed effect level (LOEL): The lowest dose of a substance in a study or group of studies that produces biologically significant increases in frequency or severity of any effects in exposed humans or animals.~~

~~Modifying factor: A factor determined by professional judgment of a toxicologist and applied to bioassay data so that the data can be safely related to humans.~~

~~Neurotoxicity: The ability of a substance to cause adverse effects on the nervous system.~~

~~No observed effect level (NOEL): The highest dose of a substance at which there are no biologically significant increases in frequency or severity of any effects in exposed humans or animals.~~

~~Permitted daily exposure (PDE): The maximum acceptable intake per day of a residual solvent in pharmaceutical products.~~

~~Reversible toxicity: The occurrence of harmful effects that are caused by a substance and that disappear after exposure to the substance ends.~~

~~Strongly suspected human carcinogen: A substance for which there is no epidemiological evidence of carcinogenesis but for which there are positive genotoxicity data and clear evidence of carcinogenesis in rodents.~~

~~Teratogenicity: The occurrence of structural malformations in a developing fetus when a substance is administered during pregnancy.~~

~~Tolerable daily intake (TDI): Tolerable daily exposure to toxic chemicals. Term used by the International Program on Chemical Safety (IPCS).~~

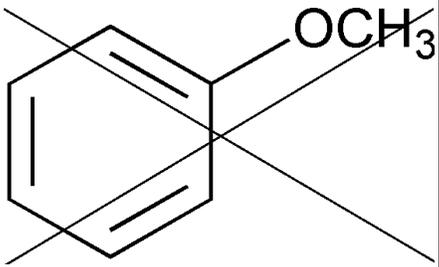
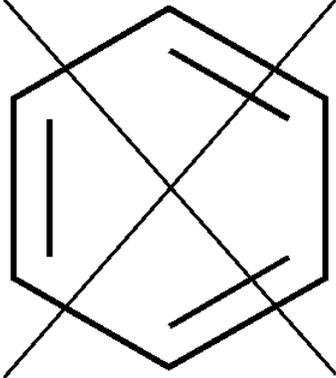
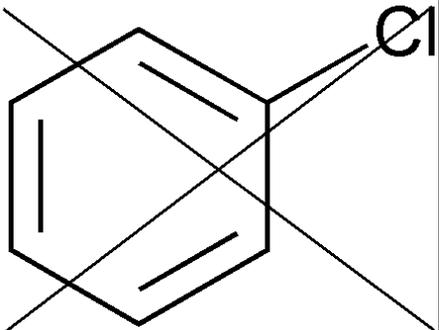
APPENDIX 1. LIST

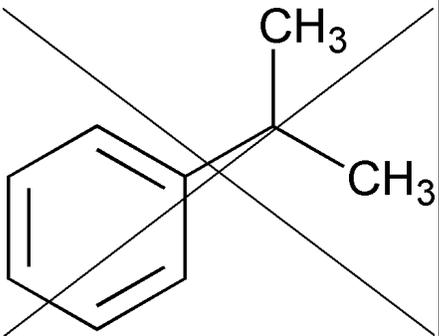
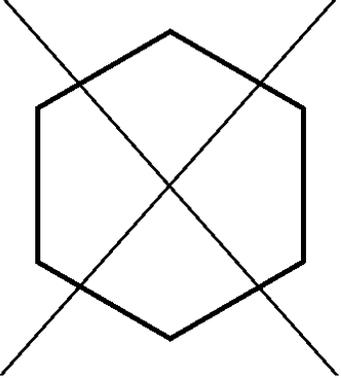
See the table *Appendix 1. List of Residual Solvents Included in This General Chapter.*

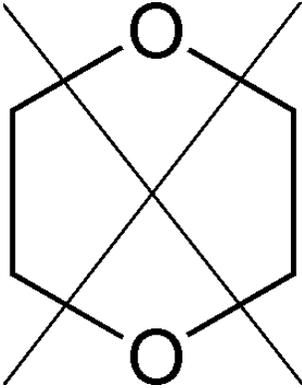
APPENDIX 1. LIST OF RESIDUAL SOLVENTS INCLUDED IN THIS GENERAL CHAPTER

Solvent	Other Names	Structure	Class
Acetic acid	Ethanoic acid	CH_3COOH	Class-3
Acetone	2-Propanone Propan-2-one	CH_3COCH_3	Class-3
Acetonitrile	-	CH_3CN	Class-2

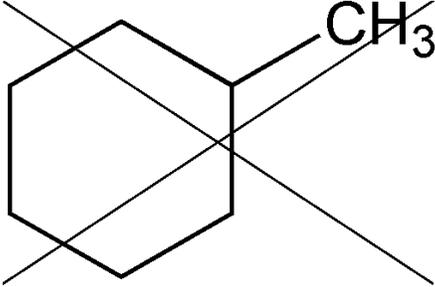
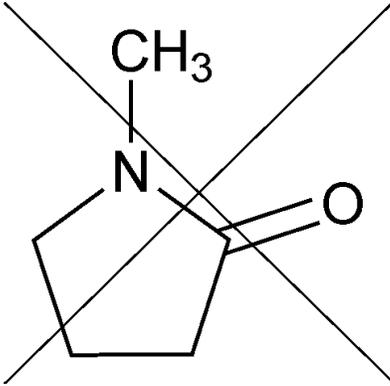
* Usually 60% *m*-xylene, 14% *p*-xylene, 9% *o*-xylene with 17% ethyl benzene.

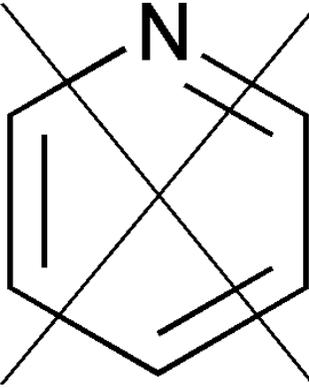
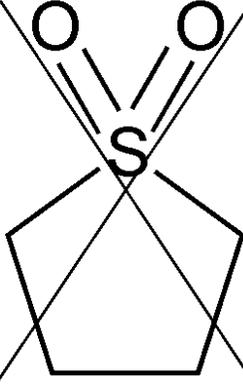
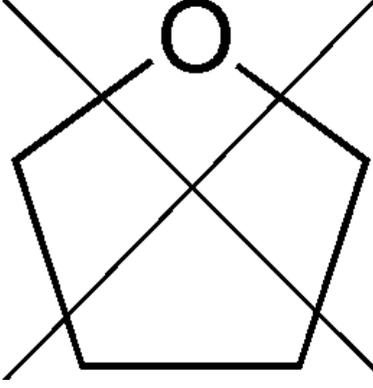
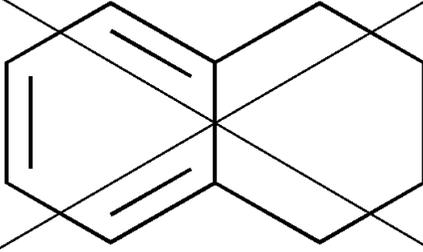
Solvent	Other Names	Structure	Class
Anisole	Methoxybenzene		Class-3
Benzene	Benzol		Class-1
1-Butanol	<i>n</i> -Butyl alcohol Butan-1-ol	$\text{CH}_3(\text{CH}_2)_3\text{OH}$	Class-3
2-Butanol	<i>sec</i> -Butyl alcohol Butan-2-ol	$\text{CH}_3\text{CH}_2\text{CH}(\text{OH})\text{CH}_3$	Class-3
Butyl acetate	Acetic acid butyl ester	$\text{CH}_3\text{COO}(\text{CH}_2)_3\text{CH}_3$	Class-3
<i>tert</i> -Butylmethyl ether	2-Methoxy-2-methylpropane	$(\text{CH}_3)_3\text{COCH}_3$	Class-3
Carbon tetrachloride	Tetrachloromethane	CCl_4	Class-1
Chlorobenzene	-		Class-2
Chloroform	Trichloromethane	CHCl_3	Class-2
* Usually 60% <i>m</i> -xylene, 14% <i>p</i> -xylene, 9% <i>o</i> -xylene with 17% ethyl benzene.			

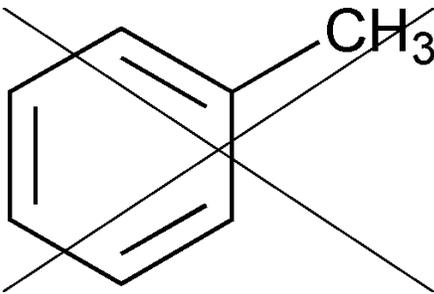
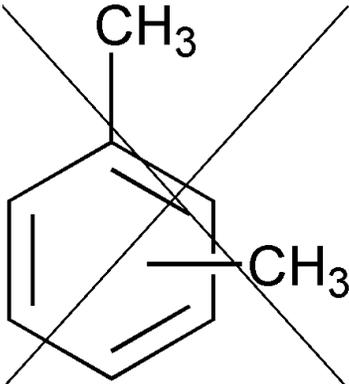
Solvent	Other Names	Structure	Class
Cumene	Isopropylbenzene (1-Methylethyl)benzene		Class-2
Cyclohexane	Hexamethylene		Class-2
1,2-Dichloroethane	<i>sym</i> -Dichloroethane Ethylene dichloride Ethylene chloride	$\text{CH}_2\text{ClCH}_2\text{Cl}$	Class-1
1,1-Dichloroethene	1,1-Dichloroethylene Vinylidene chloride	$\text{H}_2\text{C}=\text{CCl}_2$	Class-1
1,2-Dichloroethene	1,2-Dichloroethylene Acetylene dichloride	$\text{ClHC}=\text{CHCl}$	Class-2
1,2-Dimethoxyethane	Ethyleneglycol dimethyl ether Monoglyme Dimethyl cellosolve	$\text{H}_3\text{COCH}_2\text{CH}_2\text{OCH}_3$	Class-2
<i>N,N</i> -Dimethylacetamide	DMA	$\text{CH}_3\text{CON}(\text{CH}_3)_2$	Class-2
<i>N,N</i> -Dimethylformamide	dimethylformamide	$\text{HCON}(\text{CH}_3)_2$	Class-2
* Usually 60% <i>m</i> -xylene, 14% <i>p</i> -xylene, 9% <i>o</i> -xylene with 17% ethyl benzene.			

Solvent	Other Names	Structure	Class
Dimethyl sulfoxide	Methylsulfinylmethane Methyl sulfoxide dimethyl sulfoxide	$(\text{CH}_3)_2\text{SO}$	Class-3
1,4-Dioxane	<i>p</i> -Dioxane [1,4]Dioxane		Class-2
Ethanol	Ethyl alcohol	$\text{CH}_3\text{CH}_2\text{OH}$	Class-3
2-Ethoxyethanol	Cellosolve	$\text{CH}_3\text{CH}_2\text{OCH}_2\text{CH}_2\text{OH}$	Class-2
Ethyl acetate	Acetic acid ethyl ester	$\text{CH}_3\text{COOCH}_2\text{CH}_3$	Class-3
Ethylene glycol	1,2-Dihydroxyethane 1,2-Ethanediol	$\text{HOCH}_2\text{CH}_2\text{OH}$	Class-2
Ethyl ether	Diethyl ether Ethoxyethane 1,1'-Oxybisethane	$\text{CH}_3\text{CH}_2\text{OCH}_2\text{CH}_3$	Class-3
Ethyl formate	Formic acid ethyl ester	$\text{HCOOCH}_2\text{CH}_3$	Class-3
Formamide	Methanamide	HCONH_2	Class-2
Formic acid	-	HCOOH	Class-3
Heptane	<i>n</i> -Heptane	$\text{CH}_3(\text{CH}_2)_5\text{CH}_3$	Class-3
Hexane	<i>n</i> -Hexane	$\text{CH}_3(\text{CH}_2)_4\text{CH}_3$	Class-2
Isobutyl acetate	Acetic acid isobutyl ester	$\text{CH}_3\text{COOCH}_2\text{CH}(\text{CH}_3)_2$	Class-3
Isopropyl acetate	Acetic acid isopropyl ester	$\text{CH}_3\text{COOCH}(\text{CH}_3)_2$	Class-3
Methanol	Methyl alcohol	CH_3OH	Class-2
2-Methoxyethanol	Methyl cellosolve	$\text{CH}_3\text{OCH}_2\text{CH}_2\text{OH}$	Class-2
Methyl acetate	Acetic acid methyl ester	$\text{CH}_3\text{COOCH}_3$	Class-3
3-Methyl-1-butanol	Isoamyl alcohol Isopentyl alcohol 3-Methylbutan-1-ol	$(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{OH}$	Class-3
Methylbutylketone	2-Hexanone Hexan-2-one	$\text{CH}_3(\text{CH}_2)_3\text{COCH}_3$	Class-2

* Usually 60% *m*-xylene, 14% *p*-xylene, 9% *o*-xylene with 17% ethyl benzene.

Solvent	Other Names	Structure	Class
Methylcyclohexane	Cyclohexylmethane		Class-2
Methylene chloride	Dichloromethane	CH_2Cl_2	Class-2
Methylethylketone	2-Butanone MEK Butan-2-one	$\text{CH}_3\text{CH}_2\text{COCH}_3$	Class-3
Methyl isobutyl ketone	4-Methylpentan-2-one 4-Methyl-2-pentanone MIBK	$\text{CH}_3\text{COCH}_2\text{CH}(\text{CH}_3)_2$	Class-3
2-Methyl-1-propanol	Isobutyl alcohol 2-Methylpropan-1-ol	$(\text{CH}_3)_2\text{CHCH}_2\text{OH}$	Class-3
<i>N</i> -Methylpyrrolidone	1-Methylpyrrolidin-2-one 1-Methyl-2-pyrrolidinone		Class-2
Nitromethane	-	CH_3NO_2	Class-2
Pentane	<i>n</i> -Pentane	$\text{CH}_3(\text{CH}_2)_3\text{CH}_3$	Class-3
1-Pentanol	Amyl alcohol Pentan-1-ol Pentyl alcohol	$\text{CH}_3(\text{CH}_2)_3\text{CH}_2\text{OH}$	Class-3
1-Propanol	Propan-1-ol Propyl alcohol	$\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$	Class-3
2-Propanol	Propan-2-ol Isopropyl alcohol	$(\text{CH}_3)_2\text{CHOH}$	Class-3
Propyl acetate	Acetic acid propyl ester	$\text{CH}_3\text{COOCH}_2\text{CH}_2\text{CH}_3$	Class-3
* Usually 60% <i>m</i> -xylene, 14% <i>p</i> -xylene, 9% <i>o</i> -xylene with 17% ethyl benzene.			

Solvent	Other Names	Structure	Class
Pyridine	-		Class-2
Sulfolane	Tetrahydrothiophene 1,1-dioxide		Class-2
Tetrahydrofuran	Tetramethylene oxide Oxacyclopentane		Class-2
Tetralin	1,2,3,4- Tetrahydronaphthalene		Class-2
<p>* Usually 60% <i>m</i>-xylene, 14% <i>p</i>-xylene, 9% <i>o</i>-xylene with 17% ethyl benzene.</p>			

Solvent	Other Names	Structure	Class
Toluene	Methylbenzene		Class-2
1,1,1-Trichloroethane	Methylchloroform	CH_3CCl_3	Class-1
Trichloroethylene	1,1,2-Trichloroethene	$\text{HC}(\text{Cl})=\text{CCl}_2$	Class-2
Xylene*	Dimethylbenzene Xylol		Class-2
* Usually 60% <i>m</i> -xylene, 14% <i>p</i> -xylene, 9% <i>o</i> -xylene with 17% ethyl benzene.			

APPENDIX 2. ADDITIONAL BACKGROUND

A2.1. Environmental Regulation of Organic Volatile Solvents

Several of the residual solvents frequently used in the production of pharmaceuticals are listed as toxic chemicals in *Environmental Health Criteria* (EHC) monographs and in the Integrated Risk Information System (IRIS). The objectives of such groups as the International Programme on Chemical Safety (IPCS), the United States Environmental Protection Agency (EPA), and the United States Food and Drug Administration (FDA) include the determination of acceptable exposure levels. The goal is maintenance of environmental integrity and protection of human health against the possible deleterious effects of chemicals resulting from long-term environmental exposure. The procedures involved in the estimation of maximum safe exposure limits are usually based on long-term studies. When long-term study data are unavailable, shorter-term study data can be used with modification of the approach, such as use of larger safety factors. The approach described therein relates primarily to long-term or lifetime exposure of the general population in the ambient environment (i.e., ambient air, food, drinking water, and other media).

A2.2. Residual Solvents in Pharmaceuticals

Exposure limits in this general chapter are established by referring to methodologies and toxicity data described in EHC and IRIS monographs. However, the following specific assumptions about residual solvents to be used in the synthesis and formulation of pharmaceutical products should be taken into account in establishing exposure limits.

1. Patients (not the general population) use pharmaceuticals to treat their diseases or for prophylaxis to prevent infection or disease.
2. The assumption of lifetime patient exposure is not necessary for most pharmaceutical products but may be appropriate as a working hypothesis to reduce risk to human health.
3. Residual solvents are unavoidable components in pharmaceutical production and will often be a part of medicinal products.
4. Residual solvents should not exceed recommended levels except in exceptional circumstances.
5. Data from toxicological studies that are used to determine acceptable levels for residual solvents should have been generated using appropriate protocols such as those described, for example, by the Organization for Economic Cooperation and Development (OECD), EPA, and the FDA *Red Book*.

APPENDIX 3. PROCEDURES FOR ESTABLISHING EXPOSURE LIMITS

The Gaylor-Kodell method of risk assessment (Gaylor, D.W., and Kodell, R.L. Linear Interpolation Algorithm for Low Dose Assessment of Toxic Substance. *Journal of Environmental Pathology and Toxicology*, 4:305, 1980) is appropriate for Class 1 carcinogenic solvents. Only in cases where reliable carcinogenicity data are available should extrapolation by the use of mathematical models be applied to setting exposure limits. Exposure limits for Class 1 residual solvents could be determined with the use of a large safety factor (i.e., 10,000 to 100,000) with respect to the no-observed-effect level (NOEL). Detection and quantification of these residual solvents should be performed by state-of-the-art analytical techniques.

Acceptable exposure levels in this general chapter for Class 2 residual solvents were established by calculation of PDE values according to the procedures for setting exposure limits in pharmaceuticals (page 5748 of *PF 15(6)* [Nov.-Dec. 1989]), and the method adopted by IPCS for Assessing Human Health Risk of Chemicals (*Environmental Health Criteria 170*, WHO, 1994). These procedures are similar to those used by the U.S. EPA (IRIS) and the U.S. FDA (*Red Book*) and others. The method is outlined here to give a better understanding of the origin of the PDE values. It is not necessary to perform these calculations in order to use the PDE values presented in *Table 2* of this document.

PDE is derived from the no-observed-effect level (NOEL), or the lowest-observed-effect level (LOEL), in the most relevant animal study as follows:

$$\text{PDE} = (\text{NOEL} \times \text{Weight Adjustment}) / (\text{F1} \times \text{F2} \times \text{F3} \times \text{F4} \times \text{F5}) \quad (1)$$

The PDE is derived preferably from a NOEL. If no NOEL is obtained, the LOEL may be used. Modifying factors proposed here, for relating the data to humans, are the same kind of "uncertainty factors" used in *Environmental Health Criteria* (*Environmental Health Criteria 170*, WHO, Geneva, 1994) and "modifying factors" or "safety factors" in *Pharmacopoeial Forum*. The assumption of 100 percent systemic exposure is used in all calculations regardless of route

of administration.

The modifying factors are as follows:

F1 =	A factor to account for extrapolation between species	
-	F1 =	2 for extrapolation from dogs to humans
-	F1 =	2.5 for extrapolation from rabbits to humans
-	F1 =	3 for extrapolation from monkeys to humans
-	F1 =	5 for extrapolation from rats to humans
-	F1 =	10 for extrapolation from other animals to humans
-	F1 =	12 for extrapolation from mice to humans

F1 takes into account the comparative surface area to body weight ratios for the species concerned and for man. Surface area (S) is calculated as:

$$S = kM^{0.67} \text{---} (2)$$

in which M = body weight, and the constant k has been taken to be 10. The body weights used in the equation are those shown below in Table A3.1.

F2 =	A factor of 10 to account for variability between individuals. A factor of 10 is generally given for all organic solvents, and 10 is used consistently in this general chapter.
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F3 =	A variable factor to account for toxicity studies of short-term exposure	
-	F3 =	1 for studies that last at least one half-lifetime (1 year for rodents or rabbits; 7 years for cats, dogs, and monkeys)
-	F3 =	1 for reproductive studies in which the whole period of organogenesis is covered
-	F3 =	2 for a 6-month study in rodents, or a 3.5-year study in nonrodents
-	F3 =	5 for a 3-month study in rodents, or a 2-year study in nonrodents
-	F3 =	10 for studies of a shorter duration

In all cases, the higher factor has been used for study durations between the time points (e.g., a factor of 2 for a 9-month rodent study).

F4 =	A factor that may be applied in cases of severe toxicity, e.g., nongenotoxic carcinogenicity, neurotoxicity, or teratogenicity. In studies of reproductive toxicity, the following factors are used:	
-	F4 =	1 for fetal toxicity associated with maternal toxicity
-	F4 =	5 for fetal toxicity without maternal toxicity
-	F4 =	5 for a teratogenic effect with maternal toxicity
-	F4 =	10 for a teratogenic effect without maternal toxicity

F5 =	A variable factor that may be applied if the no-effect level was not established
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When only a LOEL is available, a factor of up to 10 can be used, depending on the severity of

the toxicity. The weight adjustment assumes an arbitrary adult human body weight for either sex of 50 kilograms (kg). This relatively low weight provides an additional safety factor against the standard weights of 60 kg or 70 kg that are often used in this type of calculation. It is recognized that some adult patients weigh less than 50 kg; these patients are considered to be accommodated by the built-in safety factors used to determine a PDE. If the solvent was present in a formulation specifically intended for pediatric use, an adjustment for a lower body weight would be appropriate.

As an example of the application of this equation, consider a toxicity study of acetonitrile in mice that is summarized in *Pharmeuropa*, Vol. 9, No. 1, Supplement, April 1997, page S24. The NOEL is calculated to be $50.7 \text{ mg kg}^{-1} \text{ day}^{-1}$. The PDE for acetonitrile in this study is calculated as follows:

$$\text{PDE} = (50.7 \text{ mg kg}^{-1} \text{ day}^{-1} \times 50 \text{ kg}) / (12 \times 10 \times 5 \times 1 \times 1) = 4.22 \text{ mg day}^{-1}$$

In this example,

F1 =	12 to account for the extrapolation from mice to humans
F2 =	10 to account for differences between individual humans
F3 =	5 because the duration of the study was only 13 weeks
F4 =	1 because no severe toxicity was encountered
F5 =	1 because the no-effect level was determined

A3.1. Values Used in the Calculations in This Document

Rat body weight	425 g
Pregnant rat body weight	330 g
Mouse body weight	28 g
Pregnant mouse body weight	30 g
Guinea pig body weight	500 g
Rhesus monkey body weight	2.5 kg
Rabbit body weight (pregnant or not)	4 kg
Beagle dog body weight	11.5 kg
Rat respiratory volume	290 L/day
Mouse respiratory volume	43 L/day
Rabbit respiratory volume	1440 L/day
Guinea pig respiratory volume	430 L/day
Human respiratory volume	28,800 L/day
Dog respiratory volume	9000 L/day
Monkey respiratory volume	1150 L/day
Mouse water consumption	5 mL/day
Rat water consumption	30 mL/day
Rat food consumption	30 g/day

The equation for an ideal gas, $PV = nRT$, is used to convert concentrations of gases used in inhalation studies from units of ppm to units of mg/L or mg/m^3 . Consider as an example the rat reproductive toxicity study by inhalation of carbon tetrachloride (molecular weight 153.84)

summarized in *Pharmeuropa*, Vol. 9, No. 1, Supplement, April 1997, page S9.

$$\frac{n}{V} = \frac{P}{RT} = \frac{300 \times 10^{-6} \text{ atm} \times 153\,840 \text{ mg mol}^{-1}}{0.082 \text{ L at mK}^{-1} \text{ mol}^{-1} \times 298 \text{ K}} = \frac{46.15 \text{ mg}}{24.45 \text{ L}} = 1.89 \text{ mg/L}$$

The relationship $1000 \text{ L} = 1 \text{ m}^3$ is used to convert to mg/m^3 .



INTRODUCTION

For pharmacopeial purposes, residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacturing of drug substances, excipients, or dietary supplement ingredients, or in the preparation of drug products or dietary supplement products. Appropriate selection of the solvent for the synthesis of a drug substance or an excipient may enhance the yield or determine characteristics such as crystal form, purity, and solubility. Therefore, the solvent may sometimes be a critical element in the synthetic process and may not be completely removed by the manufacturing process. Because residual solvents do not provide therapeutic benefit, they should be removed, to the extent possible, to meet safety-based limits, ingredient and product specifications, good manufacturing practices, or other quality-based requirements.

The objective of this general chapter is to define acceptable amounts of residual solvents in pharmaceutical drug products and dietary supplement products for the safety of the patient. Tests for residual solvents are not generally mentioned in specific monographs because the solvents used may vary from one manufacturer to another; however, the limits to be applied must comply with those specified in this general chapter.

This general chapter provides procedures for the analysis of residual solvents, although alternative validated methodologies may also be used to demonstrate compliance with the defined limits. The general chapter also defines requirements for the verification of USP procedures and validation requirements for alternative test methodologies.

This general chapter does not address solvents deliberately used as excipients, nor does it address solvates. However, the content of solvents in such products should be evaluated and justified. The limits specified in this general chapter do not apply directly to excipients and drug substances, except where specified in the individual monographs. However, residual solvent levels present in drug substances and excipients may be used to demonstrate compliance as an integral part of the control strategy, thereby reducing or eliminating the need for analysis in the product (see *Control Strategy*).

Throughout this general chapter, the term "likely to be present" (LTBP) refers to 1) solvents used or produced in the final manufacturing step; 2) solvents used or produced in earlier manufacturing steps that are not consistently removed by a validated process; and 3) solvents properly declared by a validated supplier of a drug substance or excipient.

CLASSIFICATION OF RESIDUAL SOLVENTS BY RISK ASSESSMENT

Residual solvents assessed in this general chapter are listed in *Appendix 1* by common names and structures. These solvents were evaluated for their possible risk to human health and placed into one of three classes based on their toxicity data and their environmental impact.

The USP is aligned with the ICH Q3C approach for the classification of residual solvents (see *Appendix 2* for additional details). The three classes of residual solvents are as follows.

Residual Solvent Class	Assessment
Class 1 (Solvents to be avoided)	Known human carcinogens
	Strongly suspected human carcinogens
	Solvents particularly known to have ozone-depleting properties
Class 2 (Solvents to be limited)	Nongenotoxic animal carcinogens or possible causative agents of other irreversible toxicity, such as neurotoxicity or teratogenicity
	Solvents suspected of other significant but reversible toxicities
Class 3 (Solvents with low toxic potential)	Solvents with low toxic potential to humans; no health-based exposure limit is needed

The limits for Class 2 solvents are based on the toxicological permitted daily exposure (PDE), calculated as defined in *Appendix 3*, whereas Class 3 solvents are considered less toxic, and control to 50 mg/day or less for each of these solvents is acceptable without justification. The lists in this chapter are not exhaustive and subject to revision. For solvents not listed, *Appendix 3* may be used to define PDE limits if sufficient toxicological data are available.

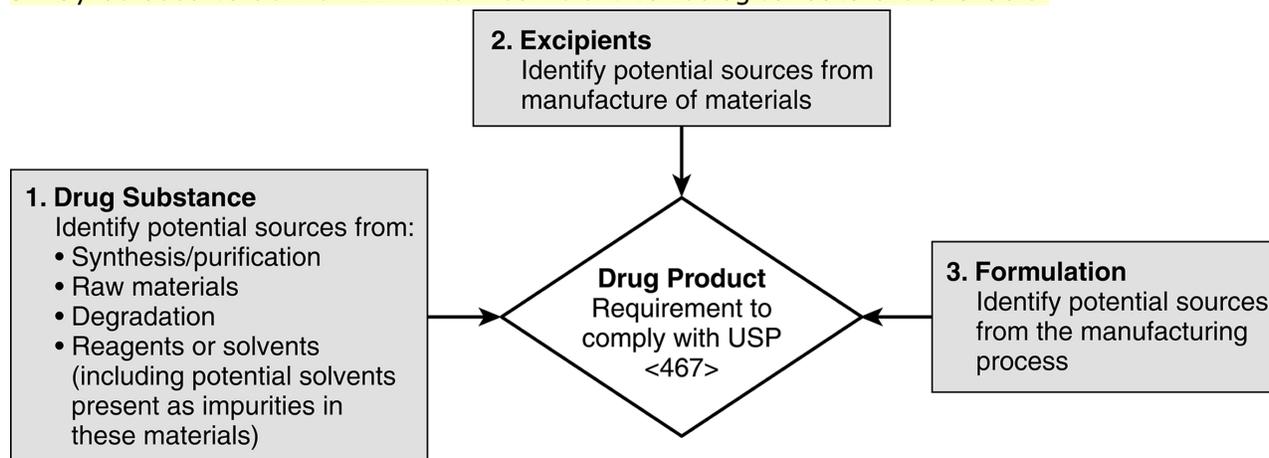


Figure 1. Potential sources of residual solvents in pharmaceutical drug products and dietary supplements.

There are three potential sources of residual solvents in the pharmaceutical drug product that should be considered (see *Figure 1*):

1. Drug substance

Potential sources of solvents include their use or formation during the synthesis or purification of the drug substance; their presence in raw materials or reagents used in their synthesis; or degradation of the drug substance.

2. Excipients

Potential solvent sources include their use or formation during the manufacture or

purification of excipients.

3. Formulation

Potential solvent sources are associated with their use or formation during the drug product manufacturing process.

If solvents are used during production, they must be of suitable quality. In addition, the potential presence of other solvents as impurities must be taken into consideration in the assessment of solvents LTBP. Because of the high toxicity of benzene and other Class 1 solvents, all likely sources of these solvents must be considered. For example, potential sources of benzene may include its presence as an impurity in a solvent used in the manufacturing process; its use in the manufacture of starting material; or its production as a reaction by-product. In the event that the user has insufficient information to complete a thorough assessment of the potential sources of residual solvents or as an alternative to performing this assessment, solvent screening may be used (see *Analytical Procedures*).

Pharmaceutical drug products and dietary supplement products should contain no higher levels of residual solvents than can be supported by safety data. All solvents included in this general chapter are listed in *Appendix 1*. Those solvents that show toxicity of special concern or carcinogenicity, and/or atmospheric ozone-depletion (Class 1, *Table 1*), should be avoided in the production of drug substances, dietary supplement ingredients, excipients, or pharmaceutical drug products/dietary supplement products unless their use can be strongly justified in a risk-benefit assessment. Those solvents associated with less severe but still significant toxicity (Class 2, *Table 2*) should be limited to protect patients from potential adverse effects. Whenever it is practicable, less toxic solvents (Class 3, *Table 3*) should be used. For the purposes of this pharmacopeia, when a manufacturer has received approval from a regulatory authority for the use of a solvent not currently listed in this general chapter, or for a level of a listed solvent higher than the limit currently given in the chapter, it is the responsibility of that manufacturer to notify USP of the identity and level of the solvent, and the appropriate test procedure. USP will then address the information in the individual monograph. A new solvent or revised limit that has been approved through the ICH process will be added to the appropriate list in this general chapter. See *Appendix 2* for additional background information related to residual solvents.

CONTROL STRATEGY

The user may choose to demonstrate compliance with this general chapter by analysis for residual solvents in the drug product or by analysis of the drug product components. These control strategy options are presented in *Figure 2*.

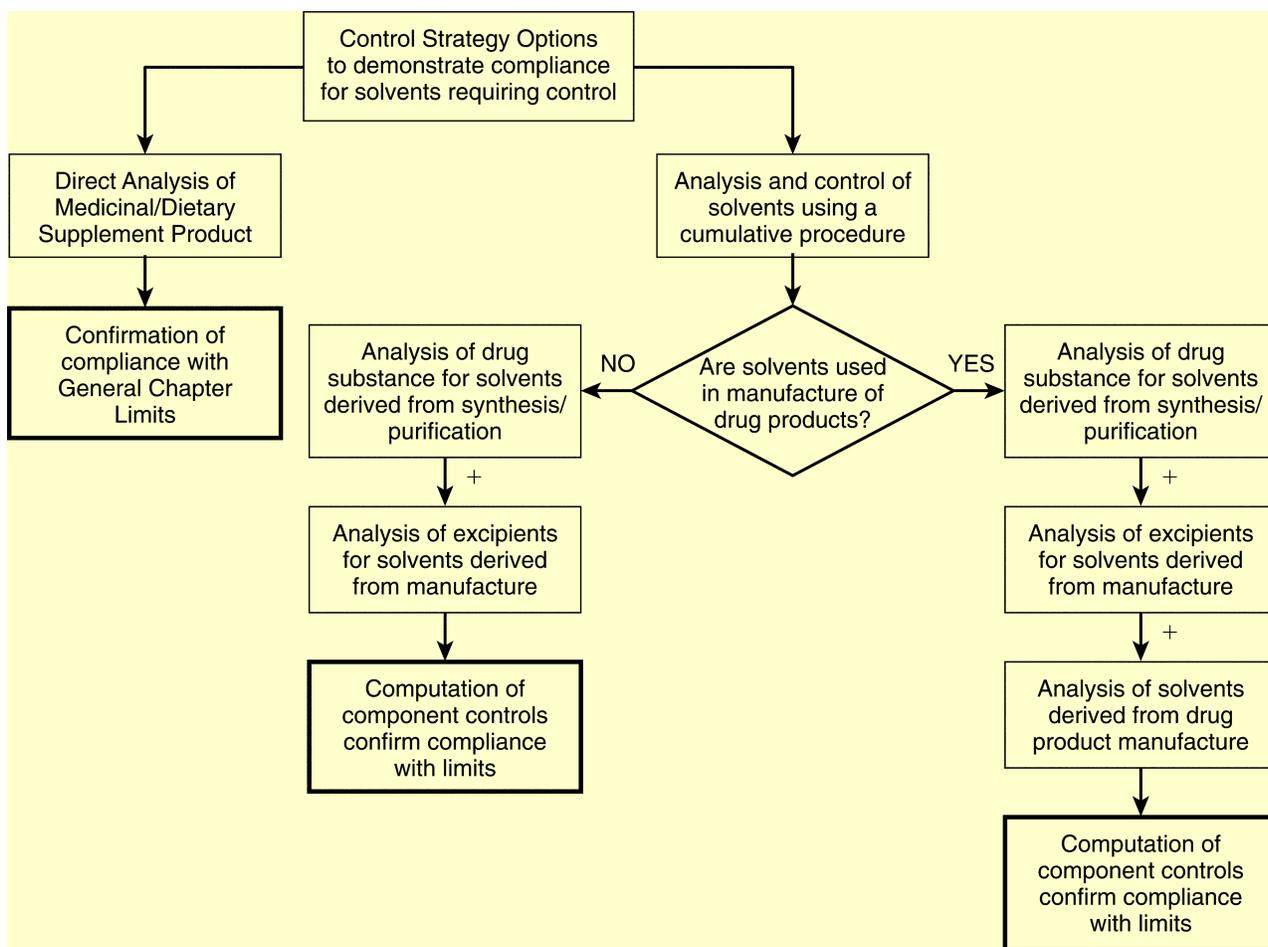


Figure 2. Control strategy options. If analysis of the drug product is needed because solvents are used in the manufacturing process, then analysis of the drug substance and excipients is not needed, provided the solvents LTBP in the drug substance and excipients are included in the test for the drug product.

The following options are to be considered when designing the control strategy.

1. **Testing the drug product:** This approach is acceptable in all cases.
2. **Analysis of the drug product components and using a cumulative approach to determine the solvent content in the drug product:**
 - a. **If no solvents are used during the manufacture of the drug product,** use the cumulative approach to determine the solvent content in API and excipients to calculate solvent levels in the drug product.
 - b. **If solvent(s) are used during the manufacture of the drug product,** use a cumulative approach by analyzing product components [API, intermediate(s), excipients] with the understanding that the solvent content in the product or product intermediate(s) will be determined at the point of use via suitable in-process testing.

In all cases the solvent levels in the final drug product must not exceed the limits defined in the general chapter.

LIMITS OF RESIDUAL SOLVENTS

Class 1: Solvents to Be Avoided

Class 1 residual solvents (*Table 1*) should not be used in the manufacture of drug substances, excipients, or drug products because of their unacceptable toxicities or deleterious environmental effects. However, if their use in order to produce a pharmaceutical drug product with a significant therapeutic advance is unavoidable, their levels should be restricted as shown in *Table 1*, unless otherwise stated in the individual monograph. The solvent 1,1,1-trichloroethane is included in *Table 1* because it is a severe environmental hazard. The stated limit of 1500 ppm is based on a review of safety data.

The procedures described in *Identification, Control, and Quantification of Residual Solvents* in this general chapter are to be applied wherever possible. Otherwise an appropriate validated procedure is to be used.

**Table 1. Control Limits for Class 1 Residual Solvents in Pharmaceutical Products:
Solvents to Be Avoided**

Solvent	Concentration Limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

Class 2: Solvents to Be Limited

Class 2 residual solvents (*Table 2*) should be limited in drug substances, excipients, and drug products because of the inherent toxicities of these residual solvents. PDEs are given to the nearest 0.1 mg/day, and concentrations are given to the nearest 10 ppm. The method used to establish PDEs for residual solvents is presented in *Appendix 3*.

Table 2. Class 2 Residual Solvents

Solvent	PDE (mg/day)	Concentration Limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cumene	0.7	70
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
1,2-Dimethoxyethane	1.0	100
<i>N,N</i> -Dimethylacetamide	10.9	1090
<i>N,N</i> -Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methylbutylketone	0.5	50
Methylcyclohexane	11.8	1180
Methylene chloride	6.0	600
<i>N</i> -Methylpyrrolidone	5.3	530
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetrahydrofuran	7.2	720
Tetralin	1.0	100
Toluene	8.9	890
Trichloroethylene	0.8	80
Xylene ^a	21.7	2170

^a Usually 60% *m*-xylene, 14% *p*-xylene, and 9% *o*-xylene with 17% ethyl benzene.

Class 3: Solvents with Low Toxic Potential

Class 3 solvents (*Table 3*) are regarded as less toxic and of lower risk to human health than Class 1 and Class 2 residual solvents. Class 3 includes no solvent known to be a human health hazard at levels normally accepted in pharmaceuticals. However, there are no long-term toxicity or carcinogenicity studies for many of the residual solvents in Class 3. Available data indicate that they are less toxic in acute or short-term studies and negative in genotoxicity studies.

It is considered that amounts of these residual solvents of 50 mg/day or less (corresponding to 5000 ppm or 0.5% in *Option 1* which is described below) would be acceptable for each solvent without justification. Higher amounts may also be acceptable, provided that they are realistic in

relation to manufacturing capability and good manufacturing practice. If a Class 3 solvent limit in an individual monograph is greater than 0.5%, that residual solvent should be identified and quantified. The procedures described in *Identification, Control, and Quantification of Residual Solvents* in this general chapter, with appropriate modifications to the standard solutions, are to be applied wherever possible. Otherwise, an appropriate validated procedure is to be used.

Table 3. Class 3 Residual Solvents

Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethylketone
<i>tert</i> -Butylmethyl ether	Methylisobutylketone
Dimethyl sulfoxide	2-Methyl-1-propanol
Ethanol	Pentane
Ethyl acetate	1-Pentanol
Ethyl ether	1-Propanol
Ethyl formate	2-Propanol
Formic acid	Propyl acetate

OPTIONS FOR DESCRIBING LIMITS FOR RESIDUAL SOLVENTS

Class 1 Solvents

All drug products must meet the concentration limits described in *Table 1*. If there are no other sources of Class 1 solvents during the drug product manufacturing process, compliance with the general chapter may be demonstrated by using a cumulative approach or by confirmation that the drug substance and excipients meet the concentration limit and no testing for these solvents in the drug product is necessary.

Class 2 and Class 3 Solvents

This general chapter provides two options for establishing compliance. Both *Option 1* and *Option 2* are based on the PDE.

Option 1

The concentration limits in ppm stated in *Table 2* for Class 2 solvents and the general requirement for Class 3 (5000 ppm) are used. They were calculated using the equation below by assuming a product weight of 10 g administered daily.

$$\text{Concentration (ppm)} = (1000 \mu\text{g/mg} \times \text{PDE})/\text{dose}$$

Here, PDE is given in terms of mg/day, and dose is given in g/day. These limits are considered acceptable for all drug substances, excipients, and drug products. Therefore, *Option 1* may be applied if the daily dose is not known or does not exceed 10 g. If all drug substances and excipients in a formulation meet the limits given in *Option 1*, these components may be used in any proportions. No further calculation is necessary, provided that the daily dose does not exceed 10 g. Products that are administered in doses greater than 10 g/day are to be

considered under *Option 2*.

Option 2

Option 2 uses the PDE and the actual maximum daily dose of the product to calculate solvent exposure and assess compliance.

Option 2 must be used to demonstrate compliance with the general chapter where the maximum daily dose of the drug product exceeds 10 g/day or where at least one component in the formulation exceeds the *Option 1* limits.

These limits are applied by adding the amounts of a residual solvent present in each of the components of the drug product. The contribution of each solvent per day should result in a total amount that does not exceed the PDE.

Consider the example of the application of *Option 1* and *Option 2* limits to acetonitrile concentration in a drug product. The PDE for acetonitrile is 4.1 mg/day, thus the *Option 1* limit is 410 ppm. The maximum administered daily mass of a drug product is 5.0 g, and the drug product contains two excipients. Assuming that there is no other source of acetonitrile in the manufacturing process, the calculated drug product content of acetonitrile and the daily exposure are given in *Table 4*.

Table 4

Component	Amount in Formulation (g)	Acetonitrile Content (ppm)	Daily Exposure (mg)
Drug substance	0.3	800	0.24
Excipient 1	0.9	400	0.36
Excipient 2	3.8	800	3.04
Drug product	5.0	728	3.64

Excipient 1 meets the *Option 1* limit, but the *Drug substance*, *Excipient 2*, and *Drug product* do not meet the *Option 1* limit. Nevertheless, the product meets the *Option 2* limit of 4.1 mg/day and thus conforms to the recommendations in this general chapter.

Consider another example using acetonitrile as a residual solvent. The maximum administered daily mass of a drug product is 5.0 g, and the drug product contains two excipients. Assuming no other source of acetonitrile in the manufacturing process, the *Drug product* content of acetonitrile and the maximum content of residual acetonitrile are given in *Table 5*.

Table 5

Component	Amount in Formulation (g)	Acetonitrile Content (ppm)	Daily Exposure (mg)
Drug substance	0.3	800	0.24
Excipient 1	0.9	2000	1.8
Excipient 2	3.8	800	3.04
Drug product	5.0	1016	5.08

In this example, the product meets neither the *Option 1* nor the *Option 2* limit according to the summation. Compliance with the general chapter may still be demonstrated via analysis of the *Drug product* as it is possible that the formulation process may have reduced the level of acetonitrile below the calculated content. If the level of acetonitrile was not reduced during

formulation to the allowed limit, then the manufacturer of the *Drug product* should take other steps to reduce the amount of acetonitrile in the drug product. If all of these steps fail to reduce the level of residual solvent, in exceptional cases the manufacturer could provide a summary of efforts made to reduce the solvent level to meet the guideline value and provide a risk benefit analysis to support allowing the product to be used with the residual solvent at a higher level.

REPORTING LEVELS OF RESIDUAL SOLVENTS

Manufacturers of pharmaceutical products need certain information about the content of residual solvents in drug substances and excipients to meet the criteria of this general chapter. The following statements are given as acceptable examples of the information that could be provided from a supplier of drug substances or excipients to a pharmaceutical manufacturer. The supplier might choose one of the following as appropriate:

- If Class 1 solvents are present, they should be identified and quantified.
- Only Class 3 solvents are LTBP. Loss on drying is less than 0.5%.
- Only Class 2 solvents X, Y,... are likely to be present. All are below the *Option 1* limit. (Here the supplier would name the Class 2 solvents represented by X, Y,...)
- Only Class 2 solvents X, Y,..., and Class 3 solvents are LTBP. Residual Class 2 solvents are below the *Option 1* limit and residual Class 3 solvents are below 0.5%.
- If other non-listed solvents are present, they should be identified and quantified.

The term LTBP as used in the above examples refers to the solvent used or produced in the final manufacturing step and to solvents used or produced in earlier manufacturing steps and not removed consistently by a validated process.

IDENTIFICATION, CONTROL, AND QUANTIFICATION OF RESIDUAL SOLVENTS

Whenever possible, the substance under test needs to be dissolved to release the residual solvent. The control strategy may involve analysis of drug products, active ingredients, and excipients and in some cases the sample does not dissolve completely. In these cases it may be acceptable to pulverize that sample into a fine powder carefully, avoiding the generation of frictional heat and in a manner to prevent the loss of volatile solvents. This operation assures that any residual solvent that may be present can be released. It should be performed as fast as possible immediately prior to dispersion in the sample solvent to prevent the loss of volatile solvents during the procedure.

[Note—The organic-free water specified in the following procedures produces no significantly interfering peaks when chromatographed.]

Class 1 and Class 2 Residual Solvents

The following procedures are recommended to identify and quantify the residual solvents that are listed in this chapter as Class 1 and Class 2 solvents and that may be present in drug substances, excipients, and drug products, with some exceptions disclosed below.

For each test matrix, verification is needed to demonstrate reliability of the compendial procedure, as described later in this chapter.

When the solvents that are present or potentially present in the sample are known, they may be determined using a limit test such as *Procedure A* and *Procedure B*, or by a quantitative test

such as *Procedure C*.

When the solvents LTBP are not known, use of the screening test of *Procedure A* and *Procedure B* is required. The combination of *Procedure A* and *Procedure B* can separate most of the solvents listed in this chapter. Additionally, *Figure 3* can be used as a decision tree to demonstrate compliance.

In a screening test, and in the case that the article does not meet the acceptance criteria of *Procedure A*, *Procedure B* is to be used to demonstrate compliance. If the article does not meet the criteria using *Procedure A* and *Procedure B*, then *Procedure C* must be used to quantify the residual solvent(s) present in the article.

When the information about solvents LTBP in the material is known through the knowledge of the process, previous testing, or the information provided from a validated vendor, only testing of those solvents is required. System suitability requirements only need to be demonstrated for the solvents expected to be present.

Several residual solvents listed in this chapter are not detected at the limit concentration using the analytical procedure described below. Those solvents include formamide, 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, *N*-methylpyrrolidone, sulfolane, *N,N*-dimethylacetamide, and *N,N*-dimethylformamide from Class 2, as well as dimethyl sulfoxide, acetic acid, and formic acid from Class 3.

Those solvents should be determined using an alternative method validated according to the procedures described in the *Verification and Validation* section of this chapter.

Class 3 Residual Solvents

These procedures can separate most solvents in Classes 1, 2, and 3. However, the procedures have not been validated for Class 3 solvents and validation will be required in accordance with the procedures described in *Figure 3* before implementation.

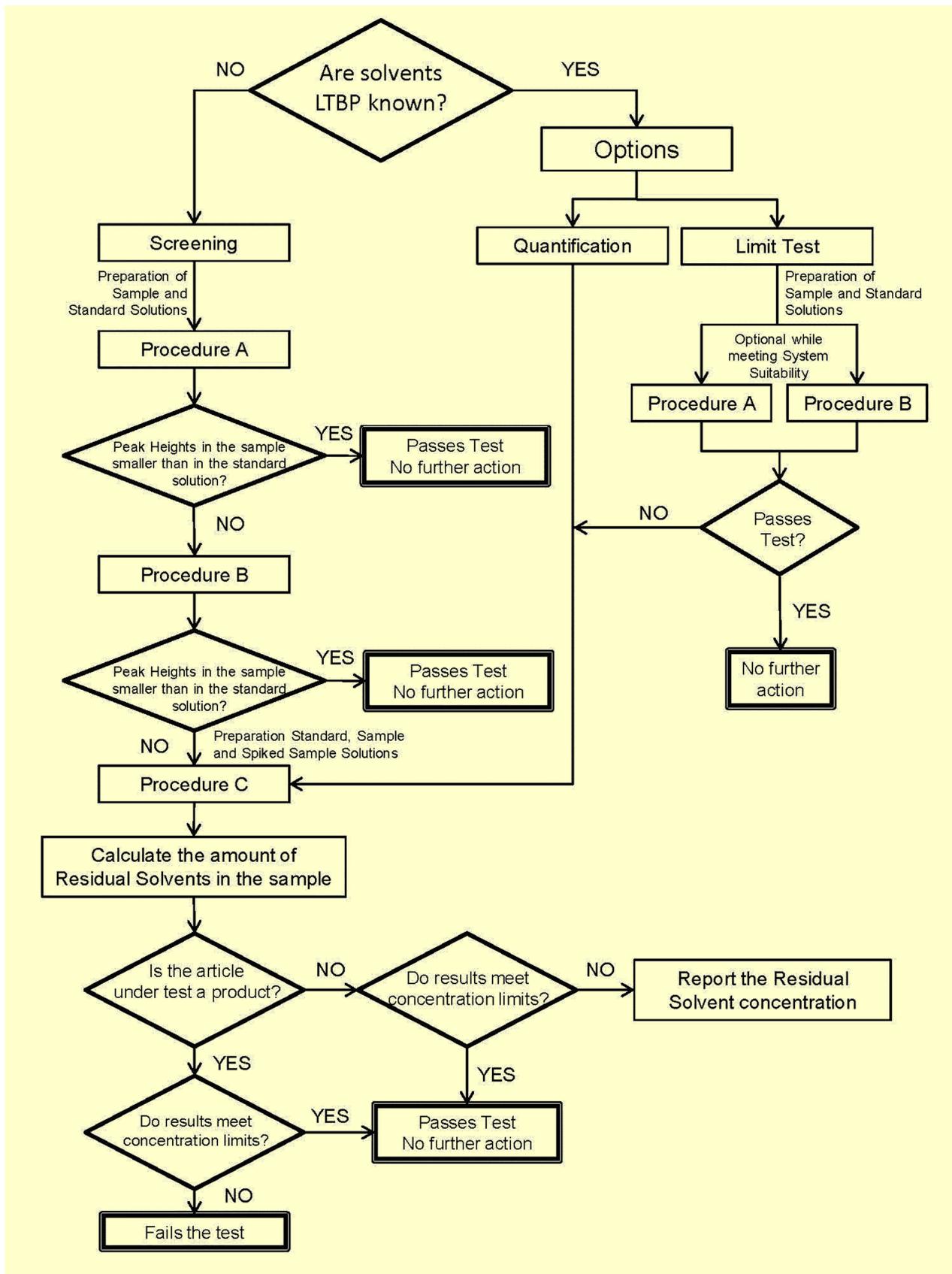


Figure 3. Determination of residual solvents and decision tree using Procedures A, B, and C.

ANALYTICAL PROCEDURES**Chromatographic systems**

(See *Chromatography (621), System Suitability.*)

Procedure A

Mode: GC

Injector: Headspace (see *Table 8* for headspace settings)

Injection type: Split

Split ratio: 1:5. [Note—Split ratio can be modified to optimize sensitivity.]

Detector: Flame ionization

Column: 0.32-mm × 30-m capillary fused silica coated with 1.8- μ m layer of phase *G43* or 0.53-mm × 30-m wide-bore coated with a 3.0- μ m layer of phase *G43*

Column temperature: See *Table 6*.

Table 6

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	20
40	10	240	—
240	0	240	20

Temperatures

Injection port: 140°

Detector: 250°

Carrier gas: Helium, nitrogen, or hydrogen

Linear velocity: About 35 cm/s for helium (adjustment and verification are necessary for other carrier gases)

Procedure B

Mode: GC

Injector: Headspace (see *Table 8* for headspace settings)

Injection type: Split

Split ratio: 1:5. [Note—Split ratio can be modified to optimize sensitivity.]

Detector: Flame ionization

Column: 0.32-mm × 30-m fused-silica coated with a 0.25- μ m layer of phase *G16* or a 0.53-mm × 30-m wide-bore coated with a 0.25- μ m layer of phase *G16*

Column temperature: See *Table 7*.

Table 7

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	20
50	6	165	—
165	0	165	20

Temperatures

Injection port: 140°

Detector: 250°

Carrier gas: Helium, nitrogen, or hydrogen

Linear velocity: About 35 cm/s for helium (adjustment and verification are necessary for other carrier gases)

Procedure C

Use the chromatographic conditions, either A or B that provide the best performance for the solvent(s) being tested.

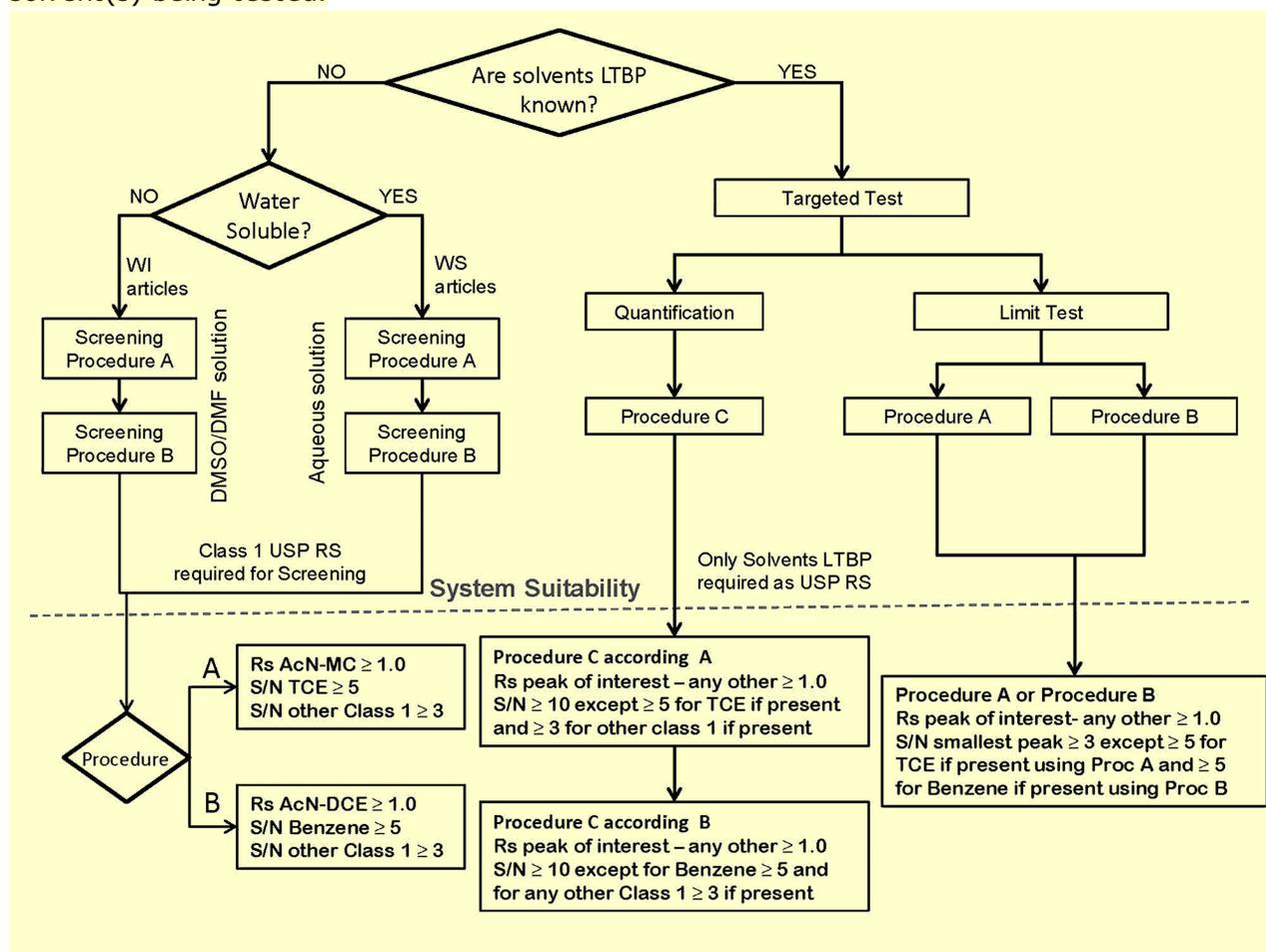


Figure 4. System suitability requirements based on the method of choice and previous

knowledge about solvents LTBP. The acceptance criteria are based on the solvents LTBP (AcN: acetonitrile, TCE: 1,1,1-trichloroethane, DCE: *cis*-dichloroethene, MC: methylene chloride).

SCREENING FOR WATER-SOLUBLE ARTICLES

Class 1 and Class 2 Residual Solvents

Stock solutions

Class 1 standard stock solution: [Note—When transferring solutions, place the tip of the pipet just below the surface of the liquid and mix.] Transfer 1.0 mL of USP Class 1 Residual Solvents Mixture RS to a 100-mL volumetric flask containing about 9 mL of dimethylsulfoxide, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask containing about 50 mL of water, dilute with water to volume, and mix. Transfer 10 mL of this solution to a 100-mL volumetric flask containing about 50 mL of water, dilute with water to volume, and mix.

Class 2 mixture A standard stock solution: Transfer 1.0 mL of USP Residual Solvents Class 2–Mixture A RS to a 100-mL volumetric flask, dilute with water to volume, and mix.

Class 2 mixture B standard stock solution: Transfer 1.0 mL of USP Residual Solvents Class 2–Mixture B RS to a 100-mL volumetric flask, dilute with water to volume, and mix.

Sample stock solution: Transfer about 250 mg of the article under test, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Standard solutions

Class 1 standard solution: [Note—When transferring solutions, place the tip of the pipet just below the surface of the liquid and mix.] Transfer 1.0 mL of *Class 1 standard stock solution* to an appropriate headspace vial containing 5.0 mL of water. Apply the stopper, cap, and mix.

Class 2 mixture A standard solution: Transfer 1.0 mL of *Class 2 mixture A standard stock solution* to an appropriate headspace vial, add 5.0 mL of water, apply the stopper, cap, and mix.

Class 2 mixture B standard solution: Transfer 5.0 mL of *Class 2 mixture B standard stock solution* to an appropriate headspace vial, add 1.0 mL of water, apply the stopper, cap, and mix.

Sample solution: Transfer 5.0 mL of *Sample stock solution* to an appropriate headspace vial, add 1.0 mL of water, apply the stopper, cap, and mix.

Class 1 system suitability solution: Transfer 1.0 mL of *Class 1 standard stock solution* to an appropriate headspace vial, add 5.0 mL of *Sample stock solution*, apply the stopper, cap, and mix.

PROCEDURE A

Table 8. Headspace Operating Parameters

	Headspace Operating Parameter Sets		
	1	2	3
Equilibration temperature (°)	80	105	80
Equilibration time (min)	60	45	45
Transfer-line temperature (°) (if appropriate) ^a	85	110	105
Syringe temperature (°) (if appropriate)	80–90	105–115	80–90
Carrier gas: helium, nitrogen ^b or hydrogen ^b at an appropriate pressure			
Pressurization time (sec) (if appropriate)	≥60	≥60	≥60
Injection volume (mL) ^c	1	1	1
<p>^a It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.</p> <p>^b The use of nitrogen or hydrogen as the carrier gas requires adjustment and verification.</p> <p>^c Injection volume may be expressed in terms of the injection time.</p>			

Analysis: Separately inject (following one of the headspace operating parameter sets described in *Table 8*) equal volumes of headspace of the *Class 1 standard solution*, *Class 1 system suitability solution*, *Class 2 mixture A standard solution*, *Class 2 mixture B standard solution*, and *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest.

System suitability requirements (see *Figure 4*): The signal-to-noise ratio (S/N) for 1,1,1-trichloroethane in the *Class 1 standard solution* is NLT 5. The S/N ratio of each peak in the *Class 1 system suitability solution* is NLT 3, and the resolution between acetonitrile and methylene chloride in the *Class 2 mixture A standard solution* is NLT 1.0.

Acceptance criteria: If a peak response of any peak, other than a peak for 1,1,1-trichloroethane, in the *Sample solution* is greater than or equal to a corresponding peak in either the *Class 1 standard solution* or either of the two *Class 2 mixture standard solutions*, or a peak response of 1,1,1-trichloroethane is greater than or equal to 150 times the peak response corresponding to 1,1,1-trichloroethane in the *Class 1 standard solution*, proceed to *Procedure B* to verify the identity of the peak; otherwise the article meets the requirements of this test.

PROCEDURE B

Analysis: Separately inject (following one of the headspace operating parameter sets described in *Table 8*) equal volumes of headspace of the *Class 1 standard solution*, *Class 1 system suitability solution*, *Class 2 mixture A standard solution*, *Class 2 mixture B standard solution*, and *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest.

System suitability requirements (see *Figure 4*): The S/N ratio for benzene in the *Class 1 standard solution* is NLT 5. The S/N ratio of each peak in the *Class 1 system suitability solution* is NLT 3, and the resolution between acetonitrile and *cis*-dichloroethene in the *Class 2 mixture A standard solution* is NLT 1.0.

Acceptance criteria: If the peak response(s) in the *Sample solution* of the peak(s) identified in *Procedure A* is/are greater than or equal to corresponding peak(s) in either the *Class 1 standard solution* or either of the two *Class 2 mixture standard solutions*, proceed to *Procedure C* to quantify the peak(s); otherwise the article meets the requirements of this test.

SCREENING FOR WATER-INSOLUBLE ARTICLES

Stock solutions

[Note—Dimethyl sulfoxide may be substituted as an alternative solvent to dimethylformamide. When transferring solutions, place the tip of the pipet just below the surface of the liquid and mix.]

Class 1 standard stock solution: Transfer 1.0 mL of USP Class 1 Residual Solvents Mixture RS to a 100-mL volumetric flask previously filled with about 80 mL of dimethylformamide, dilute with dimethylformamide to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, previously filled with about 80 mL of dimethylformamide, dilute with dimethylformamide to volume, and mix (reserve a portion of this solution for the *Class 1 system suitability solution*). Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with dimethylformamide to volume, and mix.

Class 2 mixture A standard stock solution: Transfer 1.0 mL of USP Residual Solvents Class 2–Mixture A RS to a 100-mL volumetric flask containing about 80 mL of dimethylformamide, dilute with dimethylformamide to volume, and mix.

Class 2 mixture B standard stock solution: Transfer 0.5 mL of USP Residual Solvents Class 2–Mixture B RS to a 10-mL volumetric flask, dilute with dimethylformamide to volume, and mix.

Sample stock solution: Transfer about 500 mg of the article under test, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute to volume with dimethylformamide, and mix.

Class 1 system suitability solution: Mix 5 mL of *Sample stock solution* with 0.5 mL of the intermediate dilution reserved from *Class 1 standard stock solution*. Transfer 1 mL of this solution to an appropriate headspace vial containing 5 mL of water, apply the stopper, cap and mix.

Standard solutions

Class 1 standard solution: Transfer 1.0 mL of *Class 1 standard stock solution* to an appropriate headspace vial containing 5.0 mL of water; apply the stopper, cap, and mix.

Class 2 mixture A standard solution: Transfer 1.0 mL of *Class 2 mixture A standard stock solution* to an appropriate headspace vial containing 5.0 mL of water and 1.0 mL of dimethyl sulfoxide, apply the stopper, cap, and mix.

Class 2 mixture B standard solution: Transfer 1.0 mL of *Class 2 mixture B standard stock solution* to an appropriate headspace vial containing 5.0 mL of water, apply the stopper, cap, and mix.

Sample solution: Transfer 1.0 mL of *Sample stock solution* to an appropriate headspace vial containing 5.0 mL of water, apply the stopper, cap, and mix.

PROCEDURE A

Proceed as directed for *Procedure A* in *Screening for Water-Soluble Articles* with a split ratio of 1:3. [Note—The split ratio can be modified in order to optimize sensitivity.] Separately inject (following one of the headspace operating parameter sets described in *Table 8*) equal volumes of headspace of the *Class 1 standard solution*, *Class 2 mixture A standard solution*, *Class 2 mixture B standard solution*, and *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks.

System suitability requirements (see *Figure 4*): The S/N ratio for 1,1,1-trichloroethane in the *Class 1 standard solution* is NLT 5. The S/N ratio of each peak in the *Class 1 system suitability solution* is NLT 3; and the resolution between acetonitrile and methylene chloride in the *Class 2 mixture A standard solution* is NLT 1.0.

Acceptance criteria: If a peak response of any peak, other than a peak for TCE in the *Sample solution* is greater than or equal to a corresponding peak in either the *Class 1 standard solution* or either of the two *Class 2 mixture standard solutions*, or a peak response of 1,1,1-trichloroethane is greater than or equal to 150 times the peak response corresponding to 1,1,1-trichloroethane in the *Class 1 standard solution*, proceed to *Procedure B* to verify the identity of the peak; otherwise the article meets the requirements of this test.

PROCEDURE B

Proceed as directed for *Procedure B* in *Screening for Water-Soluble Articles* with a split ratio of 1:3. Separately inject (use headspace operating parameters in column 3 of *Table 8* with a vial pressure of 10 psi) equal volumes of headspace of the *Class 1 standard solution*, *Class 2 mixture A standard solution*, *Class 2 mixture B standard solution*, *Sample solution*, and *Class 1 system suitability solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest.

System suitability requirements (see *Figure 4*): The S/N ratio for benzene in *Class 1 standard solution* is NLT 5. The S/N ratio of each peak in the *Class 1 system suitability solution* is NLT 3; and the resolution between acetonitrile and *cis*-dichloroethene in the *Class 2 mixture A standard solution* is NLT 1.0.

Acceptance criteria: If the peak response(s) in the *Sample solution* of the peak(s) identified in *Procedure A* is/are greater than or equal to corresponding peak(s) in either the *Class 1 standard solution* or either of the two *Class 2 mixture standard solutions*, proceed to *Procedure C* to quantify the peak(s); otherwise the article meets the requirements of this test.

CLASS 3 RESIDUAL SOLVENTS

If only Class 3 solvents are present and the monograph for the article under test contains a loss on drying procedure specifying an upper limit of NMT 0.5% (per *Option 1* in this general chapter), then the level of residual solvent can be determined as directed in *Loss on Drying* (731). Otherwise, the chromatographic procedure described previously or other properly validated procedure should be used. If Class 3 solvents are present with other solvents, e.g., Class 1 or Class 2 solvents, or if there is no loss on drying procedure in the monograph for the article under test, or if the limit for Class 3 solvent(s) in an individual monograph is greater than

50 mg/day (corresponding to 5000 ppm or 0.5% under *Option 1*), then the Class 3 residual solvent or solvents present in the article under test should be identified and quantified. In that case, the procedures described in this chapter, with appropriate adjustments, are to be applied wherever possible and USP Reference Standards, where available, should be used in these procedures. Such procedures should be properly validated as disclosed in the *Verification and Validation* section of this chapter. If Class 1 or Class 2 solvents are present and loss on drying is less than 0.5%, then identification of Class 3 solvents is not required and only Class 1 and Class 2 solvents should be identified and quantified.

LIMIT TESTS WHEN SOLVENTS LTBP ARE KNOWN

Prepare the *Standard solution* and the *Sample solution* as directed in *Procedure A* or *Procedure B*, in *Screening for Water-Soluble Articles* or *Screening for Water-Insoluble Articles* according to the procedure chosen based on the sample solubility. Use the USP Reference Standards of those solvents LTBP to prepare the *Standard solution*.

When solvents LTBP are known, only the standards of those solvents LTBP are needed, and determination may be done using a limit test or a quantitative test. Only quantitative tests can be used for *Option 2*.

System suitability solution: Prepare the *System suitability solution* for *Screening for Water-Soluble Articles* or for *Screening for Water-Insoluble Articles* according to the solubility of the sample, at the solvent concentration of the *Standard solution* in the screening test, using only those solvents LTBP.

PROCEDURE A

Analysis (limit test): Separately inject (following one of the headspace operating parameter sets described in *Table 8*) equal volumes of headspace of the *System suitability solution*, *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest.

System suitability requirements (see *Figure 4*): The S/N ratio for 1,1,1-trichloroethane, if present, is NLT 5. The S/N ratio is NLT 3 for any other solvent. If there is more than one solvent LTBP, the resolution between a peak of interest and any adjacent peak is NLT 1.0.

Acceptance criteria: If the peak response of every peak of interest in the *Sample solution* is smaller than the corresponding peak in the *Standard solution*, the article meets the requirements of this test.

PROCEDURE B

Analysis (limit test): Separately inject (following one of the headspace operating parameter sets described in *Table 8*) equal volumes of headspace of the *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest.

System suitability requirements (see *Figure 4*): The S/N ratio for benzene, if present, is NLT 5. The S/N ratio is NLT 3 for any other solvent. If there is more than one solvent LTBP, the resolution between a peak of interest and any other peak is NLT 1.0.

Acceptance criteria: If the peak response of every peak of interest in the *Sample solution* is smaller than the corresponding peak in the *Standard solution*, the article meets the requirements of this test.

QUANTITATIVE TESTS

Quantification for Water-Soluble Articles

Stock solutions

Prepare a *Sample stock solution* as directed in *Procedure A* for water-soluble articles. Only the Reference Standard for those solvents identified by *Procedure A* or *Procedure B* or LTBP need to be used.

Standard stock solution: [Note—Prepare a separate *Standard stock solution* for each solvent LTBP or potentially LTBP, or identified and verified by *Procedure A* and *Procedure B*. For the Class 1 solvents other than 1,1,1-trichloroethane, prepare the first dilution as directed for the first dilution under *Class 1 standard stock solution*.] Transfer an accurately measured volume of each individual USP Reference Standard corresponding to each residual solvent peak LTBP or identified and verified by *Procedure A* and *Procedure B* to a suitable container, and dilute quantitatively and stepwise if necessary, with water to obtain a solution with a final concentration of 1/20 of the value stated in *Table 1* or *Table 2* under *Concentration Limit*.

Standard solution: Transfer 1.0 mL of *Standard stock solution* to an appropriate headspace vial, add 5.0 mL of water, apply the stopper, cap, and mix.

Sample solution: Proceed as directed in *Procedure A*.

Spiked sample solution: [Note—Prepare a separate *Spiked sample solution* for each peak LTBP or potentially LTBP or identified and verified by *Procedure A* and *Procedure B*.] The use of vial containing the sample spiked with multiple standards is permitted, provided that the procedure is validated. Transfer 5.0 mL of each *Sample stock solution* to an appropriate headspace vial, add 1.0 mL of the *Standard stock solution*, apply the stopper, cap, and mix.

Analysis

Proceed as directed for *Procedure A*. [Note—If the performance of *Procedure B* is better than that shown by *Procedure A*, then *Procedure B* may be used for quantification.] Separately inject equal volumes of headspace of the *Standard solution*, *Sample solution*, and *Spiked sample solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest.

System suitability requirements (see *Figure 4*): The S/N ratio for 1,1,1-trichloroethane, if present, is NLT 5. The S/N ratio is NLT 3 for any other solvent. If there are more than one solvent LTBP, the resolution between a peak of interest and any adjacent peak is NLT 1.0.

Calculate the amount, in ppm, of each residual solvent found in the article under test:

$$\text{Result} = 5(C/W)[r_U/(r_{ST}-r_U)]$$

- C = concentration of the appropriate USP Reference Standard in the *Standard stock solution* ($\mu\text{g}/\text{mL}$)
- W = weight of the article under test taken to prepare the *Sample stock solution* (g)
- r_U = peak responses of each residual solvent obtained from the *Sample solution*

r_{ST} = peak responses of each residual solvent obtained from the *Spiked sample solution*

Quantification for Water-Insoluble Articles

Stock solutions

Prepare a *Sample stock solution* as directed in *Procedure A* for water-insoluble articles. Only the Reference Standard for those solvents LTBP need to be used.

Standard stock solution: [Note—Prepare a separate *Standard stock solution* for each solvent LTBP or potentially LTBP, or identified and verified by *Procedure A* and *Procedure B*. For the Class 1 solvents other than 1,1,1-trichloroethane, prepare the first dilution as directed for the first dilution under *Class 1 standard stock solution*.] Transfer an accurately measured volume of each individual USP Reference Standard corresponding to each residual solvent peak LTBP or identified and verified by *Procedure A* and *Procedure B* to a suitable container, and dilute quantitatively and stepwise if necessary, with water to obtain a solution with a final concentration of 1/20 of the value stated in *Table 1* or *Table 2* under *Concentration Limit*.

Standard solution: Transfer 1.0 mL of *Standard stock solution* to an appropriate headspace vial, add 5.0 mL of water, apply the stopper, cap, and mix.

Sample solution: Transfer 1.0 mL of *Standard stock solution* to an appropriate headspace vial containing 5.0 mL of water, apply the stopper, cap, and mix.

Spiked sample solution: [Note—Prepare a separate *Spiked sample solution* for each peak LTBP or potentially LTBP, or identified and verified by *Procedure A* and *Procedure B*.] Transfer 1.0 mL of *Sample stock solution* to an appropriate headspace vial, add 1.0 mL of *Standard stock solution* and 4.0 mL of water, apply the stopper, cap, and mix. The use of vial containing the sample spiked with multiple standards is permitted, provided that the procedure is validated.

PROCEDURE C

Proceed as directed in *Procedure A*. [Note—If the performance of *Procedure B* is better than that shown by *Procedure A*, then *Procedure B* may be used for quantification.] Separately inject (use headspace operating parameters in column 3 of *Table 8* with a vial pressure of 10 psi) equal volumes of headspace of the *Standard solution*, *Sample solution*, and *Spiked sample solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest. If Class 1 solvents are LTBP, also inject the *Class 1 system suitability solution*.

System suitability requirements (see *Figure 4*): The S/N ratio for 1,1,1-trichloroethane, if present, is NLT 5. The S/N ratio is NLT 3 for any other solvent. If there are more than one solvent LTBP, the resolution between a peak of interest and any adjacent peak is NLT 1.0.

Calculate the amount, in ppm, of each residual solvent found in the article under test:

$$\text{Result} = 10(C/W)[r_U/(r_{ST}-r_U)]$$

- C = concentration of the appropriate USP Reference Standard in the *Standard stock solution* ($\mu\text{g/mL}$)
- W = weight of the article under test taken to prepare the *Sample stock solution* (g)
- r_U = peak responses of each residual solvent obtained from the *Sample solution*
- r_{ST} = peak responses of each residual solvent obtained from the *Spiked sample solution*

VERIFICATION AND VALIDATION

Table 9. Summary of Verification and Validation: Requirements for Class 1 and 2 Solvents

Validation Characteristics	Verification of Compendial Procedure		Validation of Alternative Procedures	
	Limit Test Methods	Quantitative Methods	Limit Test Methods	Quantitative Methods
Specificity	Yes	Yes	Yes	Yes
Detectability	Yes	No	Yes	No
Ability to quantitate	No	Demonstrated by accuracy	No	Demonstrated by accuracy
Accuracy	No	Yes	No	Yes
Precision/repeatability	No	Yes	No	Yes
Linearity	No	Not required (implied by compendial Procedure)	No	Yes
Range	No	Demonstrated by accuracy	No	Demonstrated by accuracy
Ruggedness	No	No	No	Yes
Solution stability	Yes	Yes	Yes	Yes

Users of compendial analytical procedures should establish documented evidence that the method is suitable for its intended purpose under conditions of actual use. When using the procedures described in this chapter, verification can be accomplished using the recommendations below. There are separate recommendations for limit procedures (*Procedure A* and *Procedure B*) and quantitative procedures (*Procedure C*). These procedures have been validated for Class 1 and 2 solvents over the range of 50%–150% of the concentrations described in the standard preparation for *Procedure A*.

Alternative procedures are permitted (see *General Notices 6.30*). Alternative procedures include different analytical procedures from those described in this chapter and modifications to the procedures described herein that go beyond the stated validation of the method (e.g., different concentrations, other analytes or variations to chromatographic conditions beyond those permitted by *Chromatography* (621)). Alternative procedures must be validated. Recommendations for validation of alternative procedures (limit and quantitative) are described in *Validation of Alternative Procedures*.

VERIFICATION OF COMPENDIAL PROCEDURES

For Limit Procedures: Procedure A and Procedure B

Analytical characteristics to be verified: specificity, detectability, and solution stability.

When solvents LTBP are not known

Prepare a reagent blank.

Prepare *Standard solution(s)* as described in *Procedure A*.

Prepare the *Spiked sample solution* as described for *System suitability solution* in *Procedure A*.

Specificity: acceptance criteria

The procedure must be able to separate acetonitrile and methylene chloride (*Procedure A*), or acetonitrile and cis-dichloroethene (*Procedure B*) with a resolution of NLT 1.0.

Detectability: acceptance criteria

The mean S/N ratio for each solvent in the *Standard solution* and *Spiked sample solution* from at least three determinations from a single preparation is NLT 3.

Solution stability: acceptance criteria

Detectability should meet the requirements through the testing period (protect against significant changes across testing period).

When solvents LTBP are known

(These statements only apply to solvents LTBP.)

Prepare a reagent blank.

Prepare *Standard solution(s)* and *Sample solution(s)* as described in *Procedure A* for solvents LTBP.

Prepare *Spiked sample solution(s)* as described in *Procedure C* for solvents LTBP (corrected for native solvent content).

Prepare *75% Spiked sample solution(s)* as described in *Spiked sample solution(s)* in *Procedure C*, except at 75% of the specified concentration (corrected for native solvent content).

Specificity: acceptance criteria

The reagent blank does not produce any significant interference with any of the peaks from solvents LTBP.

The procedure must be able to separate each of the solvents in the standard solution(s) from each other and from other peaks in the *Spiked sample solution* with a resolution of NLT 1.0. If the resolution between any pair of peaks is less than 1.5, then verification must demonstrate that the method is suitable for its intended use, and appropriate resolution criteria must be incorporated in the *System suitability* test for routine use.

If the solvents present in the *Standard solution(s)* are not separated with a resolution of NLT 1.0, then *Procedure B* should be used as confirmatory.

Detectability: acceptance criteria

The mean S/N ratio for each solvent in the *Standard solution* and the *Spiked sample solution* from at least three determinations is NLT 3.

The response from the *Spiked sample solution* is within 80%–120% of the response from the *Standard solution* after correction for native solvent content. (If not, it is acceptable to consider the procedure to be verified with the stipulation that all future tests require use of the *Spiked sample solution* as the *Standard solution*.)

The response from the 75% *Spiked sample solution*, for each solvent LTBP, is less than the response from the *Spiked sample solution* (after correction for native solvent content).

Solution stability: acceptance criteria (not required if only running fresh solutions)

Detectability should meet the requirements through the testing period (without significant change across the testing period).

For Quantitative Procedures (Procedure C)

[Note—When performing *Procedure C*, solvents LTBP are typically known, either based on results from *Procedure A* or *Procedure B*, or based on available knowledge.]

Analytical characteristics to be verified are: specificity, accuracy (informs ability to quantitate, linearity, and range), precision/repeatability, and solution stability.

Specificity

See *Specificity* in *Limit Procedures (Procedure A and Procedure B)*.

Accuracy

Standard stock solution: Prepare a solution containing (at least) each Class 1 or 2 solvent LTBP or each peak identified and verified by *Procedure A* and *Procedure B*, with a concentration as described in *Procedure C* for water-soluble articles or water-insoluble articles, as appropriate.

Sample stock solution: Prepare as described in *Procedure A*.

Spiked sample solution 1: Prepare as described in *Procedure C*, using the *Standard stock solution* described here.

Spiked sample solutions A, B, C, etc.: Prepare *Spiked sample solutions* with the sample matrix and spiked with each Class 1 or 2 solvent LTBP or each peak identified and verified by *Procedure A* and *Procedure B*, in triplicate, at NLT 3 levels covering the range of interest or at least 50%–150% of the corresponding concentration of the *Standard solution* as described in *Procedure A*. [Note—*Spiked sample solution 1* may be used as one of the solutions. Recoveries should be corrected for native content of any solvent under test.]

Acceptance criteria

The mean recovery for each *Spiked sample solution A, B, and C*, when calculated against *Spiked sample solution 1*, is 80%–120%.

The mean recovery for each *Spiked sample solution A, B, and C* is 80%–120%.

Precision/Repeatability

Spiked sample solutions: At least six independent preparations from the same lot, prepared as described for *Spiked sample solution 1* in *Accuracy* or nine independent preparations as described for *Spiked sample solutions A, B, C, etc.*

Acceptance criteria

Relative standard deviation: NMT 20% for each solvent present

Solution stability

Demonstrate acceptable solution stability for the period of time to run the test.

Acceptance criteria: Solutions should exhibit peak responses of 75%–125% throughout the testing period.

VALIDATION OF ALTERNATIVE PROCEDURES

Alternative procedures, in addition to the requirements for verification of compendial procedures, should meet the following criteria. When non-chromatographic alternative procedures are validated, the analytical characteristics listed should be addressed, although it may be appropriate to use other acceptance criteria. For more information, refer to *Validation of Compendial Procedures* (1225).

For concentrations other than the nominal concentrations in this chapter, the acceptance criteria should be determined before the validation protocol is executed.

Limit procedures (*Procedure A* and *Procedure B*)

Analytical characteristics to be validated: None

Quantitative procedures (*Procedure C*)

Analytical characteristics to be validated: Linearity and intermediate precision

Linearity

Spiked sample solutions P, Q, R, etc.: Prepare sample solutions spiked with solvents LTBP, at NLT 5 levels covering the range of interest or at least 50%–150% of the corresponding concentration of the *Standard solution* as described in *Procedure A*.

Prepare *Spiked sample solutions* with the sample matrix and spiked with each Class 1 or 2 solvent LTBP at NLT 5 levels covering the range of interest. [Note—Results should be corrected for native content of any solvent under test.]

Perform linear regression analysis on the results for *Spiked sample solutions P, Q, R, etc.*

Acceptance criteria

The coefficient of determination, r^2 , is NLT 0.90.

Intermediate precision

Perform the *Repeatability* test over two independent events, for example, on different days, and/or using different instruments and/or analysts.

Acceptance criteria

Relative standard deviation: NMT 25% at the nominal concentration level for each solvent present

GLOSSARY

Abbreviations for Solvents Listed in This Chapter

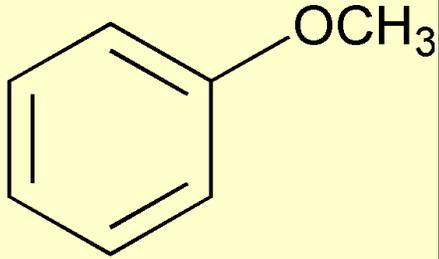
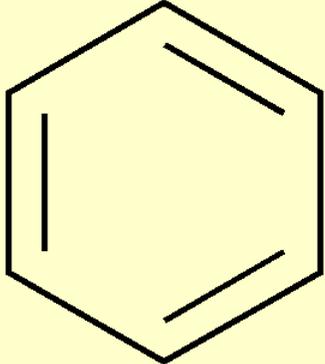
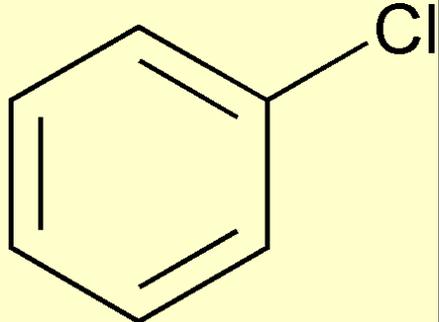
Acceptable daily intake (ADI):	The maximum acceptable intake of toxic chemicals per day. This term is used by the World Health Organization (WHO).
Genotoxic carcinogens:	Carcinogens that produce cancer by affecting genes or chromosomes.
Likely to be present (LTBP):	Those solvents used or produced in the final manufacturing step and solvents that are used or produced in earlier manufacturing steps which are not consistently removed by a validated process, or the solvents properly declared by a validated supplier of a drug substance or excipient.
Lowest-observed-effect level (LOEL):	The lowest dose of a substance in a study or group of studies that produces biologically significant increases in frequency or severity of any effects in exposed humans or animals.
Modifying factor:	A factor determined by professional judgment of a toxicologist and applied to bioassay data so that the data can be safely related to humans.
Neurotoxicity:	The ability of a substance to cause adverse effects on the nervous system.
No-observed-effect level (NOEL):	The highest dose of a substance at which there are no biologically significant increases in frequency or severity of any effects in exposed humans or animals.
Permitted daily exposure (PDE):	The maximum acceptable intake per day of a residual solvent in pharmaceutical products
Reversible toxicity:	The occurrence of harmful effects that are caused by a substance and that disappear after exposure to the substance ends.
Strongly suspected human carcinogen:	A substance for which there is no epidemiological evidence of carcinogenesis but for which there are positive genotoxicity data and clear evidence of carcinogenesis in rodents.
Teratogenicity:	The occurrence of structural malformations in a developing fetus when a substance is administered during pregnancy.
Tolerable daily intake (TDI):	Tolerable daily exposure to toxic chemicals. Term used by the International Program on Chemical Safety (IPCS).

APPENDICES

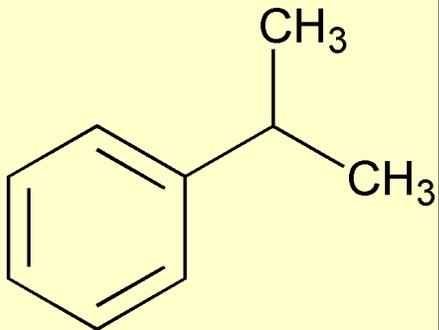
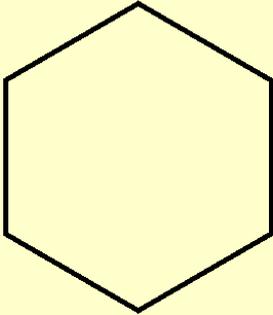
See the table *Appendix 1. List of Residual Solvents Included in This General Chapter.*

APPENDIX 1. LIST OF RESIDUAL SOLVENTS INCLUDED IN THIS GENERAL CHAPTER

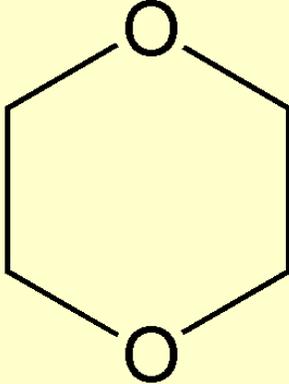
Solvent	Other Names	Structure	Class
Acetic acid	Ethanoic acid	CH ₃ COOH	Class 3
^a Usually 60% <i>m</i> -xylene, 14% <i>p</i> -xylene, and 9% <i>o</i> -xylene with 17% ethyl benzene.			

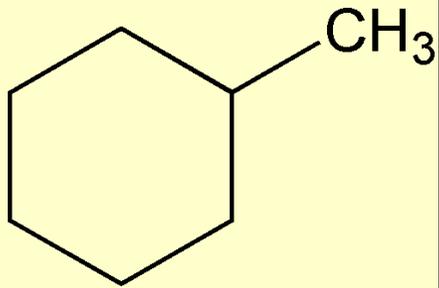
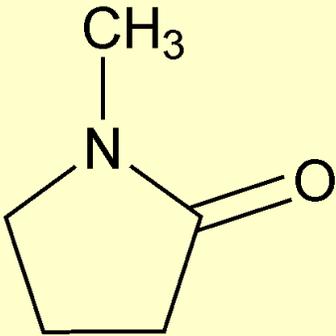
Solvent	Other Names	Structure	Class
Acetone	2-Propanone Propan-2-one	CH_3COCH_3	Class 3
Acetonitrile		CH_3CN	Class 2
Anisole	Methoxybenzene		Class 3
Benzene	Benzol		Class 1
1-Butanol	<i>n</i> -Butyl alcohol Butan-1-ol	$\text{CH}_3(\text{CH}_2)_3\text{OH}$	Class 3
2-Butanol	<i>sec</i> -Butyl alcohol Butan-2-ol	$\text{CH}_3\text{CH}_2\text{CH}(\text{OH})\text{CH}_3$	Class 3
Butyl acetate	Acetic acid butyl ester	$\text{CH}_3\text{COO}(\text{CH}_2)_3\text{CH}_3$	Class 3
<i>tert</i> -Butylmethyl ether	2-Methoxy-2-methylpropane	$(\text{CH}_3)_3\text{COCH}_3$	Class 3
Carbon tetrachloride	Tetrachloromethane	CCl_4	Class 1
Chlorobenzene			Class 2
Chloroform	Trichloromethane	CHCl_3	Class 2

^a Usually 60% *m*-xylene, 14% *p*-xylene, and 9% *o*-xylene with 17% ethyl benzene.

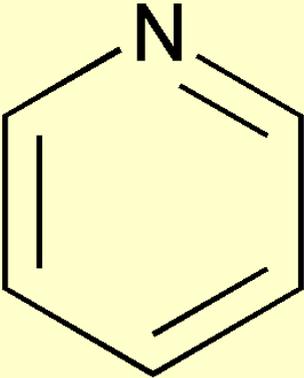
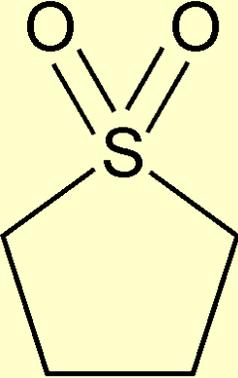
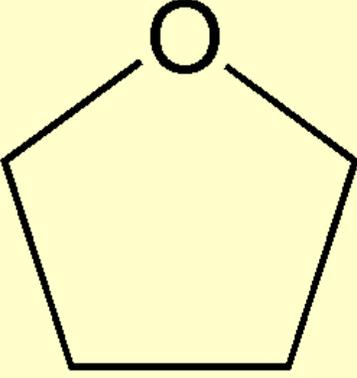
Solvent	Other Names	Structure	Class
Cumene	Isopropylbenzene (1-Methylethyl)benzene		Class 2
Cyclohexane	Hexamethylene		Class 2
1,2-Dichloroethane	<i>sym</i> -Dichloroethane Ethylene dichloride Ethylene chloride	$\text{CH}_2\text{ClCH}_2\text{Cl}$	Class 1
1,1-Dichloroethene	1,1-Dichloroethylene Vinylidene chloride	$\text{H}_2\text{C}=\text{CCl}_2$	Class 1
1,2-Dichloroethene	1,2-Dichloroethylene Acetylene dichloride	$\text{ClHC}=\text{CHCl}$	Class 2
1,2-Dimethoxyethane	Ethyleneglycol dimethyl ether Monoglyme Dimethyl cellosolve	$\text{H}_3\text{COCH}_2\text{CH}_2\text{OCH}_3$	Class 2
<i>N,N</i> -Dimethylacetamide	DMA	$\text{CH}_3\text{CON}(\text{CH}_3)_2$	Class 2
<i>N,N</i> -Dimethylformamide	Dimethylformamide	$\text{HCON}(\text{CH}_3)_2$	Class 2
Dimethyl sulfoxide	Methylsulfinylmethane Methyl sulfoxide Dimethyl sulfoxide	$(\text{CH}_3)_2\text{SO}$	Class 3

^a Usually 60% *m*-xylene, 14% *p*-xylene, and 9% *o*-xylene with 17% ethyl benzene.

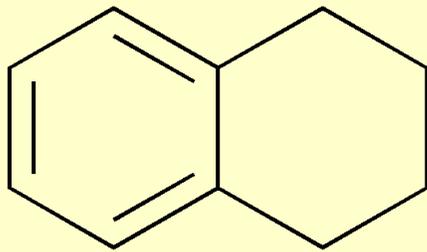
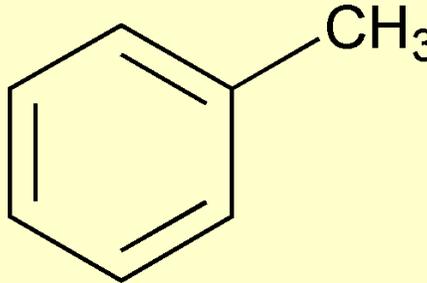
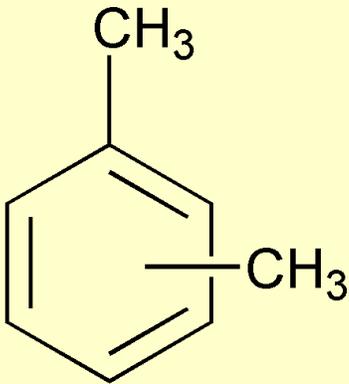
Solvent	Other Names	Structure	Class
1,4-Dioxane	<i>p</i> -Dioxane [1,4]Dioxane		Class 2
Ethanol	Ethyl alcohol	CH ₃ CH ₂ OH	Class 3
2-Ethoxyethanol	Cellosolve	CH ₃ CH ₂ OCH ₂ CH ₂ OH	Class 2
Ethyl acetate	Acetic acid ethyl ester	CH ₃ COOCH ₂ CH ₃	Class 3
Ethylene glycol	1,2-Dihydroxyethane 1,2-Ethanediol	HOCH ₂ CH ₂ OH	Class 2
Ethyl ether	Diethyl ether Ethoxyethane 1,1'-Oxybisethane	CH ₃ CH ₂ OCH ₂ CH ₃	Class 3
Ethyl formate	Formic acid ethyl ester	HCOOCH ₂ CH ₃	Class 3
Formamide	Methanamide	HCONH ₂	Class 2
Formic acid		HCOOH	Class 3
Heptane	<i>n</i> -Heptane	CH ₃ (CH ₂) ₅ CH ₃	Class 3
Hexane	<i>n</i> -Hexane	CH ₃ (CH ₂) ₄ CH ₃	Class 2
Isobutyl acetate	Acetic acid isobutyl ester	CH ₃ COOCH ₂ CH(CH ₃) ₂	Class 3
Isopropyl acetate	Acetic acid isopropyl ester	CH ₃ COOCH(CH ₃) ₂	Class 3
Methanol	Methyl alcohol	CH ₃ OH	Class 2
2-Methoxyethanol	Methyl cellosolve	CH ₃ OCH ₂ CH ₂ OH	Class 2
Methyl acetate	Acetic acid methyl ester	CH ₃ COOCH ₃	Class 3
3-Methyl-1-butanol	Isoamyl alcohol Isopentyl alcohol 3-Methylbutan-1-ol	(CH ₃) ₂ CHCH ₂ CH ₂ OH	Class 3
Methylbutylketone	2-Hexanone Hexan-2-one	CH ₃ (CH ₂) ₃ COCH ₃	Class 2
^a Usually 60% <i>m</i> -xylene, 14% <i>p</i> -xylene, and 9% <i>o</i> -xylene with 17% ethyl benzene.			

Solvent	Other Names	Structure	Class
Methylcyclohexane	Cyclohexylmethane		Class 2
Methylene chloride	Dichloromethane	CH_2Cl_2	Class 2
Methylethylketone	2-Butanone MEK Butan-2-one	$\text{CH}_3\text{CH}_2\text{COCH}_3$	Class 3
Methyl isobutyl ketone	4-Methylpentan-2-one 4-Methyl-2-pentanone MIBK	$\text{CH}_3\text{COCH}_2\text{CH}(\text{CH}_3)_2$	Class 3
2-Methyl-1-propanol	Isobutyl alcohol 2-Methylpropan-1-ol	$(\text{CH}_3)_2\text{CHCH}_2\text{OH}$	Class 3
<i>N</i> -Methylpyrrolidone	1-Methylpyrrolidin-2-one 1-Methyl-2-pyrrolidinone		Class 2
Nitromethane		CH_3NO_2	Class 2
Pentane	<i>n</i> -Pentane	$\text{CH}_3(\text{CH}_2)_3\text{CH}_3$	Class 3
1-Pentanol	Amyl alcohol Pentan-1-ol Pentyl alcohol	$\text{CH}_3(\text{CH}_2)_3\text{CH}_2\text{OH}$	Class 3

^a Usually 60% *m*-xylene, 14% *p*-xylene, and 9% *o*-xylene with 17% ethyl benzene.

Solvent	Other Names	Structure	Class
1-Propanol	Propan-1-ol Propyl alcohol	$\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$	Class 3
2-Propanol	Propan-2-ol Isopropyl alcohol	$(\text{CH}_3)_2\text{CHOH}$	Class 3
Propyl acetate	Acetic acid propyl ester	$\text{CH}_3\text{COOCH}_2\text{CH}_2\text{CH}_3$	Class 3
Pyridine			Class 2
Sulfolane	Tetrahydrothiophene 1,1-dioxide		Class 2
Tetrahydrofuran	Tetramethylene oxide Oxacyclopentane		Class 2

^a Usually 60% *m*-xylene, 14% *p*-xylene, and 9% *o*-xylene with 17% ethyl benzene.

Solvent	Other Names	Structure	Class
Tetralin	1,2,3,4-Tetrahydronaphthalene		Class 2
Toluene	Methylbenzene		Class 2
1,1,1-Trichloroethane	Methylchloroform	CH_3CCl_3	Class 1
Trichloroethylene	1,1,2-Trichloroethene	$\text{HC}(\text{Cl})=\text{CCl}_2$	Class 2
Xylene ^a	Dimethylbenzene Xylol		Class 2
^a Usually 60% <i>m</i> -xylene, 14% <i>p</i> -xylene, and 9% <i>o</i> -xylene with 17% ethyl benzene.			

APPENDIX 2. ADDITIONAL BACKGROUND

A2.1. Environmental Regulation of Organic Volatile Solvents

Several of the residual solvents frequently used in the production of pharmaceuticals are listed as toxic chemicals in *Environmental Health Criteria* (EHC) monographs and in the *Integrated Risk Information System* (IRIS) and other sources. The objectives of such groups as the International Programme on Chemical Safety (IPCS), the U.S. Environmental Protection Agency (EPA), and the U.S. Food and Drug Administration (FDA) include the determination of acceptable exposure levels. The goal is maintenance of environmental integrity and protection

of human health against the possible deleterious effects of chemicals resulting from long-term environmental exposure. The procedures involved in the estimation of maximum safe exposure limits are usually based on long-term studies. When long-term study data are unavailable, shorter-term study data can be used with modification of the approach, such as use of larger safety factors. The approach described therein relates primarily to long-term or lifetime exposure of the general population via the ambient environment (i.e., ambient air, food, drinking water, and other sources).

Residual Solvents in Pharmaceuticals

Exposure limits in this general chapter are established by referring to methodologies and toxicity data described in EHC and IRIS monographs. However, the following specific assumptions about residual solvents to be used in the synthesis and formulation of pharmaceutical products should be taken into account in establishing exposure limits.

1. Patients (not the general population) use pharmaceuticals to treat their diseases or for prophylaxis to prevent infection or disease.
2. The assumption of lifetime patient exposure is not necessary for most pharmaceutical products but may be appropriate as a working hypothesis to reduce risk to human health.
3. Residual solvents are unavoidable components in pharmaceutical production and will often be a part of pharmaceutical drug products.
4. Residual solvents should not exceed recommended levels except in exceptional circumstances.
5. Data from toxicological studies that are used to determine acceptable levels for residual solvents should have been generated using appropriate protocols such as those described by the Organization for Economic Cooperation and Development (OECD), the EPA, and the FDA *Redbook* or derived from alternative sources also considered to be adequate to support PDE limit setting.

APPENDIX 3. PROCEDURES FOR ESTABLISHING EXPOSURE LIMITS

The Gaylor-Kodell method of risk assessment (Gaylor, DW, and Kodell, RL. Linear interpolation algorithm for low dose risk assessment of toxic substances. *J Environ Pathol and Toxycol*, 1980;4:305) is appropriate for Class 1 carcinogenic solvents. Only in cases where reliable carcinogenicity data are available should extrapolation by the use of mathematical models be applied to setting exposure limits. Exposure limits for Class 1 residual solvents could be determined with the use of a large safety factor (i.e., 10,000–100,000) with respect to the no-observed-effect level (NOEL). Detection and quantification of these residual solvents should be performed using state-of-the-art analytical techniques.

Acceptable exposure levels in this general chapter for Class 2 residual solvents were established by calculation of PDE values according to the procedures for setting exposure limits in pharmaceuticals (page 5748 of PF 15(6) [Nov.–Dec. 1989]), and the method adopted by IPCS for *Assessing Human Health Risks of Chemicals (Environmental Health Criteria 170, WHO, 1994)*, detailed calculations are published in *Pharmeuropa*, Vol. 9, No. 1, Supplement, April 1997. These procedures are similar to those used by the U.S. EPA (IRIS) and the U.S. FDA (*Redbook*) and others. The method is outlined here to give a better understanding of the origin of the PDE values. It is not necessary to perform these calculations in order to use the PDE

values presented in *Table 2* of this document.

PDE is derived from the NOEL, or the lowest-observed-effect level (LOEL), in the most relevant animal study as follows:

$$\text{PDE} = (\text{NOEL} \times \text{Weight Adjustment}) / (F1 \times F2 \times F3 \times F4 \times F5)$$

The PDE is derived preferably from a NOEL. If no NOEL is obtained, the LOEL may be used. Modifying factors proposed here, for relating the data to humans, are the same kind of "uncertainty factors" used in *Environmental Health Criteria (Environmental Health Criteria 170, WHO, 1994)* and "modifying factors" or "safety factors" used in *Pharmacopeial Forum*. The assumption of 100% systemic exposure is used in all calculations regardless of the route of administration.

The modifying factors are as follows:

F1 =	A factor to account for extrapolation between species
F1 =	2 for extrapolation from dogs to humans
F1 =	2.5 for extrapolation from rabbits to humans
F1 =	3 for extrapolation from monkeys to humans
F1 =	5 for extrapolation from rats to humans
F1 =	10 for extrapolation from other animals to humans
F1 =	12 for extrapolation from mice to humans

F1 takes into account the comparative surface area to body weight ratios for the species concerned and for humans. Surface area (*S*) is calculated as:

$$S = kM^{0.67}$$

k = a constant that has been taken to be 10

M = body weight

The body weights used in the equation are those shown below in *Table A3.1*.

F2 =	A factor of 10 to account for variability between individuals. A factor of 10 is generally given for all organic solvents, and 10 is used consistently in this general chapter.
F3 =	A variable factor to account for toxicity studies of short-term exposure
F3 =	1 for studies that last at least one half-lifetime (1 year for rodents or rabbits; 7 years for cats, dogs, and monkeys)
F3 =	1 for reproductive studies in which the whole period of organogenesis is covered
F3 =	2 for a 6-month study in rodents, or a 3.5-year study in nonrodents
F3 =	5 for a 3-month study in rodents, or a 2-year study in nonrodents
F3 =	10 for studies of a shorter duration

In all cases, the higher factor has been used for study durations between the time points (e.g., a factor of 2 for a 9-month rodent study).

F4 =	A factor that may be applied in cases of severe toxicity, e.g., nongenotoxic carcinogenicity, neurotoxicity, or teratogenicity. In studies of reproductive toxicity, the following factors are used:	
F4 =	1	for fetal toxicity associated with maternal toxicity
F4 =	5	for fetal toxicity without maternal toxicity
F4 =	5	for a teratogenic effect with maternal toxicity
F4 =	10	for a teratogenic effect without maternal toxicity
F5 =	A variable factor that may be applied if the no-effect level was not established	

When only a LOEL is available, a factor of up to 10 can be used, depending on the severity of the toxicity. The weight adjustment assumes an arbitrary adult human body weight for either sex of 50 kg. This relatively low weight provides an additional safety factor against the standard weights of 60 or 70 kg that are often used in this type of calculation. It is recognized that some adult patients weigh less than 50 kg; these patients are considered to be accommodated by the built-in safety factors used to determine a PDE. If the solvent was present in a formulation specifically intended for pediatric use, an adjustment for a lower body weight would be appropriate.

As an example of the application of this equation, consider a toxicity study of acetonitrile in mice that is summarized in *Pharmeuropa*, 1997;9(1): S24. The NOEL is calculated to be 50.7 mg kg⁻¹ day⁻¹. The PDE for acetonitrile in this study is calculated as follows:

$$\text{PDE} = (50.7 \text{ mg kg}^{-1} \text{ day}^{-1} \times 50 \text{ kg}) / (12 \times 10 \times 5 \times 1 \times 1) = 4.22 \text{ mg day}^{-1}$$

In this example,

F1 =	12	to account for the extrapolation from mice to humans
F2 =	10	to account for differences between individual humans
F3 =	5	because the duration of the study was only 13 weeks
F4 =	1	because no severe toxicity was encountered
F5 =	1	because the no-effect level was determined

A3.1. Values Used in the Calculations in This Document

Rat body weight	425 g
Pregnant rat body weight	330 g
Mouse body weight	28 g
Pregnant mouse body weight	30 g
Guinea-pig body weight	500 g
Rhesus monkey body weight	2.5 kg
Rabbit body weight (pregnant or not)	4 kg
Beagle dog body weight	11.5 kg
Rat respiratory volume	290 L/day
Mouse respiratory volume	43 L/day
Rabbit respiratory volume	1440 L/day
Guinea-pig respiratory volume	430 L/day
Human respiratory volume	28,800 L/day
Dog respiratory volume	9000 L/day
Monkey respiratory volume	1150 L/day
Mouse water consumption	5 mL/day
Rat water consumption	30 mL/day
Rat food consumption	30 g/day

The equation for an ideal gas, $PV = nRT$, is used to convert concentrations of ga

■ 2S (USP39)

BRIEFING

⟨771⟩ **Ophthalmic Ointments**, *USP 38* page 537 and *PF 40(6)* [Nov.–Dec. 2015]. There are five *USP* general chapters that link with *USP*'s taxonomy for pharmaceutical dosage forms: *Injections and Implanted Drug Products* ⟨1⟩, *Oral Drug Products—Product Quality Tests* ⟨2⟩, *Topical and Transdermal Drug Products—Product Quality Tests* ⟨3⟩, *Mucosal Drug Products—Product Quality Tests* ⟨4⟩, and *Inhalation and Nasal Drug Products—General Information and Product Quality Tests* ⟨5⟩. This taxonomy describes five routes of administration: injection or parenteral; gastrointestinal or oral; topical (dermal and transdermal); mucosal; and lungs or inhalational (for more information, see the *Stimuli* article titled *Product Quality and Product Performance General Chapters* in *PF 39(3)* [May–June 2013]). The five general chapters provide critical product quality attributes for the various dosage forms by the various routes of administration. In addition to these five chapters, *Ophthalmic Ointments* ⟨771⟩ outlines the critical product quality attributes for the various dosage forms that can be applied to the eye. Product quality tests assess physical, chemical, and microbial attributes. Taken together, the tests help ensure identity, strength, quality, and purity of a dosage form. The General Chapters—Dosage Forms Expert Committee is proposing the following revision to ⟨1⟩, ⟨2⟩, ⟨3⟩, ⟨4⟩, ⟨5⟩, and ⟨771⟩ in this issue of *PF* to clarify the applicability of these chapters. On the basis of comments received, it is proposed to make the following revisions to this chapter.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCDF: M. Marques.)

Correspondence Number—C160869

Comment deadline: November 30, 2015

Change to read:

(771) OPHTHALMIC OINTMENTS ▲ PRODUCTS—QUALITY TESTS ▲^{USP39}

Change to read:

Added Substances—Suitable substances may be added to ophthalmic ointments to increase stability or usefulness, unless proscribed in the individual monograph, provided they are harmless in the amounts administered and do not interfere with the therapeutic efficacy or with the responses to the specified assays and tests. No coloring agent may be added, solely for the purpose of coloring the finished preparation, to an article intended for ophthalmic use (see also *Added Substances* under *General Notices* and under *Antimicrobial Effectiveness Testing* (51)).

A suitable substance or mixture of substances to prevent the growth of microorganisms must be added to ophthalmic ointments that are packaged in multiple-use containers, regardless of the method of sterilization employed, unless otherwise directed in the individual monograph, or unless the formula itself is bacteriostatic. Such substances are used in concentrations that will prevent the growth of or kill microorganisms in the ophthalmic ointments (see also *Antimicrobial Effectiveness Testing* (51) and *Antimicrobial Agents—Content* (341)). Sterilization processes are employed for the finished ointment or for all ingredients, if the ointment is manufactured under rigidly aseptic conditions, even though such substances are used (see also *Parenteral and Topical Preparations* in the section *Added Substances*, under *General Notices*, and *Sterilization and Sterility Assurance of Compendial Articles* (1211)). Ophthalmic ointments that are packaged in single-use containers are not required to contain antibacterial agents; however, they meet the requirements for *Sterility Tests* (71).

Containers—Containers, including the closures, for ophthalmic ointments do not interact physically or chemically with the preparation in any manner to alter the strength, quality, or purity beyond the official requirements under the ordinary or customary conditions of handling, shipment, storage, sale, and use.

Metal Particles—Follow the *Procedure* set forth under *Metal Particles in Ophthalmic Ointments* (751).

Leakage—Select 10 tubes of the Ointment, with seals applied when specified. Thoroughly clean and dry the exterior surfaces of each tube with an absorbent cloth. Place the tubes in a horizontal position on a sheet of absorbent blotting paper in an oven maintained at a temperature of $60 \pm 3^\circ$ for 8 hours. No significant leakage occurs during or at the completion of the test (disregard traces of ointment presumed to originate externally from within the crimp of the tube or from the thread of the cap). If leakage is observed from one, but not more than one, of the tubes, repeat the test with 20 additional tubes of the Ointment. The requirement is met if no leakage is observed from the first 10 tubes tested, or if leakage is observed from not more than one of 30 tubes tested.



INTRODUCTION

Ophthalmic products are sterile products that are intended for application to any ocular

structure, including any space adjacent to an ocular structure and its immediate surrounding spaces.

The routes of administration of ophthalmic products fall into three general categories: topical, intraocular injections, and extraocular injections. Topical drug products are intended to be administered to an ocular surface component, such as the eyelid, conjunctiva, or cornea, and can produce local or systemic effects. Intraocular and extraocular injections are administered through external boundary tissue. The ophthalmic routes of administration include, but are not limited to: topical, subconjunctival, sub-tenons, subretinal, subchoroidal, intracorneal, intrascleral, suprachoroidal, intravitreal, intracameral, juxtasclear, and retrobulbar routes (see *Figure 1*). Ophthalmic products are administered to the eye in a wide variety of dosage forms, including but not restricted to: solutions, suspensions, ointments, gels, emulsions, strips, injections, inserts, and implants.

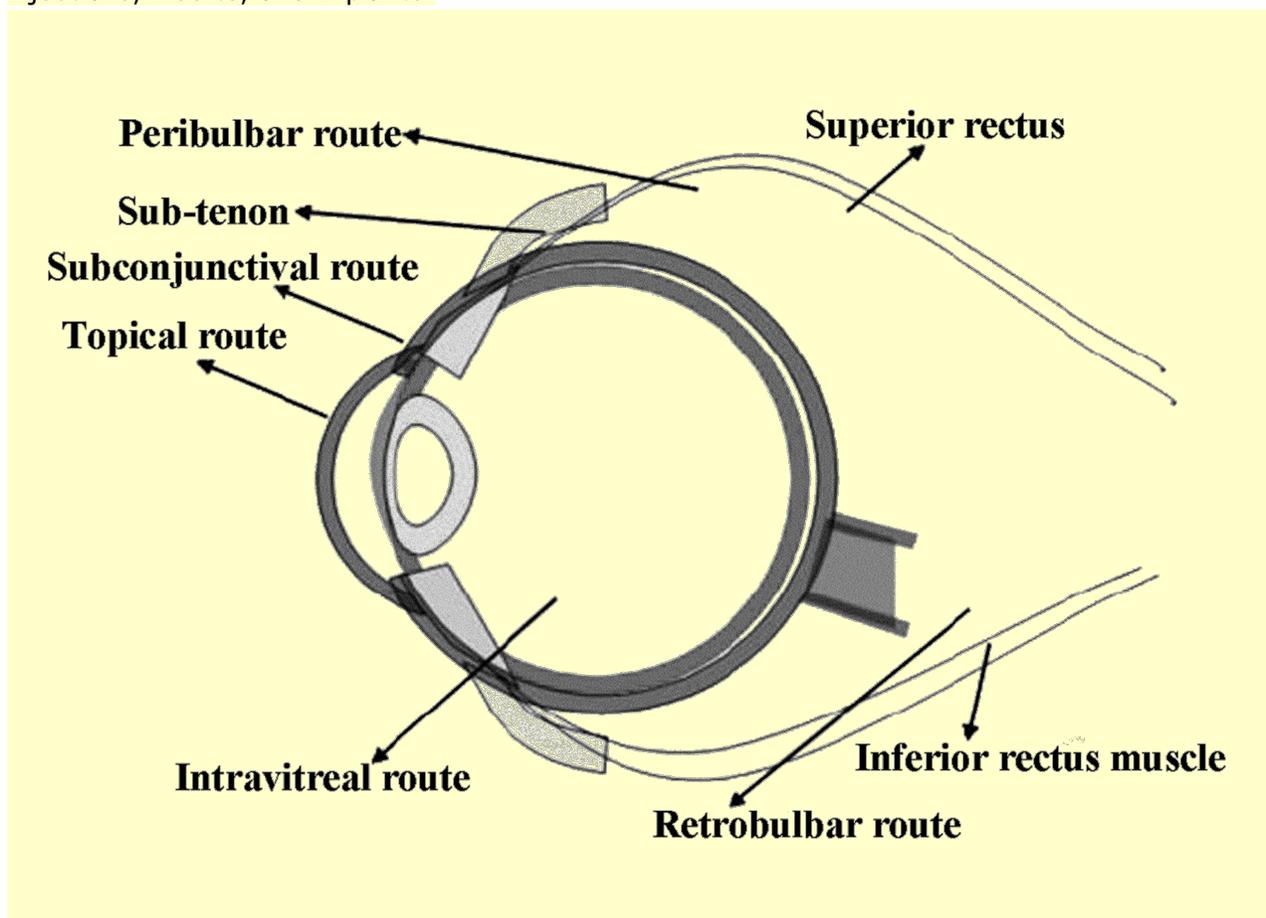


Figure 1. Some of the routes of administration in the eye.

■ General chapter <771> provides lists of consolidated common product quality test requirements in a concise and coherent fashion. This general chapter applies, in part or in its entirety, when referenced in a drug product monograph. The general chapter includes the quality tests for the specific route of administration. The quality tests listed can be used, as appropriate, by manufacturers toward the development of new drug product monographs for submission to the USP.

■ 2S (USP39)

Note—All references to chapters above 1000 are for information purposes only, for use as a helpful resource. These chapters are not mandatory unless explicitly called out for application.

OPHTHALMIC DOSAGE FORMS

This chapter discusses the specific characteristics of dosage forms that are administered to any structure in the eye. See *Pharmaceutical Dosage Forms* (1151) for additional information pertaining to the description and manufacturing of pharmaceutical dosage forms.

Ophthalmic products have the same or similar requirements as injectables and implants. For more information on the requirements for these dosage forms, see *Injections and Implanted Drug Products* (1).

Depending on the formulation, container–closure system, and sterilization process, degradation and/or morphological changes can occur during the sterilization step of the manufacturing process and should be minimized. Temperatures required for autoclaving can cause irreversible damage to certain dosage forms such as suspensions, semisolids, emulsions, and others, whereas filtration is applicable only to formulations containing particulates with particle sizes $<0.2 \mu\text{m}$. An alternative is to manufacture the product from sterile ingredients in an aseptic environment.

For multidose products, a suitable antimicrobial preservative is required, and its effectiveness over the shelf life of the product must be verified.

Solutions

[Note—Included in this section are those solid products that, when reconstituted according to the label instructions, result in a solution.]

The corneal contact time of topical ophthalmic solutions increases with the viscosity of the formulations. Several synthetic polymers, including but not limited to polyvinylalcohol, polyvinylpyrrolidone, polyethylene glycol, polyacrylic acid, and many cellulose derivatives such as hypromellose and hydroxyethylcellulose, are commonly used as viscosity enhancers because of their physiologic compatibility and satisfactory physicochemical properties.

Another approach to increase the corneal contact time involves the use of polymers that provide the liquid formulation with semisolid consistency only when it is placed in the conjunctival or corneal area. In this way, easy instillation of the solution is followed by prolonged permanence as a result of the viscoelastic properties of the formed hydrogel. "Hydrogel," or "water-based gels," are three-dimensional networks of polymer chains containing water within the network. The network can be either physically or chemically cross-linked (a covalent bonding) structures between polymer chains. The water content of hydrogels can be adjusted by modulating the composition and conformation of polymers, such as the hydrophilic/hydrophobic balance of polymer chains and pendant groups, and the degree of cross-linking. The in situ gelling phenomenon is caused by a change in the conformation of the polymer(s) that can be triggered by external stimuli such as temperature, pH, ionic content, and lacrimal fluid, upon delivery to the eye. Additionally, some polymers can interact, via noncovalent bonds, with conjunctival mucin and maintain the formulation in contact with corneal tissues.

Solutions can be injected not only by the intravitreal route but also by other routes, such as subconjunctival, sub-tenons, retrobulbar, suprachoroidal, and subretinal routes.

Suspensions

[Note—Included in this section are those solid products that, when reconstituted according to

the label instructions, result in a suspension.]

Use of aqueous or oily suspensions may be considered for a number of reasons such as the following: 1) for drugs that are poorly water soluble, 2) for drugs that have poor aqueous stability, and 3) in cases where there is a need to increase contact time with the eye and to increase wetting of the eye. The drug particle size is often reduced to levels $<10\ \mu\text{m}$ in an attempt to avoid excessive lacrimation.

After topical instillation, particles are expected to be retained in the cul-de-sac, and the drug slowly dissolves or is released from the polymeric structures by diffusion, dissolution, polymer degradation, or ion-exchange.

Suspensions can be injected not only by the intravitreal route but also through other routes, such as subconjunctival, sub-tenons, retrobulbar, suprachoroidal, and subretinal.

Ointments

Eye ointments are semisolid products usually intended for application to the conjunctiva, cornea, or eyelid. They have longer contact time when compared to many solutions. A suitable ophthalmic ointment base is nonirritating to the eye and permits diffusion of the drug substance throughout the secretions bathing the eye.

The majority of water-free, oleaginous eye ointment bases are composed of white petrolatum and liquid petrolatum (mineral oil), or the eye ointment base is a modification of the petrolatum-based formula. They are designed to melt at body temperature. Other types of lipophilic ointment bases can also be used. An anhydrous vehicle can be advantageous for moisture-sensitive drugs.

Gels

Semisolid gel-type products are an alternative to traditional ointments and are based on the effect of increasing the viscosity to prolong the retention of the drug in the eye. Several types of gelling agents can be used, such as polyacrylic acid derivatives, carbomer, and hypromellose.

Emulsions

Topical ophthalmic emulsions are generally prepared by dissolving or dispersing the drug substance into an oil phase, adding suitable emulsifying and suspending agents, and mixing with water vigorously to form a uniform oil-in-water emulsion. Each phase is typically sterilized before or during charging into the mixing vessel. High-shear homogenation may be used to reduce oil droplet size to submicron size, which may improve the physical stability of the oil micelles so that they do not coalesce. The resulting dosage form should contain small oil droplets, uniformly suspended.

Limited aqueous solubility of the drug substance(s) is the most common rationale for developing an ophthalmic emulsion. The drug substance(s) can be added to the phase in which it is soluble at the beginning of the manufacturing process, or the drug substance can be added after the emulsion is prepared by a suitable dispersion process.

The physical stability of the emulsion can be measured by light-scattering techniques that characterize oil-phase, globule-size distribution. Suitable surfactants may be added to improve emulsion stability.

Strips

Ophthalmic strips can have different ophthalmic uses including, but not limited to, use as a diagnostic tool to visualize defects or aberrations in the corneal epithelium or to measure the amount of tear production. They are made of filter paper, and they can contain compounds such as fluorescein sodium. They are individually packed to preserve sterility until the moment of use.

Injections

Although injections are considered a dosage form for nomenclature purposes, they are not treated as a dosage form in this chapter. Instead, refer to the appropriate physical form, such as solution, suspension, or others, for general information.

Inserts

Ophthalmic inserts and ocular systems are solid dosage forms of appropriate size and shape that are placed in the conjunctival fornix, in the lacrimal punctum (*Figure 2*), or on the cornea. Inserts can usually be removed, if adverse effects develop, and provide extended release of the drug over a certain period of time. Inserts can be classified as erodible (soluble) or nonerodible (insoluble). Drug release from soluble inserts involves two steps: 1) fast release of a portion of the drug as the tear fluid penetrates into the system, and 2) slow release as a gel layer is formed on the surface of the insert. Collagen shields made from porcine sclera collagen or bovine corium tissue—and devices obtained by molding, extrusion, or compression (minitables) of gelling polymers—belong to this category of soluble inserts. Bioerodible polymers (e.g., cross-linked gelatin derivatives and polyesters) can be used to prepare erodible inserts; these matrices act as reservoirs or interact with the drug molecules through labile bonds.

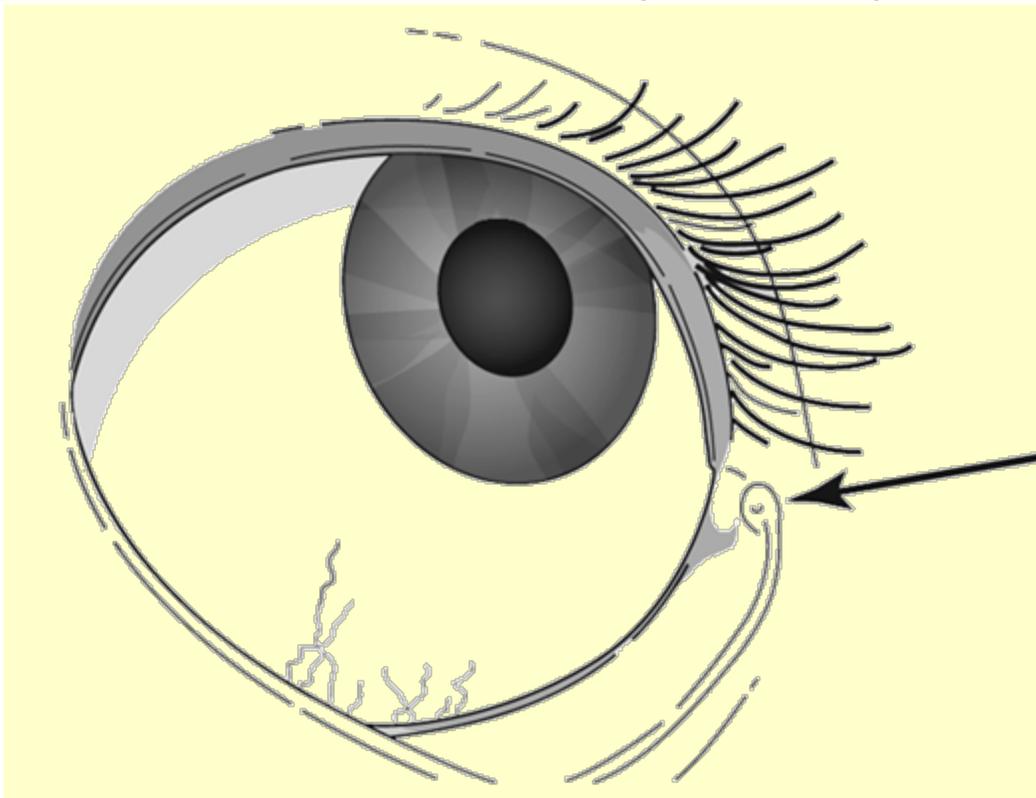


Figure 2. Lacrimal punctum.

Soluble inserts can have the drug incorporated into an erodible matrix, such as hydroxypropyl cellulose, hyaluronic acid, carbomer, or polyacrylic acid. Soluble inserts may be placed in the lower cul-de-sac and generally dissolve within 12–24 h. Erodible polymeric products undergo gradual dissolution while releasing the drug, and the patient does not have to remove the erodible polymeric insert after use.

Insoluble inserts can have a reservoir or matrix structure. Their mechanism of action is based on diffusion of a fluid into the device, dissolving of the drug, and the creation of a saturated solution released to the medium by diffusion out of the insert. These insoluble inserts need to be removed after a certain period of time.

Implants

Implants are injected or implanted into the anterior segment (intracameral, subconjunctival, sub-tenon, or other parts of the anterior segment) or intravitreal cavities, and the implants can be in different shapes, such as a disc or a thin rod. Implants can be made of nonbiodegradable or biodegradable polymers.

Among the types of nonbiodegradable solid (polymer) implants are reservoir-type implants anchored to the sclera, in which the drug is released across a nonbiodegradable, semipermeable polymer. The reservoir-type implants can be made of a variety of materials, including polyvinyl alcohol–ethylene vinyl acetate. Biodegradable polymers can be used to form injectable solid implants.

Drug–Device Combination Products

An ophthalmic drug–device combination product is constituted, in most cases, of two components. One is a pharmaceutical dosage form containing the drug substance(s), and the other is a device that will activate or facilitate the penetration of the drug substance(s) from the dosage form into a particular region of the eye. Some examples of the devices are those that generate waveforms (heat or light).

This chapter is applicable only to the pharmaceutical dosage form component of the ophthalmic drug–device combination product. The appropriate FDA regulations regarding medical devices should be used for the device component.

DRUG PRODUCT QUALITY

Procedures and acceptance criteria for testing ophthalmic products are divided into two categories: 1) those that assess general quality attributes, e.g., identification, potency, purity (and impurities), sterility, and particulate matter; and 2) those that assess in vitro product performance, i.e., dissolution or drug release of the drug substance from the drug product. Quality tests assess the integrity of the dosage form, whereas the performance tests assess drug release and other attributes that relate to in vivo drug performance. Taken together, quality and performance tests ensure the identity, strength, quality, purity, and efficacy of the drug product. This chapter addresses the quality tests for ophthalmic products. The performance tests (dissolution/drug release) are addressed by *Ophthalmic*

Products—Performance Tests (1771).

Universal Tests

In this chapter, the division of the product quality tests into universal and specific tests does not strictly follow the ICH guidance *Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances* (available at www.ich.org). Universal tests in this chapter mean the tests that are applicable to all ophthalmic products, regardless of the dosage form type.

DESCRIPTION

A qualitative description of the drug product should be provided. The acceptance criteria should contain the final acceptable appearance, including clarity and color, of the dosage form and packaging. If the color changes during storage, a quantitative procedure may be appropriate. This is not a compendial test but is part of the manufacturer's specification of the drug product.

IDENTIFICATION

Identification tests are discussed in *General Notices, 5.40 Identity*. Identification tests should establish the identity of the drug or drugs present in the article and should discriminate between compounds of closely related structures that are likely to be present. Identity tests should be specific for the drug substance(s) [e.g., infrared (IR) spectroscopy]. Near-infrared (NIR) or Raman spectrophotometric methods could be acceptable for the identification of the drug product (see *Near-Infrared Spectroscopy—Theory and Practice* (1119) and *Raman Spectroscopy—Theory and Practice* (1120)). The most widely used identification procedures for drug substance(s) contained in pharmaceutical dosage forms are chromatographic procedures with comparison to the appropriate standards (see *Chromatography* (621) and *Thin-Layer Chromatographic Identification Test* (201)). Identification by use of a single chromatographic retention time does not constitute a specific test.

ASSAY

A specific and stability-indicating test should be used to determine the strength (content) of the drug product. In cases where the use of a nonspecific assay test is justified, other supporting analytical procedures should be used to achieve overall specificity. A specific procedure should be used when there is evidence of excipient interference with the nonspecific assay test. Additional information on specific assays may be found in *Antibiotics—Microbial Assays* (81), (621), *Spectrophotometry and Light-Scattering* (851), and *Ion Chromatography* (1065).

IMPURITIES

Process impurities, synthetic byproducts, and other inorganic and organic impurities may be present in the drug substance and excipients used in the manufacture of the drug product. These impurities are controlled by the drug substance and excipient monographs. Organic impurities arising from the degradation of the drug substance in the drug product and those impurities arising during the manufacturing process of the drug product should be monitored. All articles meet the requirements in *Elemental Impurities—Limits* (232) and *Residual Solvents* (467).

See *pH* (791). Normal tears have a pH of about 7.4. The eye can tolerate products over a range of pH values from about 3.0 to about 8.6, depending on the buffering capacity of the formulation. The pH value of the formulation should be the one where the drug product is the most stable. Formulations that target the extremes of the acceptable pH range will have better patient acceptability if the formulations have a low buffering capacity.

OSMOLARITY

See *Osmolality and Osmolarity* (785). Ophthalmic products may be tolerated over a fairly wide range of tonicity (0.5%–5% sodium chloride, equivalent to about 171–1711 mOsm/kg). Hypotonic solutions are better tolerated than hypertonic solutions. Precautions should be taken to ensure that the product maintains its osmolarity during shelf life. Any possible contributions or interferences from the packaging system should be considered.

PARTICULATE AND FOREIGN MATTER

All ophthalmic products should be inspected for package integrity and, to the extent possible, for the presence of observable foreign and particulate matter (visible particulates). These unwanted particles arise from two sources: extrinsic (i.e., foreign matter); and intrinsic, or product-related matter. Extrinsic matter cannot be associated with the product or process. Intrinsic particles are added during assembly of the product or result from a change over time. A third category, inherent matter, describes a physical state or particles that are an expected attribute of the product.

One hundred percent inspection of injectable products in clear packages is required to remove final packages with visible particles. When 100% inspection of the injectable product in the final package is difficult, such as in opaque containers, alternative methods may be used to determine acceptability. *Visible Particulates in Injections* (790) provides further guidance for general inspection methods and a definition of “essentially free of visible particles” for batch compliance.

Two general categories apply for product administration to the tissues in the eye. Intraocular administration includes all ophthalmic products that cross (penetrate) boundary tissue, such as the cornea and sclera. Extraocular administration of ophthalmic products includes all other ocular components and spaces. For subvisible particle content, USP guidance is followed. Products for intraocular use must comply with *Particulate Matter in Ophthalmic Solutions* (789). Products for extraocular use must comply with *Particulate Matter in Injections* (788). Considerations for product evaluation and the background for both subvisible methods are found in *Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions* (1788).

STERILITY

Ophthalmic dosage forms must meet the requirements of *Sterility Tests* (71). If the specific ingredients used in the formulation do not lend themselves to routine sterilization techniques, ingredients that meet the sterility requirements described in (71), along with aseptic manufacture, may be used. The immediate container for ophthalmic products shall be sterile at the time of filling and closing. It is mandatory that the immediate containers for ophthalmic products be sealed and tamper proof so that sterility is ensured at the time of first use.

ANTIMICROBIAL PRESERVATIVES

Antimicrobial agents must be added to products that are packaged in containers that allow for the withdrawal or administration of multiple doses, unless one of the following conditions

prevails: 1) there are different directions in the individual monograph; 2) the substance contains a radionuclide with a physical half-life of <24 h; and 3) the drug product, without additional agents, is sufficiently microbicidal to meet the requirements of *Antimicrobial Effectiveness Testing* (51). Substances must meet the requirements of (51) and *Antimicrobial Agents—Content* (341). Acceptance criteria for antimicrobial preservative content in multiple-unit products should be established.

BACTERIAL ENDOTOXINS

All injected ophthalmic drug products shall be prepared in a manner designed to minimize bacterial endotoxins as defined in *Bacterial Endotoxins Test* (85). The limits are NMT 0.5 EU/mL for ophthalmic irrigation products and NMT 2.0 EU/dose/eye for injected or implanted drug products. Typically, this test is not required for topically applied ophthalmic products. This chapter does not address the endotoxin limits for devices that are injected or implanted.

UNIFORMITY OF DOSAGE UNITS

This test is applicable to dosage forms packaged in single-unit containers. It includes both the mass of the dosage form and the content of the drug substance(s) in the dosage form. The test can be performed by either content uniformity or weight variation (see *Uniformity of Dosage Units* (905)).

CONTAINER CONTENTS

Container contents of ophthalmic products should be determined (see *Minimum Fill* (755)).

LEACHABLES AND EXTRACTABLES

The packaging system should not interact physically or chemically with the product in any manner to alter the strength, quality, or purity of the drug product. The packaging system should meet the requirements in *Elastomeric Closures for Injections* (381), *Containers—Glass* (660), *Plastic Materials of Construction* (661.1), and *Plastic Packaging Systems for Pharmaceutical Use* (661.2). Further information regarding packaging systems testing can be found in *Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems* (1663) and *Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems* (1664). The assessment of possible leachables and/or extractables and the establishment of any acceptance criteria for these compounds should take into account the risk assessment of the product, its indication, and its packaging system. Furthermore, it should be noted that a risk assessment of extractable/leachable impact on topical or intraocular route of administration is a challenging undertaking. Toxicological or safety assessments of primary or secondary packaging component extractables and leachables are not typically available for ophthalmic routes of administration. A risk assessment may include evaluation of toxicology and safety from other routes of administration and an assessment of the Total Daily Intake of the extractable/leachable being evaluated. The preponderance of such assessments leads to an estimate of extractable/leachable risk via ocular delivery to the patient.

CONTAINER—CLOSURE INTEGRITY

The packaging system should be closed or sealed in such a manner as to prevent contamination or loss of contents and should provide evidence of being tamper proof. Validation of container integrity must demonstrate no penetration of microbial contamination or of chemical or physical impurities (see *Sterile Product Packaging—Integrity Evaluation* (1207)).

Specific Tests

VISCOSITY

An increase in viscosity increases the residence time in the eye. However, drug diffusion out of the formulation into the eye may be inhibited due to high product viscosity. Ophthalmic ointments are designed to be of very high viscosity to prolong the residence time in the eye. The inclusion of viscosity evaluation in the specification of the product should be based on the type of dosage form and if changes in product viscosity will affect its performance. This is not a compendial test but is part of the manufacturer's specification of the drug product. See *Viscosity—Capillary Methods* (911), *Viscosity—Rotational Methods* (912), and *Viscosity—Rolling Ball Method* (913).

ANTIOXIDANT CONTENT

If antioxidants are present in the drug product, tests of their content should be established, unless oxidative degradation can be detected by another test method such as impurity testing. Acceptance criteria for antioxidant content should be established. They should be based on the levels of antioxidant necessary to maintain the product's stability at all stages throughout its proposed usage and shelf life.

RESUSPENDABILITY/REDISPERSIBILITY

Consideration must be given to establishing good physical stability of a suspension. If the particles settle and eventually produce a cake at the bottom of the container, they must redisperse readily at the time of use to achieve dosage uniformity.

PARTICLE SIZE AND PARTICLE SIZE DISTRIBUTION

The potential for any changes in the particle size of ophthalmic suspensions and emulsions needs to be evaluated through stability testing (see *Light Diffraction Measurement of Particle Size* (429)).

DROP SIZE

For ophthalmic drug products dispensed as drops, drop sizes may typically range from 20 to 70 μL . Drop size can be controlled by weight or by volume, and it is typically evaluated during product development.

ADDED SUBSTANCES

Suitable substances may be added to ophthalmic products to increase stability, unless proscribed in the individual monograph, provided they are harmless in the amounts administered and do not interfere with therapeutic efficacy or with responses to the specified assays and tests. Typically, they should be evaluated during product development. The use of ingredients solely to impart a color, odor, or flavor is prohibited.

▲USP39

BRIEFING

(782) **Vibrational Circular Dichroism Spectroscopy.** The General Chapters—Chemical Analysis Expert Committee proposes this new general chapter about vibrational circular dichroism (VCD) spectroscopy. VCD is an extension of electronic circular dichroism (ECD) from the region of electronic transitions in the UV and visible regions of the spectrum to vibrational transitions in the infrared (IR) region. The principal chapter in *USP* that addresses molecular chirality is *Optical Rotation* (781). Optical rotation (OR) and ECD are the traditional forms of chiroptical spectroscopy, whereas VCD is a newer form of chiroptical spectroscopy that contains more molecular structure information and recently has been adopted more broadly for use in the pharmaceutical industry. For discussion of the theory and principles of VCD

measurements, as well as a more detailed discussion of the application of VCD to problems of pharmaceutical interest, see *Vibrational Circular Dichroism Spectroscopy—Theory and Practice* (1782), which also appears in this *PF*. A related *Stimuli* article titled *Vibrational Circular Dichroism as a New Technology for Determining the Absolute Configuration, Conformation, and Enantiomeric Purity of Chiral Pharmaceutical Ingredients* appeared in *PF* 39(4) and provides insight into the rationale for this proposed chapter.

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Comment deadline: November 30, 2015

Add the following:

■ (782) **VIBRATIONAL CIRCULAR DICHROISM SPECTROSCOPY**

1. INTRODUCTION
2. QUALIFICATION OF VCD SPECTROMETERS
 - 2.1 Installation Qualification
 - 2.2 Operational Qualification
 - 2.3 Performance Qualification
3. PROCEDURE
 - 3.1 Sample
 - 3.2 Standard
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4. VALIDATION AND VERIFICATION
 - 4.1 Validation
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 - 4.3 Verification

1. INTRODUCTION

Vibrational circular dichroism (VCD) is a form of chiroptical spectroscopy for which chiral molecules have non-zero spectra with intensities that are identical for mirror-image pairs (enantiomers) of chiral molecules but with signs that are opposite with respect to zero intensity. For a chiral molecular substance, two important properties are: 1) absolute configuration (AC), which indicates which of two mirror-image forms of the molecule is present; and 2) enantiomeric excess (EE, also called enantiomeric purity), the fractional excess of one enantiomer of a chiral molecule over its mirror-image enantiomer. The principal chapter in *USP* that addresses molecular chirality is *Optical Rotation* (781). Optical rotation (OR) and electronic circular dichroism (ECD) are the traditional forms of chiroptical spectroscopy, whereas VCD is a newer form of chiroptical spectroscopy that contains more molecular structure information and recently has been adopted more broadly for use in the pharmaceutical industry.

In this chapter, the required methodology for the use of VCD is described in terms of instrument qualification; sign and intensity calibration; procedures, validation, and verification of VCD measurement; calculation; and statistical analysis as needed for the determination of AC and EE of chiral pharmaceutical products. VCD is the extension of ECD from the region of electronic transitions in the UV and visible regions of the spectrum to vibrational transitions in the infrared (IR) region.

VCD is defined as:

$$\Delta A = A_L - A_R$$

ΔA = difference in the IR absorbance

A_L = sample absorbance for left circularly polarized (LCP) radiation

A_R = sample absorbance for right circularly polarized (RCP) radiation

Unpolarized IR intensity is defined as the average of LCP and RCP intensities:

$$A = (A_L + A_R)/2$$

A = IR absorbance

A_L = sample absorbance for left circularly polarized (LCP) radiation

A_R = sample absorbance for right circularly polarized (RCP) radiation

The IR intensities of enantiomers are identical, whereas enantiomers have equal and opposite-signed VCD intensities.

For discussion of the theory and principles of VCD measurements, as well as a more detailed explanation of the application of VCD to problems of pharmaceutical interest, see *Vibrational Circular Dichroism Spectroscopy—Theory and Practice* (1782) in this issue of *PF*.

2. QUALIFICATION OF VCD SPECTROMETERS

This section on qualification is divided into three subsections: 1) Installation Qualification (IQ), 2) Operational Qualification (OQ), and 3) Performance Qualification (PQ) of a Fourier transform (FT)-VCD instrument. Although a VCD instrument can be based on a scanning dispersive spectrometer, no such VCD spectrometers are commercially available, and hence they will not be considered. Additional details on the qualification of instruments can be found in *Analytical Instrument Qualification* (1058).

2.1 Installation Qualification

The IQ requirements provide evidence that the hardware and software are properly installed in the desired location. An FT-VCD instrument is a sensitive, circular-polarization-difference, FT-infrared (FT-IR) spectrometer. Its installation requires a stable bench-top surface, access to dry air or nitrogen for purging of water vapor, and standard electrical outlets. Evidence for IQ involves ensuring that the IR interferometer is scanning, that its photoelastic modulator(s) [PEM(s)] is operating, and that the electronics hardware is interacting with both the FT-IR main bench and the PEM. The final evidence for IQ is that the software display for acquisition of a VCD spectrum is ready for operator use.

2.2 Operational Qualification

In OQ, an instrument's performance is characterized using a qualification standard of known spectral properties to verify that the system operates within target specifications. The purpose of OQ is to demonstrate that instrument performance is suitable. OQ is a check of the key operational parameters and should be performed after installation and after repairs and/or maintenance.

The OQ tests described in the following sections are typical examples only. Other tests and samples can be used to establish specifications for OQ. Instrument vendors often have samples and test parameters available as part of the IQ/OQ package. The acceptance criteria provided in this section are applicable for general use, whereas specifications for particular instruments and applications may vary, depending on analytical methods used and desired accuracy of the final result.

CHARACTERIZING INSTRUMENT PERFORMANCE

FT-VCD spectrometers measure simultaneously the VCD spectrum and the parent IR spectrum. The VCD spectrum is obtained from a VCD interferogram arising from the combined high-frequency PEM polarization modulation and the Fourier frequencies, whereas the parent IR spectrum arises from the conventional FT-IR interferogram based only on the instrument's Fourier frequencies. Part of the instrument performance is from the underlying FT-IR operation, and the remaining part of the instrument performance is from those components that are uniquely associated with the FT-VCD operation. The combined FT-IR and FT-VCD criteria are wavenumber accuracy and absorbance linearity, whereas those uniquely associated with the VCD operation are: VCD sign and intensity calibration, empty-beam baseline accuracy, sample-associated baseline deviations and artifact levels, baseline stability and spectral reproducibility, and signal-to-noise ratio. As described in (1782), two standard samples are commonly used for specifying VCD performance. For sign and intensity calibration, neat α -pinene is used as a validation standard, whereas for baseline characteristics, a solution of camphor in carbon tetrachloride (CCl_4) is used as the qualification standard. VCD spectra presented in (1782) serve as examples of performance characteristics of typical spectra for VCD sign and intensity calibration, baseline stability, and signal-to-noise ratio.

WAVENUMBER ACCURACY

The wavenumber accuracy of FT-VCD instruments is based on the corresponding wavenumber accuracy of the parent FT-IR operation. This accuracy is determined by fringe counting of the visible laser co-operating with the FT-IR interferometer. The frequency calibration is accurate to within the spectral resolution of the instrument and requires no calibration standard. The accuracy of wavenumber frequency should be checked by measuring the IR spectrum of a 35- μm -thick polystyrene film, as described in the *USP* chapters on mid-IR spectroscopy, *Mid-Infrared Spectroscopy* (854) and *Mid-Infrared Spectroscopy—Theory and Practice* (1854). Because the FT-VCD and FT-IR spectra are simultaneously measured by the action of the same interferometer, the wavenumber accuracy of the VCD spectrum is the same as that of the FT-IR operation of the instrument.

LINEARITY AND STABILITY

Commercial FT-IR instruments have excellent linearity, provided that the infrared detector remains unsaturated. FT-IR spectra are particularly prone to detector saturation, because the

intensity of the entire spectrum is present simultaneously at the detector. Detector saturation can be avoided by the use of a long-wavelength pass filter or appropriate adjustment of the gain of the preamplifier to ensure that the maximum of the FT-IR interferogram lies within the range of preamplifier voltage capability. The hallmark of the onset of nonlinearity is distortion of the FT-IR interferogram that can lead to negative FT-IR absorbance values at the endpoint frequency of the spectral coverage, which is clearly incorrect for an absorbing sample. Aperture reduction, insertion of screens, or neutral density filters can be used to avoid detector saturation.

These same considerations apply to the linearity of FT-VCD measurements. The linearity of VCD is more sensitive to the onset of detector saturation than is the parent IR spectrum. VCD spectra are unstable and distorted when the parent FT-IR spectrum has any saturation, and in some cases linearity in the VCD is not restored until the FT-IR intensity is well below saturation. Saturation can be tested by varying the throughput of the instrument by changing the sample path length and making sure that all of the points across the spectrum are changed by the same factor, i.e., there are no relative shape changes in the spectrum. These relative shape changes should be less than the apparent noise level or, at most, 2% (1.00 ± 0.02), depending on the accuracy needed for the VCD measurements.

PHASE CORRECTION, SIGN, AND INTENSITY CALIBRATION

The raw VCD spectrum is measured as the ratio of the FT of the VCD interferogram to that of the FT of the IR interferogram. Both of these interferograms must be phase corrected before performing the FT. The phase correction for the IR is straightforward, but the phase correction of the VCD interferogram requires transfer of a phase file from a VCD interferogram representing only positive intensities. Most commercial FT-VCD instruments provide such phase files for the measurement of VCD.

Next, the measured raw VCD spectrum must be calibrated such that VCD spectra with the correct signs and intensities are produced. The signs can be determined by measuring the qualification standard of (-)-camphor or (+)-camphor in carbon tetrachloride solution and comparing it to a previously published standard VCD spectrum of this molecule, such as that provided in (1782). Either all of the signs agree, in which case the sign is correctly set, or all of the signs are opposite, in which case the phase of the VCD synchronous detection (lock-in amplifier or numerical processing) must be changed by 180° to reverse the signs of all VCD bands, positive-to-negative and negative-to-positive.

Calibration of VCD intensities is performed by dividing the raw VCD by a calibration curve representing the magnitude of unit VCD intensity for the VCD spectrometer. The shape of the calibration curve follows a first-order Bessel function, which in turn depends on the settings of the PEM generating LCP and RCP radiation. The maximum of the calibration curve corresponds to the maximum wavenumber value set on the PEM controller. The calibration curve for a given PEM setting can be obtained from the crossing points of curves generated by placing a multiple-wave plate followed by a polarizer in place of the sample and measuring the VCD spectrum in the usual manner. Commercial VCD instruments provide such curves, and the correct calibration curve must be used for each wavenumber setting of the PEM. For an example of a calibration curve for the PEM setting at 1200 cm^{-1} , see (1782).

After calibration of the intensities of the VCD instrument, a measurement of a 0.9 M solution of (+)-*R*-camphor in carbon tetrachloride measured in a 100- μm path-length cell should be

approximately 2×10^{-4} . After baseline subtraction, described below and in (1782), this difference should be a positive peak ΔA value of $+1 \times 10^{-4}$ absorbance units at 1240 cm^{-1} and a negative ΔA peak value of -1×10^{-4} at 1040 cm^{-1} .

EMPTY BEAM BASELINE CHARACTERISTICS

To avoid significant levels of baseline deviations from the absorbance features of a sample, the empty beam baseline for a VCD instrument should be as close as possible to the true zero of the measurement. A minimum standard for FT-VCD spectrometers is to have empty beam baselines sufficiently close to zero so that deviations from true zero are <25% of the span of maximum positive-to-negative absorbance intensity of a 0.9 M solution of (+)-*R*-camphor in carbon tetrachloride, measured in a 100- μm path-length cell. This magnitude, noted above, is approximately 2×10^{-4} as determined from a positive peak value of $+1 \times 10^{-4}$ at 1240 cm^{-1} and a negative peak value of -1×10^{-4} at 1040 cm^{-1} . The value of 25% of this intensity range is approximately 5×10^{-5} . VCD empty beam baseline deviations (maximum to minimum), over the mid-IR range from 900 to 1800 cm^{-1} , should not exceed $\pm 5 \times 10^{-4}$ and should not deviate more than $+5 \times 10^{-4}$ or -5×10^{-4} from the zero of VCD intensity over this wavenumber range.

SAMPLE BASELINES AND ABSORPTION ARTIFACTS

An important test for a VCD measurement is the VCD spectrum of a racemic sample relative to that of the baseline of the empty instrument or the VCD spectrum of a nonchiral solvent, such as carbon tetrachloride. Because of possible baseline changes due to the sample cell, it is preferable to compare the VCD of the solvent with that of a solution of the racemic sample of the chiral molecule in the same solvent. Both should have zero VCD, but the racemic solution may have baseline artifacts (offsets) at the location of the absorption bands. Absorption bands change the index of refraction of the sample and can cause baseline deviations that are particular to the sample molecule. If such absorption artifacts are present, the only way to recover the artifact-free VCD spectrum is by subtraction of the racemic mixture (equal quantities of both enantiomers) from that of the measured VCD spectrum. Alternatively, subtraction of the VCD spectrum of the opposite enantiomer is divided by 2, because the VCD intensity doubles if the VCD spectrum of one enantiomer is subtracted from that of the other. The qualification standard used for measuring the VCD baseline with an absorbing sample present is racemic camphor in carbon tetrachloride solution versus the VCD spectrum of neat carbon tetrachloride in the range $900\text{--}1800 \text{ cm}^{-1}$, where there are only absorption bands from camphor and none for carbon tetrachloride. Both samples should have zero VCD and a baseline that is featureless with respect to the camphor absorption bands.

SIGNAL-TO-NOISE RATIO

The signal-to-noise ratio of a VCD measurement can be determined from the noise curve generated by subtracting one-half of the FT scans of a measurement from the other half, thus cancelling the signal and exposing the noise level. The noise level varies across the spectrum and increases when the light level reaching the detector decreases, either due to the overall throughput of the instrument in a particular spectral region or directly within the absorption

bands of the sample. The level of noise in the noise curve can be reduced by increasing the measurement time of the spectrum. The noise level is inversely proportional to the square root of the measurement time. This is illustrated with a particular example in *Figures 9 and 10 of (1782)*.

Smoothing a VCD spectrum without increasing the measurement time will reduce the size of the peak-to-peak noise excursions but will not reduce the overall accuracy of a VCD measurement, because the VCD baseline, and hence the VCD intensity relative to the zero baseline, will undergo the same level of variations, although the VCD spectrum appears to have less noise after smoothing. To know the true level of the noise and hence the uncertainty level of a VCD spectrum, all smoothing functions, if any, should be turned off. Noise can legitimately be reduced by lowering the spectral resolution. In evaluating the noise level of the VCD measurement, the resolution and interferogram apodization must be specified.

2.3 Performance Qualification

PQ determines that the instrument is capable of meeting the user's requirements for all the parameters that may affect the quality of the measurement. Depending on typical use, the specifications for PQ may be different from the manufacturer's OQ specifications; however, for general use across a range of typical chiral molecules and sampling conditions, the specifications for OQ will be the same as those needed for PQ. For validated methods, specific PQ tests, also known as system suitability tests, can be used in lieu of PQ requirements. Specific procedures, acceptance criteria, and time intervals for characterizing performance depend on the instrument and intended application. Demonstrating stable instrument performance over extended periods of time provides some assurance that reliable measurements can be taken from test sample spectra.

Particular long-term stability tests that can be carried out for PQ evaluation beyond those carried out for IQ or OQ are as follows. It is recommended that a 60-min measurement of a 0.9 M solution of (+)-*R*-camphor in carbon tetrachloride, measured in a 100- μm path-length cell, be performed between different applications of the VCD spectrometer to ensure that the signs, intensities, and signal-to-noise ratio of the VCD spectrum established as a qualification test during OQ are maintained. This is particularly important if there has been an interruption in power or a long hiatus between measurements.

If averaging over long periods of time, such as overnight, it is important that the stability of both the VCD baseline and the sample VCD spectrum are stable over time and that the signal-to-noise ratio improves as expected with the square-root of measurement time or number of interferometer scans, as illustrated by the example in (1782). In general, stability should be maintained to within the noise level of the VCD measurement. Because VCD intensities are generally three to four orders of magnitude smaller than the parent IR intensity, the instrument stability for the FT-IR performance of the instrument is typically larger over time relative to the noise level of the VCD spectrum.

3. PROCEDURE

The measurement of the VCD spectrum of a sample follows the same general rules as the measurement of an IR spectrum as for mid-IR spectroscopy described in (854).

3.1 Sample

A VCD spectrum can be measured in all types of standard IR transmission cells, either for neat liquids or solutions. For solids, such as in potassium bromide (KBr) pellets, mulls, or thin films, it is recommended that additional steps be taken to ensure that artifacts arising from sample linear birefringence are not present as described in (1782) for advanced instrumentation methods, such as dual PEM modulation or rotating sample cells.

Because of the importance of noise control in a VCD measurement, the absorbance of a sample should not exceed $A = 1.0$ in the region of measurement interest. For absorbance >1.0 , insufficient radiation is present at the detector, and noise dominates the VCD spectrum. The optimum level of absorbance relative to the sample reference (empty beam or solvent) is approximately 0.4. One should adjust the path length and/or concentration of the sample such that the average absorbance is approximately 0.4–0.5 over the region of interest, with limits of 0.1–1.0. For samples with a wide range of absorbance, more than one measurement can be carried out with differing average absorbance values to ensure that a VCD spectrum of adequate quality is obtained over the entire range of interest.

To perform a solution-state VCD measurement, first find a solvent that dissolves an adequate amount of solute to achieve a desired absorbance level. Solvents without CH bonds are the most desirable for this purpose; these include carbon tetrachloride, deuterated chloroform (CDCl_3), or deuterated dimethyl sulfoxide (DMSO-d_6). The IR cell should have windows that are transparent in the mid-IR region, with generally low levels of linear birefringence. Commonly used materials are barium fluoride (BaF_2), which has good transmission to 800 cm^{-1} , and calcium fluoride (CaF_2), which is used if coverage to only $1100\text{--}1200\text{ cm}^{-1}$ is needed, such as for aqueous solutions.

3.2 Standard

To ensure that the analyst can use an IR cell for a transmission measurement of a solution, it is recommended that a standard OQ measurement of a 0.9 M solution of (+)-*R*-camphor in carbon tetrachloride using a 100- μm barium fluoride cell be performed. For different choices of concentration or path length, one can use the linear dependence of these quantities on the measured ΔA to ensure that an accurate measurement with the correct signs and intensities has been achieved.

3.3 Analysis

VCD spectra, once measured, can be analyzed in a variety of ways, depending on the intended application. The principal application of VCD in the pharmaceutical industry is the determination of absolute configuration (AC) in conjunction with a VCD calculation using a quantum chemistry computation software package. A second application is the determination of EE of a sample, either for the purpose of determining the EE of a sample or for monitoring the EE of one or more species during the course of the chemical reaction involving chiral species. Achievement of the highest level of accuracy in the determination of EE requires the use of a chemometric software package that uses the entire range of the VCD spectrum, as opposed to a single band or wavenumber frequency. Other applications of VCD are its use with a standard database of VCD and IR spectra to simultaneously ensure the correct identity, AC, and EE of a chiral substance, for example as a chiral raw material identification. VCD can also be used to specify the AC and EE of a drug product in the presence of either achiral or chiral excipients, such as

carbohydrates. These applications are described in more detail in (1782).

4. VALIDATION AND VERIFICATION

4.1 Validation

The objective of VCD procedure validation, as with the validation of any analytical process, is to demonstrate that the measurement is suitable for its intended purpose. The application of VCD spectroscopy is somewhat different from that of conventional vibrational spectroscopic techniques because it is not a primary technique. Rather, it is the polarization difference spectroscopy of the parent IR spectroscopy that has been used by itself for decades as a primary identification and quantitation technique as described for mid-IR spectroscopy in (854). The differential VCD spectrum supplements the parent IR spectrum with a new set of intensities at each position of an IR absorption band. The new set of intensities then reveals stereospecific properties of the sample molecules that must be chiral to have a non-zero VCD spectrum. There are two primary properties of a sample of a chiral molecule; one is the AC of the dominant chiral species (dominant enantiomer), and the second property is the EE of the dominant enantiomer over its lesser (mirror-image) enantiomer. In addition, the VCD spectrum carries additional information about the solution-state conformation, or conformational distribution, of the molecule that may be available in only lesser detail, if any, using the parent IR spectrum alone. Applications that require validation for the use of VCD spectroscopy also exist for the combined use of the measurement of the VCD of a substance for which there exists a reference spectrum with known AC and EE. Any subsequent measurement of the VCD spectrum, together with the simultaneous measurement of its parent IR spectrum, confirms in one measurement the molecular identity of the sample as well as the AC and EE. Such applications are useful, for example, for chiral raw-material identification of AC and EE, for which there is currently no established methodology for a single measurement. VCD can also confirm, in a single measurement, the AC and EE of a final drug product, including the chirality (if any) of the excipients.

Before validation of VCD for AC determination, an overall instrument validation should be carried out by the measurement of the VCD spectrum of neat (-)-*S*- α -pinene or (+)-*R*- α -pinene using a 75- μ m cell. The calibrated, baseline-corrected VCD spectrum should yield a positive peak maximum of ΔA equal to approximately $+5 \times 10^{-4}$ absorbance units at 1220 cm^{-1} , and a negative peak value of approximately -4×10^{-4} at 1130 cm^{-1} . The noise level should be comparable to that established for the instrument during the OQ phase of instrumental qualification described above. Because the VCD spectrum of α -pinene is relatively large, sufficient signal-to-noise ratio is available with a 20-min collection to verify that the instrument is operating with correct VCD intensities, signs, and noise levels for the applications of VCD described below.

Validation for AC determination by VCD can be achieved by the successful comparison of the measured VCD spectrum of the validation standard, neat (-)-*S*- α -pinene, to its quantum chemistry calculated VCD spectrum. Success is achieved by measuring the baseline-corrected VCD spectrum with sufficient signal-to-noise ratio that the signs, frequencies, and relative intensities of the major VCD bands can be compared clearly to the corresponding VCD signs, frequencies, and relative intensities of the calculated VCD spectrum. If the signs, frequencies, and relative intensities agree, then the AC of the measured sample of (-)- α -pinene is the same

as that for the model of *S*- α -pinene that has been constructed for the calculation of this standard molecule. If the frequencies and relative VCD intensities are opposite after instrument sign calibration, then the AC of the sample is opposite (the mirror image) of the enantiomer used for the calculation. A successful comparison requires pretreatment of the VCD data for baseline subtraction and intensity calibration. It also requires a computational procedure that is known to be accurate in calculating VCD spectra. Finally, a program should be used to impartially assess the degree of agreement between measured and calculated VCD spectra such that one relies on more than an analyst's visual assessment of the agreement, or lack of agreement, between a measured spectrum and a calculated spectrum. An example of this procedure can be found in (1782) as well as in the references cited in (1782).

Validation of VCD spectroscopy for the determination of EE is achieved by plotting the EE predicted by VCD versus the EE for a sample of neat (–)-*S*- α -pinene for a series of samples with differing values of %EE over the range from 100% EE to 0% EE. The EE predicted by VCD involves measurement of the VCD spectra at a chosen spectral resolution and time of collection to achieve a desired level of signal-to-noise ratio, followed by pretreatment of the measured VCD spectra for baseline subtraction. The prediction of EE could be based on a size of a single VCD band for the samples with differing VCD, normalized for constant IR intensity; but more generally and with better accuracy, the entire range of VCD bands across the spectrum is used for each spectrum by means of a chemometrics software package. Validation is accompanied by a measure of the %EE accuracy through a cross-correlation analysis, yielding a root mean square (RMS). An example of such a determination of %EE by VCD for neat (–)-*S*- α -pinene is provided, along with literature references, in (1782).

More generally, validation is required when a method is intended for use as an alternative to the official procedure for testing an official article. The objective is to demonstrate that the measurement of VCD is suitable for its intended purpose as described for various types of measurements in *Validation of Compendial Procedures* (1225). For %EE determination, creation of a linear plot of actual versus VCD-predicted values of %EE establishes that the magnitude of IR-normalized VCD spectra, as evaluated with chemometrics, exhibits sufficient linearity, range, accuracy, specificity, precision, detection limit, quantitation limit, and robustness with respect to the detection of %EE.

Chapter (1225) provides definitions and general guidance on analytical procedures validation without indicating specific validation criteria for each characteristic. The intention of the following sections is to provide the user with specific validation criteria that represent the minimum expectations for VCD technology. For each particular application, tighter criteria may be needed to demonstrate suitability for the intended use.

ACCURACY

Accuracy can be determined by the measurement of a VCD spectrum of the established validation standard of neat (–)- α -pinene or (+)- α -pinene, as presented, for example, in (1782), and verifying that the measured signs and intensities of the VCD spectrum agree with the standard VCD spectrum to within the noise level of the measurement. In particular, the VCD spectrum should yield a positive peak maximum of ΔA equal to approximately $+5 \times 10^{-4}$ absorbance units at 1220 cm^{-1} and a negative peak value of approximately -4×10^{-4} at 1130 cm^{-1} . This validation ensures that the VCD spectrometer is accurately calibrated and has the correct signs for the VCD bands.

4.2 Precision

REPEATABILITY

Validation criteria: Measurement of VCD spectra of the validation standard sample, neat (+)- α -pinene or (-)- α -pinene, in 20-min blocks with variations in the VCD spectra at or less than the noise level of each 20-min block that is approximately ΔA equal to or less than 1×10^{-5} .

INTERMEDIATE PRECISION

The effect of random events on the analytical precision of the validation criteria of VCD measurement should be established. Typical variables include performing the analysis on different days, using different instrumentation, or having the method performed by two or more analysts. As a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision. (For example, this could be two analysts on each of 3 days, or two analysts on two sets of equipment on 2 days for each analyst–equipment combination.)

Validation criteria: The *Validation criteria* are the same as those for *Repeatability*, above.

SPECIFICITY

Specificity is not a required metric for the determination of AC. For the measurement of EE for more than one chiral species in a single set of measurements, the analytical procedure, including chemometric analysis, must be able to unequivocally assess the EE of chiral species in the presence of components that may be expected to be present, i.e., solvent and other nonchiral species.

Validation criteria: The *Validation criteria* are demonstrated by meeting the *Accuracy* requirement.

QUANTITATION LIMIT

For the determination of AC, there is no quantitation limit (QL). The QL for EE can be determined by carrying out a determination of quantitation of %EE for the VCD validation standard of neat α -pinene for a series of EE values, performing a chemometric analysis of VCD-predicted EE values and performing a determination of RMS error, as illustrated, for example, in (1782).

Validation criteria: The analytical procedure should be capable of determining the analyte precisely and accurately at a level equivalent to approximately 1% EE for neat α -pinene.

LINEARITY

A linear relationship between the analyte concentration and response should be demonstrated by preparing NLT 5 standard solutions at concentrations encompassing the anticipated concentration of the test solution. The standard curve should then be evaluated using appropriate statistical methods, such as a least-squares regression, for the area of peak height of a selected VCD band, or for the entire spectrum, using chemometric analysis such as partial least squares. For experiments that do not yield a linear relationship between analyte concentration and response, appropriate statistical methods must be applied to describe the analytical response.

Validation criteria: The *Validation criteria* find the correlation coefficient above 0.95 for a

linear plot of actual EE versus VCD-predicted EE.

ROBUSTNESS

The reliability of an analytical measurement should be demonstrated by deliberate changes to experimental parameters, such as changes in spectral resolution, concentration, or path length for measurement of VCD.

4.3 Verification

U.S. Current Good Manufacturing Practices regulations [21 CFR 211.194(a)(2)] indicate that users of the analytical procedures, as described in *USP-NF*, are not required to validate these procedures if provided in a monograph. Instead, they must simply verify their suitability under actual conditions of use.

The objective of procedure verification is to demonstrate that the procedure, as prescribed in a specific monograph, can be executed by the analysts with suitable accuracy, specificity, and precision using the instruments, and sample solutions of chiral molecules available. According to *Verification of Compendial Procedures* (1226), if the verification of the compendial procedure by following the monograph is not successful, the procedure may not be suitable for use with the article under test. It may be necessary to develop and validate an alternative procedure as allowed in *General Notices* 6.30.

Verification of compendial methods should, at a minimum, include the execution of the validation parameters for specificity, accuracy, precision, and limit of quantitation, when appropriate, as indicated in *Validation*. ■ 2S (*USP39*)

BRIEFING

(821) **Radioactivity**, *USP 38* page 616. This chapter was introduced in its current form in 1975 and has not undergone any major revision since its first publication. Currently, the chapter contains definitions, special considerations, and procedures with respect to the monographs for radiopharmaceuticals (radioactive drugs). The majority of this chapter is informational. USP has launched an initiative to minimize nonprocedural information in general chapters with numbers below 1000. The USP Council of Experts believes that (821) should be revised as part of this initiative. To achieve this objective, several members of the USP Council of Experts jointly published a *Stimuli* article in *PF 38(4)* [July–Aug. 2012], *Revision of General Chapter Radioactivity* (821). The objectives of this *Stimuli* article were threefold: (1) provide background information about the need for the proposed revision, (2) initiate discussion about this topic, and (3) solicit public comments for review and discussion by the relevant Expert Committee and Expert Panel members and USP staff.

The current chapter is divided into two major sections, *General Considerations* and *Identification and Assay of Radionuclides*, both of which contain several subsections with information about aspects of radioactivity. Because of the nature of the information presented in these subsections, these can be moved to the proposed *Radioactivity—Theory and Practice* (1821). The *Terms and Definitions* section is now listed as *Glossary* and has been revised to reflect current practices. The revised chapter will contain procedures and information about instrumentation used in the identification and assay of the radionuclides, along with appropriate information about calibration and maintenance of instrumentation. To make the chapter more useful, we propose to include quantitative requirements such as minimum resolution for the detector, allowed variation for instrument performance, and appropriate interval for

performance checks, as well as other useful parameters that can ensure the suitability of the equipment for its intended use. Interested parties are invited to submit their comments to the proposed revisions of the chapter.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

The following Briefing list includes monographs and/or chapters that both reference the General Chapter under revision and require revision to keep references to the General Chapter accurate. Other monographs and/or chapters may also be listed, even where the reference to the General Chapter remains unchanged, as additional notice to stakeholders where there is believed to be potential for the change in the general chapter itself to affect pass-fail determinations for particular monograph articles.

Chapter Dependencies (click to expand).

- 1059 EXCIPIENT PERFORMANCE
- Ammonia N 13 Injection
- Chromic Phosphate P 32 Suspension
- Chromium Cr 51 Edetate Injection
- Cyanocobalamin Co 57 Capsules
- Cyanocobalamin Co 57 Oral Solution
- Cyanocobalamin Co 58 Capsules
- Fludeoxyglucose F 18 Injection
- Gallium Citrate Ga 67 Injection
- Indium In 111 Capromab Pendetide Injection
- Indium In 111 Chloride Solution
- Indium In 111 Ibritumomab Tiuxetan Injection
- Indium In 111 Oxyquinoline Solution
- Indium In 111 Pentetate Injection
- Indium In 111 Pentetreotide Injection
- Indium In 111 Satumomab Pendetide Injection
- Iobenguane I 123 Injection
- Iobenguane I 131 Injection
- Iodinated I 125 Albumin Injection
- Iodinated I 131 Albumin Aggregated Injection
- Iodinated I 131 Albumin Injection
- Iodohippurate Sodium I 123 Injection
- Iothalamate Sodium I 125 Injection
- Iodohippurate Sodium I 131 Injection
- Rose Bengal Sodium I 131 Injection
- Krypton Kr 81m
- Samarium Sm 153 Lexidronam Injection
- Rubidium Chloride Rb 82 Injection
- Sodium Fluoride F 18 Injection
- Sodium Chromate Cr 51 Injection
- Sodium Iodide I 131 Solution
- Sodium Iodide I 123 Solution
- Sodium Pertechetate Tc 99m Injection
- Sodium Phosphate P 32 Solution
- Strontium Chloride Sr 89 Injection
- Technetium ^{99m}Tc Fanolesomab Injection
- Technetium Tc 99m Albumin Aggregated Injection
- Technetium Tc 99m Albumin Colloid Injection
- Technetium Tc 99m Albumin Injection
- Technetium Tc 99m Apcitide Injection
- Technetium Tc 99m Arcitumomab Injection
- Technetium Tc 99m Bicisate Injection
- Technetium Tc 99m Depreotide Injection
- Technetium Tc 99m Disofenin Injection
- Technetium Tc 99m Exametazime Injection
- Technetium Tc 99m Gluceptate Injection
- Technetium Tc 99m Lidofenin Injection
- Technetium Tc 99m Mebrofenin Injection
- Technetium Tc 99m Mertiatide Injection
- Technetium Tc 99m Nofetumomab Merpentan Injection
- Technetium Tc 99m Pentetate Injection
- Technetium Tc 99m Pyrophosphate Injection
- Technetium Tc 99m Red Blood Cells Injection
- Technetium Tc 99m Sestamibi Injection
- Technetium Tc 99m Succimer Injection
- Technetium Tc 99m Sulfur Colloid Injection
- Technetium Tc 99m Tetrafosmin Injection
- Thallous Chloride Tl 201 Injection
- Urea C 14 Capsules
- Xenon Xe 127

- Xenon Xe 133 Injection

- Yttrium Y 90 Ibritumomab Tiuxetan Injection

(CHM4: R. Ravichandran.)

Correspondence Number—C152699

Comment deadline: November 30, 2015

(821) RADIOACTIVITY

Change to read:

~~Radioactive pharmaceuticals require specialized techniques in their handling and testing in order that correct results may be obtained and hazards to personnel be minimized. All operations should be carried out or supervised by personnel having had expert training in handling radioactive materials.~~

~~The facilities for the production, use, and storage of radioactive pharmaceuticals are generally subject to licensing by the federal Nuclear Regulatory Commission, although in certain cases this authority has been delegated to state agencies. The federal Department of Transportation regulates the conditions of shipment of radioactive materials. State and local agencies often have additional special regulations. Each producer or user must be thoroughly cognizant of the applicable regulations of the federal Food, Drug, and Cosmetic Act, and any additional requirements of the U. S. Public Health Service and of state and local agencies pertaining to the articles concerned.~~

~~Definitions, special considerations, and procedures with respect to the Pharmacopeial monographs on radioactive drugs are set forth in this chapter.~~

GENERAL CONSIDERATIONS

Fundamental Decay Law

~~The decay of a radioactive source is described by the equation:~~

$$N_t = N_0 e^{-\Lambda t}$$

~~in which N_t is the number of atoms of a radioactive substance at elapsed time t , N_0 is the number of those atoms when $t = 0$, and Λ is the transformation or decay constant, which has a characteristic value for each radionuclide. The *half-life*, $T_{1/2}$, is the time interval required for a given activity of a radionuclide to decay to one-half of its initial value, and is related to the decay constant by the equation:~~

$$T_{1/2} = 0.69315/\Lambda$$

~~The activity of a radioactive source (A) is related to the number of radioactive atoms present by the equation:~~

$$A = \Lambda N$$

~~from which the number of radioactive atoms at time t can be computed, and hence the mass of the radioactive material can be determined.~~

~~The activity of a pure radioactive substance as a function of time can be obtained from the exponential equation or from decay tables, or by graphical means based on the half-life (see *Normalized Decay Chart, Figure 1*).~~

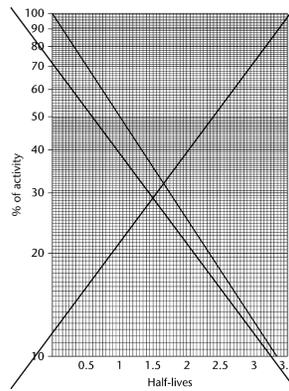


Fig. 1. Normalized Decay Chart.

The activity of a radioactive material is expressed as the number of nuclear transformations per unit time. The fundamental unit of radioactivity, the *curie* (Ci), is defined as 3.700×10^{10} nuclear transformations per second. The *millicurie* (mCi) and *microcurie* (μ Ci) are commonly used subunits. The "number of nuclear transformations per unit time" is the sum of rates of decay from all competing modes of disintegration of the parent nuclide. Before the activity of any given radionuclide in a measured specimen can be expressed in curies, it is often necessary to know the abundance(s) of the emitted radiation(s) measured.

Geometry

The validity of relative calibration and measurement of radionuclides is dependent upon the reproducibility of the relationship of the source to the detector and its surroundings. Appropriate allowance must be made for source configuration.

Background

Cosmic rays, radioactivity present in the detector and shielding materials, and radiation from nearby radioactive sources not properly shielded from the measuring equipment, all contribute to the background count rate. All radioactivity measurements must be corrected by subtracting the background count rate from the gross count rate in the test specimen.

Statistics of Counting

Since the process of radioactive decay is a random phenomenon, the events being counted form a random sequence in time. Therefore, counting for any finite time can yield only an estimate of the true counting rate. The precision of this estimate, being subject to statistical fluctuations, is dependent upon the number of counts accumulated in a given measurement and can be expressed in terms of the standard deviation Σ . An *estimate* for Σ is

$$\sqrt{n}$$

where n is the number of counts accumulated in a given measurement. The probability of a single measurement falling within

$$\pm 100/\sqrt{n}\%$$

of the mean of a great many measurements is 0.68. That is, if many measurements of n counts each were to be made, approximately two thirds of the observations would lie within

$$\pm 100/\sqrt{n}\%$$

of the mean, and the remainder outside.

Because of the statistical nature of radioactive decay, repeated counting of an undisturbed source in a counting assembly will yield count rate values in accordance with the frequency of a normal distribution. Deviations in these values from the normal distribution conform to the χ^2 test. For this reason, the χ^2 test is frequently applied to determine the performance and correct operation of a counting assembly. In the selection of instruments and conditions for assay of radioactive sources, the figure of merit ϵ^2/B should be maximized (where ϵ = counter efficiency = observed count rate/sample disintegration rate, and B = background count rate).

Counting Losses

The minimum time interval that is required for the counter to resolve two consecutive signal pulses is known as the dead time. The dead time varies typically from the order of microseconds for proportional and scintillation counters, to hundreds of microseconds for Geiger-Müller counters. Nuclear events occurring within the dead time of the counter will not be registered. To obtain the corrected count rate, R , from the observed count rate, r , it is necessary to use the formula:

$$R = r / (1 - r\tau)$$

in which τ is the dead time. The foregoing correction formula assumes a nonextendable dead time. Thus, for general validity, the value of $r\tau$ should not exceed 0.1. The observed count rate, r , refers to the gross specimen count rate and is not to be corrected for background before use in the foregoing equation.

Calibration Standards

Perform all radioactivity assays using measurement systems calibrated with appropriately certified radioactivity standards. Such calibration standards may be purchased either direct from the National Institute of Standards and Technology or from other sources that have established traceability to the National Institute of Standards and Technology through participation in a program of inter-comparative measurements. Where such calibration standards are unavailable, the Pharmacopeia provides the nuclear decay data required for calibration. These data, as well as half-life values, are obtained from the Evaluated Nuclear Structure Data File of the Oak Ridge Nuclear Data Project, and reflect the most recent values at the time of publication.

Carrier

The total mass of radioactive atoms or molecules in any given radioactive source is directly proportional to the activity of the radionuclide for a given half-life, and the amount present in radiopharmaceuticals is usually too small to be measured by ordinary chemical or physical methods. For example, the mass of ^{131}I having an activity of 100 mCi is 8×10^{-7} g. Since such small amounts of material behave chemically in an anomalous manner, carriers in the form of nonradioactive isotopes of the same radionuclide may be added during processing to permit ready handling. In many cases, adsorption can be prevented merely by increasing the hydrogen ion concentration of the solution. Amounts of such material, however, must be sufficiently small that undesirable physiological effects are not produced. The term "carrier-free" refers only to radioactive preparations in which nonradioactive isotopes of the radionuclide are absent. This implies that radioactive pharmaceuticals produced by means of (n, f) reactions cannot be considered carrier-free.

The activity per unit volume or weight of a medium or vehicle containing a radionuclide either in the carrier-free state or in the presence of carrier is referred to as the radioactive concentration, whereas the term specific activity is used to express the activity of a radionuclide per gram of its element.

Radiochemical Purity

Radiochemical purity of a radiopharmaceutical preparation refers to the fraction of the stated radionuclide present in the stated chemical form. Radiochemical impurities in radiopharmaceuticals may result from decomposition and from improper preparative procedures. Radiation causes decomposition of water, a main ingredient of most radiopharmaceuticals, leading to the production of reactive hydrogen atoms and hydroxyl radicals, hydrated electrons, hydrogen, hydrogen ions, and hydrogen peroxide. The last mentioned is formed in the presence of oxygen radicals, originating from the radiolytic decomposition of dissolved oxygen. Many radiopharmaceuticals show improved stability if oxygen is excluded. Radiation may also affect the radiopharmaceutical itself, giving rise to ions, radicals, and excited states. These species may combine with one another and/or with the active species formed from water. Radiation decomposition may be minimized by the use of chemical agents that act as electron or radical scavengers. Electrons trapped in solids cause discoloration due to formation of F-centers and the darkening of glass containers for radiopharmaceuticals, a situation that typifies the case. The radiochemical purity of radiopharmaceuticals is determined by column, paper, and thin-layer chromatography or other suitable analytical separation techniques as specified in the individual monograph.

Radionuclidic Purity

Radionuclidic purity of a radiopharmaceutical preparation refers to the proportion of radioactivity due to the desired radionuclide in the total radioactivity measured. Radionuclidic purity is important in the estimation of the radiation dose received by the patient when the preparation is administered. Radionuclidic impurities may arise from impurities in the target materials, differences in the values of various competing production cross-sections, and excitation functions at the energy or energies of the bombarding particles during production.

Terms and Definitions

The *date of manufacture* is the date on which the manufacturing cycle for the finished product is completed.

The *date of assay* is the date (and time, if appropriate) when the actual assay for radioactivity is performed.

The *date of calibration* is an arbitrary assigned date and time to which the radioactivity of the product is calculated for the convenience of the user.

The *expiration date* is the date that establishes a limit for the use of the product. The expiration period (i.e., the period of time between the date of manufacture and the expiration date) is based on a knowledge of the radioactive properties of the product and the results of stability studies on the finished dosage form.

Labeling

Individual radiopharmaceutical monographs indicate the expiration date, the calibration date, and the statement, "Caution—Radioactive Material." The labeling indicates that in making

dosage calculations, correction is to be made for radioactive decay, and also indicates the radioactive half-life of the radionuclide. Articles that are Injections comply with the requirements for *Labeling under Injections* (1), and those that are Biologics comply with the requirements for *Labeling under Biologics* (1041).

IDENTIFICATION AND ASSAY OF RADIONUCLIDES

Instrumentation

IONIZATION CHAMBERS

An ionization chamber is an instrument in which an electric field is applied across a volume of gas for the purpose of collecting ions produced by a radiation field. The positive ions and negative electrons drift along the lines of force of the electric field, and are collected on electrodes, producing an ionization current. In a properly designed well-type ionization chamber, the ionization current should not be too dependent on the position of the radioactive specimen, and the value of the current per unit activity, known as the calibration factor, is characteristic of each gamma-ray emitting radionuclide.

The ionization current produced in an ionization chamber is related to the mean energy of the emitted radiation and is proportional to the intensity of the radiation. If standard sources of known disintegration rates are used for efficiency calibration, the ionization chamber may then be used for activity determinations between several microcuries and several hundred millicuries or more. The upper limit of activity that may be measured in an ionization chamber usually is not sharply defined and may be limited by saturation considerations, range of the amplifier, and design of the chamber itself. The data supplied with or obtained from a particular instrument should be reviewed to ascertain the useful ranges of energies and intensities of the device.

Reproducibility within approximately 5% or less can be readily obtained in about 10 seconds, with a deep re-entrant well-type chamber. The most commonly used form of ionization chamber for measurement of the activities of radiopharmaceuticals is known as a dose calibrator.

Although the calibration factor for a radionuclide may be interpolated from an ionization chamber energy-response curve, there are a number of sources of error possible in such a procedure. It is therefore recommended that all ionization chamber calibrations be performed with the use of authentic reference sources of the individual radionuclides, as described hereinafter.

The calibration of a dose calibrator should be maintained by relating the measured response of a standard to that of a long-lived performance standard, such as radium-226 in equilibrium with its daughters. The instrument must be checked daily with the ^{226}Ra or other source to ascertain the stability over a long period of time. This check should include performance standard readings at all radionuclide settings employed. To obtain the activity (A_x) of the radionuclide being measured, use the relationship:

$$A_x = R_x R / R_n$$

in which R_n is the new reading for the radium or other source, R_c is the reading for the same source obtained during the initial calibration procedure, and R is the observed reading for the radionuclide specimen. Obviously, any necessary corrections for radioactive decay of the reference source must first be applied. Use of this procedure should minimize any effects due to

drift in the response of the instrument. The recommended activity of the ^{226}Ra or other monitor used in the procedure described above is 75 to 150 μCi . It is recommended also that the reproducibility and/or stability of multirange instruments be checked for all ranges with the use of appropriate standards.

The size and shape of a radioactive source may affect the response of a dose calibrator, and it is often necessary to apply a small correction when measuring a bulky specimen.

SCINTILLATION AND SEMICONDUCTOR DETECTORS

When all or part of the energy of beta or gamma radiation is dissipated within scintillators, photons of intensity proportional to the amount of dissipated energy are produced. These pulses are detected by an electron multiplier phototube and converted to electrical pulses, which are subsequently analyzed with a pulse height analyzer to yield a pulse height spectrum related to the energy spectrum of the radiation emitted by the source. In general, a beta-particle scintillation pulse height spectrum approximates the true beta energy spectrum, provided that the beta-particle source is prepared in such a manner that self-absorption is minimized. Beta ray spectra may be obtained by using calcium fluoride or anthracene as the scintillator, whereas gamma ray spectra are usually obtained with a thallium-activated sodium iodide crystal or a large volume lithium-drifted germanium semiconductor detector. The spectra of charged particles also may be obtained using silicon semiconductor detectors and/or gas proportional counters. Semiconductor detectors are in essence solid-state ionization chambers, but the energy required to create an electron-hole pair or to promote an electron from the valence band to the conduction band in the semiconductor is about one-tenth the energy required for creation of an ion pair in a gas-filled ionization chamber or proportional counter and is far less than the energy needed to produce a photon in a NaI(Tl) scintillation crystal. In gamma ray spectrometry, a Ge(Li) detector can yield an energy resolution of 0.33% for 1.33 MeV gamma rays from ^{60}Co , while a 3 × 3 inch NaI(Tl) crystal can give a value of 5.9% for the same gamma ray energy. The energy resolution is a measure of the ability to distinguish the presence of two gamma rays closely spaced in energy and is defined by convention as the full width of the photopeak at its half maximum (FWHM), expressed in percentage of the photopeak energy.

Gamma ray spectra exhibit one or more sharp, characteristic photopeaks, or full-energy peaks, as a result of total absorption in the detector of the full energy of gamma radiations from the source; these photopeaks are useful for identification purposes. Other secondary peaks are observed as a consequence of backscatter, annihilation radiation, coincidence summing, fluorescent X-rays, etc., accompanied by a broad band known as the Compton continuum arising from scattering of the photons in the detector and from surrounding materials. Since the photopeak response varies with gamma ray energy, calibration of a gamma ray spectrometer should be achieved with radionuclide standards having well-known gamma ray energies and emission rates. The shape of the gamma ray spectrum is dependent upon the shape and size of the detector and the types of shielding materials used.

When confirming the identity of a radionuclide by gamma ray spectrometry, it is necessary to make a comparison of the specimen spectrum with that of a specimen of known purity of the same radionuclide obtained under *identical instrument parameters and specimen geometry*. Where the radionuclides emit coincident X- or gamma radiations, the character of the pulse-height distribution often changes quite dramatically because of the summing effect of these coincident radiations in the detector as the efficiency of detection is increased (e.g., by

bringing the source closer to the detector). Such an effect is particularly evident in the case of iodine-125. Among the more useful applications of gamma-ray spectrometry are those for the identification of radionuclides and the determination of radionuclidic impurities.

Where confirmation of the identity of a given radionuclide by means of a direct comparison with the spectrum of a specimen of the same radionuclide of known purity is not possible, the identity of the radionuclide in question must then be established by the following method. Two or more of the following nuclear decay scheme parameters of the radionuclide specimen to be identified shall be measured, and agreement shall be within $\pm 10\%$: (1) half-life, (2) energy of each gamma- or X-ray emitted, (3) the abundance of each emission, and (4) E_{\max} for those radionuclides that decay with beta-particle emissions. Such measurements are to be performed as directed in the *Identification* and *Assay* sections of this chapter. Agreement of two or more of the measured parameters with the corresponding published nuclear decay scheme data constitutes confirmation of the identity of the radionuclide.

LIQUID-SCINTILLATION COUNTERS

Alpha- and beta-emitting radionuclides may be assayed with the use of a liquid-scintillation detector system. In the liquid scintillator, the radiation energy is ultimately converted into light quanta that are usually detected by two multiplier phototubes so arranged as to count only coincidence radiation. The liquid scintillator is a solution consisting of a solvent, primary and secondary solutes, and additives. The charged particle dissipates its energy in the solvent, and a fraction of this energy is converted into fluorescence in the primary solute. The function of the secondary solute is to shift the fluorescence radiation to longer wavelengths that are more efficiently detected by the multiplier phototubes. Frequently used solvents are toluene and *p*-xylene; primary solutes are 2,5-diphenyloxazole (PPO) and 2-(4'-*tert*-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole (butyl-PBD); and secondary solutes are 2,2'-*p*-phenylenebis[4-methyl-5-phenyloxazole] (dimethyl-POPOP) and *p*-bis(*o*-methylstyryl)benzene (bis-MSB). As a means of attaining compatibility and miscibility with aqueous specimens to be assayed, many additives, such as surfactants and solubilizing agents, are also incorporated into the scintillator. For an accurate determination of radioactivity of the specimen, care must be exercised to prepare a specimen that is truly homogeneous. The presence of impurities or color in solution causes a decrease in photon output of the scintillator; such a decrease is known as quenching. Accurate radioactivity measurement requires correcting for count-rate loss due to quenching.

The disintegration rate of a beta-particle source may be determined by a procedure in which the integral count rate of the specimen is measured as a function of the pulse-height discriminator bias, and the emission rate is then obtained by extrapolation to zero bias. Energetic alpha-particle emitters may be similarly measured by this method.

Identification

A radionuclide can be identified by its mode of decay, its half-life, and the energies of its nuclear emissions.

The radioactive half-life is readily determined by successive counting of a given source of the radionuclide over a period of time that is long compared to its half-life. The response of the counting assembly when employed for the decay measurement of long-lived radionuclides should be monitored with an even longer-lived reference source to assess and compensate for errors arising from electronic drift. In the case of short-lived radionuclides, when the counting period constitutes a significant fraction of the half-life of the radionuclide, the recorded count

rate must be corrected to the time when the count is initiated, as follows:

$$R_t = rAt/(1 - e^{-\Lambda t})$$

in which R_t is the count rate at the beginning of a counting period, r is the count rate observed over the entire counting period, t is the duration of the counting period, Λ is the decay constant of the radionuclide, and e is the base of the natural logarithm. When t is small compared to the half-life of the radionuclide under study so that $\Lambda t < 0.05$, then $(1 - e^{-\Lambda t})$ approaches Λt , and no such correction is necessary.

The energy of nuclear emissions is often determined by the maximum range of penetration of the radiation in matter (in the case of alpha and beta particles) and by the full-energy peak or photopeak in the gamma-ray spectrum (in the case of X and gamma rays). Since beta particles are emitted with a continuous energy spectrum, the maximum beta energy, E_{\max} , is a unique index for each beta-emitting radionuclide. In addition to the maximum range and energy spectrum of the beta particles, the absorption coefficient, when obtained under reproducible counting conditions, can serve as a reliable index for identification of a beta emitter. Fortunately, beta particles are absorbed in matter in an approximately exponential manner, and a plot of the logarithm of the beta-particle count rate as a function of the absorber thickness is known as the absorption curve. The initial portion of the absorption curve shows linearity from which the absorption coefficient can be obtained. The maximum range is determined by the use of absorbers of varying thickness, and the energy spectrum is measured by beta-ray scintillation spectrometry.

The absorption of gamma rays in matter is strictly exponential, but the half-value layers of attenuation have not been very useful for the purpose of radionuclide characterization. Gamma rays from each isomeric transition are mono-energetic; their energy can be directly measured by gamma-ray spectrometry. Because of their high energy resolution, solid-state detectors [Ge(Li)] are vastly superior to scintillation detectors [NaI(Tl)] in gamma-ray spectrometry.

The activities of radiopharmaceutical solutions are frequently in the range of millicuries per mL. Such solutions usually must be extensively diluted before they can be accurately assayed. The diluent should be compatible with the radiopharmaceutical with respect to factors such as pH and redox potentials, so that no hydrolysis or change in oxidation state occurs upon dilution, which could lead to adsorption and separation of the radionuclide from solution.

BETA-EMITTING RADIONUCLIDES

Mass Absorption Coefficient Procedure—Deposit and dry an aliquot of the radioactive phosphorus-32 solution on a thin plastic film to minimize backscattering, and place it under a suitable counter. Determine the counting rates successively, using not less than six different "thicknesses" of aluminum each between 20 and 50 mg/cm² and a single absorber thicker than 800 mg/cm², which is used to measure the background. (The absorbers are inserted between the test specimen and the counter but are placed nearer the counter window to minimize scattering.) Net beta-particle count rates are obtained after subtraction of the count rate found with the absorber having a thickness of 800 mg/cm² or greater. Plot the logarithm of the net beta-particle count rate as a function of the total absorber "thickness." The total absorber "thickness" is the "thickness" of the aluminum absorbers plus the "thickness" of the counter window (as stated by the manufacturer) plus the air-equivalent "thickness" (the distance in

centimeters of the specimen from the counter window multiplied by 1.205 mg/cm³ at 20° and 76 cm of mercury), all expressed in mg/cm². An approximately straight line results.

Choose two total absorber "thicknesses" that differ by 20 mg/cm² or more and that fall on the linear plot, and calculate the mass absorption coefficient, μ , by the equation:

$$\mu = 1/(t_2 - t_1) \cdot \ln(N_{t_1}/N_{t_2}) = (2.303/(t_2 - t_1)) \times (\log N_{t_1} - \log N_{t_2})$$

in which t_1 and t_2 represent the total absorber "thicknesses," in mg/cm², t_2 being the thicker absorber, and N_{t_1} and N_{t_2} being the net beta particle rates with the t_1 and t_2 absorbers, respectively.

For characterization of the radionuclide, the mass absorption coefficient should be within $\pm 5\%$ of the value found for a pure specimen of the same radionuclide when determined under identical counting conditions and geometry.

Other Methods of Identification—Other methods for determining the identity of a beta emitter also rely upon the determination of E_{\max} . This may be accomplished in several ways. For example, (1) utilization of the range energy relationships of beta particles in an absorber, or (2) determination of E_{\max} from a beta particle spectrum obtained on an energy calibrated beta spectrometer using a thin source of the radionuclide (see *Scintillation and Semiconductor Detectors* in this chapter).

GAMMA-EMITTING RADIONUCLIDES

The gamma ray spectrum of a radionuclide is a valuable tool for the qualitative identification of gamma ray emitting radionuclides. The full energy peak, or the photopeak, is identified with the gamma ray transition energy that is given in the decay scheme of the radionuclide.

In determining radionuclidic identity and purity, the gamma ray spectrum of a radioactive substance is obtained with either a NaI(Tl) crystal or a semiconductor Ge(Li) detector. The latter has an energy resolution more than an order of magnitude better than the former and is highly preferred for analytical purposes. The spectrum obtained shall be identical in shape to that of a specimen of the pure radionuclide, measured with the same detection system and in the same geometry. The gamma ray spectrum of the radiopharmaceutical shall contain only photopeaks identifiable with the gamma ray transition energies found in the decay scheme of the same radionuclide. For low geometrical efficiencies, the areas under the photopeaks, after correction for the measured detector efficiency, shall be proportional to the abundances or emission rates of the respective gamma rays in the radionuclide.

RADIONUCLIDIC IMPURITIES

Because they are extremely toxic, alpha emitting nuclides must be strictly limited in radiopharmaceutical preparations. Procedures for identifying beta and gamma active radionuclides as given in the foregoing text are applicable to the detection of gamma and usually beta contaminants.

The gross alpha particle activity in radiopharmaceutical preparations can be measured by the use of a windowless proportional counter or a scintillation detector employing a silver activated zinc sulfide phosphor or by the techniques of liquid scintillation counting.

The heavy ionization caused by alpha particles allows the measurement of alpha emitting radionuclides in the presence of large quantities of beta and gamma active nuclides by the

use of appropriate techniques for discriminating the amplitudes of signal pulses. In proportional counting, the operating voltage region for counting alpha particles, referred to as the "alpha plateau," is considerably lower than the "beta plateau" for counting beta and gamma radiations. Typical "alpha plateau" and "beta plateau" voltage settings with P-10 counting gas are 900 to 1300 and 1600 to 2000 volts, respectively.

When silver-activated zinc sulfide phosphor is employed for alpha-particle detection, the alpha particles can be distinguished from other interfering radiation by pulse-height discrimination. Care must be exercised to minimize self-absorption at the source whenever specimens are prepared for alpha-particle counting.

Assay

BETA-EMITTING RADIONUCLIDES

Procedure—The disintegration rate (A) of a beta-particle-emitting specimen is obtained by counting a quantitatively deposited aliquot in a fixed geometry according to the formula:

$$A = R/(\epsilon \times f_r \times f_b \times f_s)$$

in which ϵ is the counting efficiency of the counter; f_r is the correction factor for counter dead time; f_b is the correction factor for backscatter; and f_s is the correction factor for self-absorption. The count rate for zero absorber is obtained by extrapolation of the initial linear portion of the absorption curve to zero absorber "thickness," taking into consideration the mg/cm^2 "thickness" of specimen coverings, counter window, and the intervening air space between specimen and the counter window. The counter efficiency, ϵ , is determined by use of a long-lived secondary standard with similar spectral characteristics. $\text{RaD} + \text{E}$ has frequently been used for efficiency calibration of counters for phosphorus-32. By the use of identical measurement conditions for the specimen and the standard (and extrapolation to zero absorber), the ratio of the values of f_r , f_b , and f_s for the standard and the specimen approaches unity.

The previous relationship is valid also when the counter has been calibrated with a standard of the radionuclide to be assayed. In this case, however, the extrapolations to zero absorber "thickness" for the specimen and standard are not required, as the two absorption corrections cancel for a given geometry.

Another useful and frequently employed method for the determination of the disintegration rate of beta-emitting radionuclides is liquid-scintillation counting, which also utilizes an extrapolation of the specimen count rate to zero pulse-height discriminator bias.

GAMMA-EMITTING RADIONUCLIDES

For the assay of gamma-emitting radionuclides, three methods are provided. The selection of the preferred method is dictated by the availability of a calibration standard of the radionuclide to be assayed and the radionuclidic purity of the article itself.

Direct comparison with a calibration standard is required if a calibration standard of the radionuclide to be assayed is available and if the upper limit of conceivable error in the activity determination arising from the presence of radionuclidic impurities has been determined to be less than 3%. If the required calibration standard is not routinely available, as would probably be the case for a short-lived radionuclide, but was available at some time prior to the performance of the assay for determination of efficiency of the counting system for the

radionuclide to be assayed, use a calibrated counting system, provided the radionuclidic impurity content of the specimen meets the requirements stated for the direct comparison method. If the requirements for either of the first two methods cannot be met, use the method for determination of activity from a calibration curve.

With the exception of the first method, the counting systems used are monitored for stability. This requirement is met by daily checks with a long-lived performance check source and weekly checks with at least three sources covering a broad range of gamma-ray emission energies (e.g., ^{57}Co , ^{137}Cs , and ^{60}Co). If a discrepancy for any of the aforementioned measurements is found, either completely recalibrate or repair and recalibrate the system prior to further use.

Assay by Direct Comparison with a Calibration Standard—An energy selective measurement system (e.g., pulse height analyzer) is not required for this procedure. Use either an ionization chamber or an integral counting system with a NaI(Tl) detector. A consistently reproducible geometrical factor from specimen to specimen is essential for accurate results. With proper precautions, the accuracy of this method approaches the accuracy with which the disintegration rate of the calibration standard is known.

Determine the counting rate of the detector system for a calibration standard of the radionuclide to be assayed (e.g., active enough to give good measurement statistics in a reasonable time, but not so active as to cause serious dead-time problems), selecting such a standard as to provide optimum accuracy with the particular assembly used. Place an accurately measured aliquot of the unknown assay specimen (diluted, if necessary) in a container identical to that used for the standard, and measure this specimen at approximately the same time and under the same geometrical conditions as for the standard. If the elapsed time between the measurements of the calibration standard and the specimen exceeds 12 hours, check the stability of the measurement system within 8 hours of the specimen measurement time with a long-lived performance check source. Record the system response with respect to the same check source at the time of calibration, and if subsequent checks exceed the original recorded response by more than $\pm 3\%$, recalibration is required. Correct both activity determinations for background, and calculate the activity, in $\mu\text{Ci per mL}$, by the formula:

$$SD(g/b)$$

in which S is the μCi strength of the standard, D is the dilution factor, and g and b are the measured values of counting rate for the specimen and the standard, respectively.

Assay with a Calibrated Integral Counting System—The procedure and precautions given for the preceding direct comparison method apply, except that the efficiency of the detector system is determined and recorded for each radionuclide to be assayed, rather than simply recording the counting rate of the standard. Thus, the efficiency for a given radionuclide, x , is determined by $\epsilon_x = b_x/s_x$, in which b_x is the counting rate, corrected for background and dead-time, for the calibration standard of the radionuclide, x , and s_x is the corresponding activity of the certified calibration standard in nuclear transformations per second. For subsequent specimen assays, the activity is given by the formula:

$$A_x = Dg_x/\epsilon_x$$

in which D is the dilution factor, g_x is the specimen counting rate (corrected for background and dead-time), and ϵ_x is the corresponding efficiency for the radionuclide.

Determination of Activity from a Calibration Curve—Versatility in absolute gamma-ray intensity measurements can be achieved by employing multi-channel pulse height analysis. The photopeak efficiency of a detector system can be determined as a function of gamma-ray energy by means of a series of gamma-ray emission rate standard specimens, and the gamma-ray emission rate of any radionuclide for which no standard is available can be determined by interpolation from this efficiency curve. However, exercise care to ensure that the efficiency curve for the detector system is adequately defined over the entire region of interest by using a sufficient number of calibration points along the photopeak-energy axis.

Selection of a Counting Assembly—A gamma-ray spectrometer is used for the identification of radionuclides that emit X-rays or gamma rays in their decay. Requirements for an assembly suitable for identification and assay of the radionuclides used in radiopharmaceuticals are that (a) the resolution of the detector based on the 662-keV photopeak of ^{137}Cs - $^{137\text{m}}\text{Ba}$ must be 8.0% or better, (b) the detector must be equipped with a specimen holder designed to facilitate exact duplication of counting geometry, and (c) the pulse height analyzer must have enough channels to delineate clearly the photopeak being observed.

Procedure—Minimal requirements for the maintenance of instrument calibrations shall consist of weekly performance checks with a suitable reference source and a complete recalibration semi-annually. Should the weekly performance check deviate from the value determined at the time of calibration by more than 4.0%, a complete recalibration of the instrument is required at that time.

This method involves three basic steps, namely photopeak integration, determination of the photopeak efficiency curve, and calculation of the activity of the specimen.

photopeak integration—The method for the determination of the required photopeak area utilizes a Gaussian approximation for fitting the photopeak. A fixed fraction of the total number of photopeak counts can be obtained by taking the peak width, a , at some fraction of the maximum, where the shape has been experimentally found to be very close to Gaussian, and multiplying by the counting rate of the peak channel, P , after correction for any Compton and background contributions to the peak channel count rate. This background usually can be adequately determined by linear interpolation. This is illustrated in *Figure 2*.

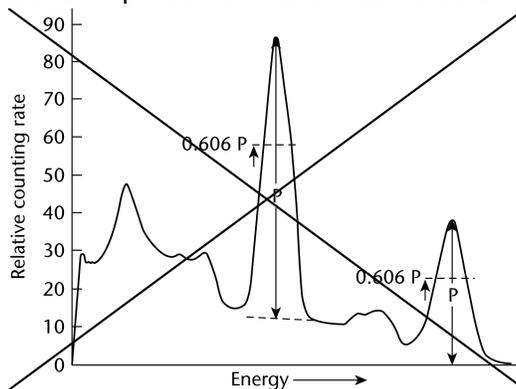


Fig. 2. Typical Gamma-ray Spectrum Showing the Selection of the Peak Channel Counting Rate, P , after the Correction for Compton and Background Contributions.

The photopeak curve shape is closest to a straight line at $0.606P$, and the contribution of the fractional channels to a can be accurately estimated by interpolation. Calculate a by the equation:

$$a = D - D' + \left[\frac{(d - 0.606P)}{(d - c)} \right] + \left[\frac{(d' - 0.606P)}{(d' - c')} \right]$$

in which c and d and also c' and d' are the single channel counting rates on either side of $0.606P$, and D and D' are the channel numbers (locations) of d and d' , respectively. The location of the required variables on the photopeak is illustrated in Figure 3.

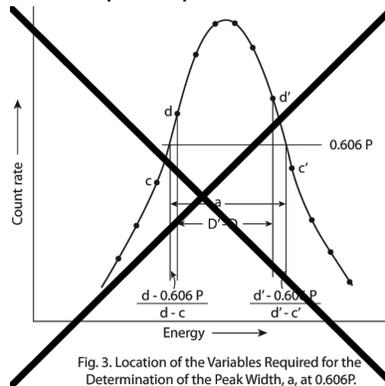


Fig. 3. Location of the Variables Required for the Determination of the Peak Width, a , at $0.606P$.

From the known values for the counting rate in the peak channel of the photopeak, P , and the width of the peak at $0.606P$, a , a calibrated fraction of the photopeak area is then obtained from the product, (aP) .

To summarize the procedures involved in obtaining a calibrated fraction of a photopeak area using this method, the necessary steps or calculations are presented below in a stepwise manner:

- (1) Subtract any Compton and background contributions from the photopeak to be measured.
- (2) Determine the counting rate of the peak channel (maximum channel counting rate after subtracting Compton and background), P .
- (3) Multiply P by 0.606 , and locate the horizontal line corresponding to the peak width, a .
- (4) Obtain the peak width, a , by inserting the values of variables (obtained as shown in the preceding figure) into the equation defining a .
- (5) The desired calibrated fraction of the peak area is then equal to the product of a times P or $F = aP$, where F is a fractional area of the peak proportional to the emission rate of the source.

This method provides a quick and accurate means of determining the gamma-ray emission rate of sources while avoiding, to a large extent, subjective estimates of the detailed shape of the tails of the peaks. The error due to using the maximum channel counting rate, rather than the theoretical maximum or peak channel rate, is of the order of 1.0% if a is 6 or greater.

photopeak efficiency calibration—Radionuclides such as those listed in the accompanying table together with some of their nuclear decay data are available as certified reference standards.* A sufficient number of radioactive standard reference sources should be selected in order to obtain the calibration curve over the desired range. Where possible, standard sources of those radionuclides that are to be assayed should be included.

Nuclear Properties of Selected Calibration Standards^(1,2)

Principal Photon Emissions	Energy (ke V)	Photons per 100 Disintegrations
^{133}Ba ($T_{1/2} = 10.5$ years)	-	-
$K_{\alpha 1}$	30.97	63.4
$K_{\alpha 2}$	30.62	34.2
K_{β}	35.0	22.8
F_{\pm}	53.15	2.14
F_2	79.62	2.55
F_3	80.99	33.0
F_6	276.39	6.9
F_7	302.83	17.8
F_8	356.0	60.0
F_9	383.85	8.7
^{137}Cs - ^{137m}Ba ($T_{1/2} = 30.17$ years)	-	-
$K_{\alpha 1}$	32.19	3.82
$K_{\alpha 2}$	31.82	2.07
K_{β}	36.4	1.39
Weighted Mean ⁽⁴⁾	(32.9)	(7.28)
F_{\pm}	661.6	89.98
^{22}Na ($T_{1/2} = 2.60$ years)	-	-
h ν	511	179.80 ⁽⁵⁾
F_{\pm}	1274.54	99.94
^{60}Co ($T_{1/2} = 5.27$ years)	-	-
F_{\pm}	1173.2 ⁽⁶⁾	100.0
F_2	1332.5 ⁽⁶⁾	100.0

(1) In measurements for gamma- (or X-) ray assay purposes, fluorescent radiation from lead shielding (specifically, lead K X rays - 76 ke V) may interfere with quantitative results. Allowance must be made for these effects, or the radiation suppressed; a satisfactory means of absorbing this radiation is covering the exposed lead with cadmium sheet 0.06 to 0.08 inch thick, and then covering the cadmium with copper 0.02 to 0.04 inch thick.

(2) Only those photon emissions having an abundance $\geq 1\%$ are normally included.

(3) The K notation refers to X-ray emissions.

(4) The weighted mean energies and total intensities are given for groups of photons that would not be resolved by a NaI(Tl) detector.

(5) For this photon intensity to be usable, all emitted positrons must be annihilated in the source material.

(6) Cascade.

$^{57}\text{Co}(T_{1/2} = 270.9 \text{ days})$	-	-
ΣX_k	7.0	56.0
F_{\pm}	14.4	9.5
F_2	122.06	85.51
F_3	136.47	10.60
Weighted Mean	(125.0)	(96.11)
$(F_2 + F_3)^{(4)}$	-	-
$^{54}\text{Mn}(T_{1/2} = 312.7 \text{ days})$	-	-
ΣX_k	6.0	25.0
F_{\pm}	834.83	99.98
$^{109}\text{Cd}-^{109}\text{Ag}(T_{1/2} = 464 \text{ days})$	-	-
$K_{\alpha 1}$	22.16	35.3
$K_{\alpha 2}$	21.99	18.6
K_{β}	24.9	11.4
Weighted Mean ⁽⁴⁾	-	63.5
F_{\pm}	88.0	3.72
$^{129}\text{I}(T_{1/2} = 1.57 \times 10^7 \text{ years})$	-	-
$K_{\alpha 1}^{(3)}$	29.78	37.0
$K_{\alpha 2}$	29.46	20.0
K_{β}	13.2	37.0
F_{\pm}	39.58	7.52
Weighted Mean ⁽⁴⁾	(31.3)	(77.80)

(1) In measurements for gamma- (or X-) ray assay purposes, fluorescent radiation from lead shielding (specifically, lead K X-rays ~76 keV) may interfere with quantitative results. Allowance must be made for these effects, or the radiation suppressed; a satisfactory means of absorbing this radiation is covering the exposed lead with cadmium sheet 0.06 to 0.08 inch thick, and then covering the cadmium with copper 0.02 to 0.04 inch thick.

(2) Only those photon emissions having an abundance $\geq 1\%$ are normally included.

(3) The K notation refers to X-ray emissions.

(4) The weighted mean energies and total intensities are given for groups of photons that would not be resolved by a NaI(Tl) detector.

(5) For this photon intensity to be usable, all emitted positrons must be annihilated in the source material.

(6) Cascade.

Calculate the gamma-ray emission rate from the equation:

$$F = A_s b$$

in which A_s is the activity, in disintegrations per second, of the standard used, and b is the number of gamma rays per disintegration at that energy. Accurately measure quantities of standard solutions of each radionuclide into identical containers, and determine the fractional photopeak area (F) for each of the standards.

Using the equation $\epsilon_p = F/\Gamma$, calculate the photopeak efficiency, ϵ_p , and construct a log-log plot of ϵ_p versus the gamma ray energy as shown in Figure 4.

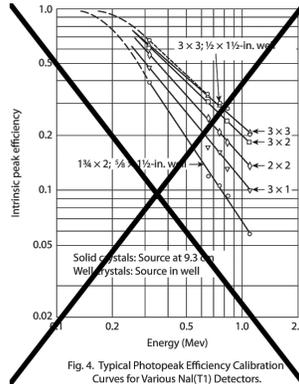


Fig. 4. Typical Photopeak Efficiency Calibration Curves for Various NaI(Tl) Detectors.

determination of specimen activity—In the same manner as in the preparation of the calibration curve, determine the fractional area (F) of the principal photopeak of the specimen under assay or an accurately measured aliquot adjusted to the same volume in an identical container as used for the standards. From the calibration curve, find the value of ϵ_p for this radionuclide.

Using the equation $\Gamma = F/\epsilon_p$, calculate the gamma ray emission rate (Γ). Calculate the activity (A), in disintegrations per second, of the specimen using the equation $A = (\Gamma/b)(D)$, in which b is the number of gamma rays per disintegration and D is the dilution factor. To obtain the activity, in μCi or mCi , divide A by 3.7×10^4 or 3.7×10^7 , respectively. The above relationship is equally valid for obtaining the activity of an undiluted specimen or capsule; in this case, the dilution factor, D , is unity.



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1. INTRODUCTION

Radiopharmaceuticals and radioactive devices require specialized techniques in their production, testing, handling, dispensing, and administration in order to ensure optimal effectiveness and maintain safety for workers, patients, and the public. Hence, all operations involving these articles should be carried out by or under the supervision of personnel who have been appropriately trained in the handling of radioactive materials and appropriate instruments.

The facilities for the production, storage, and use of radioactive drugs and radioactive devices are generally subject to licensing by the U.S. Nuclear Regulatory Commission or Agreement State agency, or similar governmental agency outside the United States. Most radioactive drugs and radioactive devices are subject to other regulations relating to transportation, environmental release, and workplace safety.

USP monographs for radioactive articles include specifications for radionuclide identification and assay for radioactivity. Moreover, radioactivity counting assemblies or radiation detectors are required instruments for the determination of radionuclidic purity and radiochemical purity. The purpose of this general chapter is to provide standards for measurement of radioactivity including instrument qualification, performance checks, identification of radionuclides and radionuclidic impurities; and assay of radionuclides.

Information regarding radioactivity is described in *Radioactivity—Theory and Practice* (1821), which includes definitions, types of decay, general considerations relating to radioactive decay, counting, radionuclide production, purity, labeling, and instrumentation for detection and measurement of radioactive emissions; and a glossary.

2. PERSONNEL TRAINING AND DOCUMENTATION

Qualification of instruments and personnel training should be completed in accordance with written policies and/or procedures. Documentation of the qualification of instruments and personnel training should be included in the Quality Assurance Program. The length of time to retain the documentation varies depending on the appropriate regulatory requirements.

3. QUALIFICATION OF INSTRUMENTS

3.1 Installation Qualification

Installation Qualification (IQ) is to be performed upon installation of an instrument and after major repair. There are two categories in an IQ—visual inspection and operating environment. Visual inspection involves a check of the condition of the instrument to determine if it is ready to use. The inspection confirms that all cables are intact, the instrument is intact with no broken pieces/parts, and all components of the equipment are in working order. The operating environment for the device is checked for proper temperature, humidity, and power requirements. The operating environment should also be checked to avoid high background radioactivity. Temperatures are typically 10°–30° C and relative humidity ranges are generally 20%–90%. Shielding may be necessary to ensure that the background radiation has no negative effect on the instrument performance.

3.2 Operational Qualification

Operational Qualification (OQ) is a check of operational specifications for the equipment, including equipment set-up, functional testing of subsystems, and proper overall operation. OQ should describe operational procedures for the equipment and is performed after IQ and after major repair. OQ should include all appropriate tests to show that the instrument is capable of functioning properly. Typical examples of OQ include clock accuracy, high voltage, zero adjust, background response, and contamination check.

3.3 Performance Qualification

Performance Qualification (PQ) demonstrates that the equipment is capable of performing tasks required in the operating environment and provides the intended results. PQ should describe the required performance tasks for the equipment and include all appropriate tests to demonstrate that the instrument is performing within its operating parameters. These PQ tests are done following the OQ tests. Additionally, some or all the PQ tests are performed routinely (e.g., daily, quarterly, semiannually, annually) to demonstrate the instruments continual acceptance for use. Typical PQ tests include constancy and relative response, accuracy, reproducibility (precision), system linearity, determination of “quench” curves in liquid scintillation counters, and supplier equivalence.

3.4 Geometry Testing

The purpose of the geometry test is to demonstrate correct readings of the test sample, accounting for differences in the container, volume, or position of the test sample compared to the radioactive calibration standard. Geometry testing should be performed at installation of the dose calibrator and for each type of vial and syringe used to contain the test sample. Testing should include the test sample holder. Corrections factors may be developed as needed.

4. ONGOING PERFORMANCE TESTS OF INSTRUMENTS

Performance and operational tests are completed periodically to ensure the instrument is capable of meeting specified criteria for accurate assays and that no changes have occurred since initial testing. Tests include physical inspection, measurement of system parameters, and operational tests with radioactive calibration sources. These tests should also be completed

after repair. Regulatory agencies may require specific tests at a regular frequency. The following sections describe the performance tests using a dose calibrator as an example. The same principles will apply to other instruments used for radiation measurements.

4.1 Physical Inspections

Ensure that the dose calibrator and associated accessories, such as source holders and well liners, are undamaged and that foreign objects are not present in the chamber. Prior to use, repair or replace any damaged components as required and remove all foreign objects.

4.2 System Parameters

The system electronics should be tested according to the manufacturer's diagnostic testing procedures. The results should meet the acceptance criteria detailed in the manufacturer's manual. These parameters typically include the high voltage on the chamber, the zero adjustment on the electrometer, verification of communication between components of the system, and the accuracy of the system clock.

If the manufacturer's diagnostic procedures are not available, then at a minimum the user should check, record, and trend the chamber high voltage and the measured value for the electrometer zero check. Small changes in the high voltage will identify system malfunctions that might not be apparent with the use of radioactive calibration sources, but will begin impacting higher activity readings. The system clock should be checked and should be accurate within one min.

4.3 Operational Tests

BACKGROUND AND CONTAMINATION TEST

The purpose of this test is to determine the background radiation level and to ensure the absence of contamination that may affect measurements on the dose calibrator. Background radiation may fluctuate due to movement of sources near the chamber, as well as by contamination in or near the chamber, the liner, or the source holder. For this reason, this test should be performed with the liner and the source holder in the chamber. All radioactive sources should be well shielded or at a sufficient distance from the chamber. The background should be recorded at least once on each day of use, or more frequently if necessary, using the most common radionuclide setting. The background radiation level should be determined and recorded at initial installation, and periodically verified. It is especially important to verify if the minimum assayed quantity of radioactivity decreases.

CONSTANCY TEST

The purpose of this test is to ensure the constancy of the dose calibrator over time. This test should be performed on each day of use, and completed after the system tests outlined above. Typical radioactive calibration sources used in this test are Cesium-137 or Cobalt-60 contained within a solid matrix. Cobalt-57 might not be suitable due to its nominal half-life of less than one year. The radioactive calibration source is not required to have the same geometry as routine test samples. A single radioactive calibration source is sufficient for this check. The source is placed in the source holder and the reading recorded for the dedicated radionuclide setting, as well as for all other commonly used radionuclide settings. This ensures no drift in response for any setting. Results should be recorded and be within the appropriate range of the decay corrected value recorded during IQ. Control charts or other data representations should

be used to identify and document trends.

ACCURACY TEST

The purpose of this test is to demonstrate the stability of the dose calibrator over the energy range used. This test should be performed annually or after the dose calibrator is repaired or moved. If the dose calibrator is used for multiple radionuclides, two or three different radioactive calibration sources should be included to cover the energy range of the radionuclides. These calibration sources are typically contained in a solid matrix and do not necessarily match the geometry of routine test samples. If the dose calibrator is only used for a single radionuclide, or only for position emission tomography (PET) radionuclides, a single calibration source may be adequate. The quantity of radioactivity in the calibration source should be greater than 100 μCi (3.7×10^6 Bq). Sources used for this test should be traceable to a National Metrology Institute (NMI). Measurements of the calibration source activity are recorded and the average measured value is compared to the expected value. The measured value should be within the appropriate range of the expected value.

REPRODUCIBILITY TEST

The purpose of this test is to measure the precision of the dose calibrator and should be performed at least annually. The radioactive calibration source should have a half-life such that decay corrections are not required over the period of the test. The quantity of radioactivity in the calibration source should be greater than 100 μCi (3.7×10^6 Bq). An appropriate number of consecutive readings are recorded using the same geometry. Each of the measurements taken should be within an appropriate range of the mean of all the measurements.

LINEARITY TEST

The purpose of this test is to show that the response of the dose calibrator is linear across the range of radioactivity levels to be measured. This is especially critical at high levels of radioactivity where the measured radioactivity may differ from the true radioactivity due to saturation effects, and at low levels of radioactivity due to variations on the background radiation and changes in the source positioning. This test should be performed annually or after the dose calibrator has been repaired or moved.

The test is considered successful if the ratio of the measured radioactivity to the expected activity is within the appropriate range of the expected values. Three techniques may be employed for this test: decay, graded shield, and graded source. Use of graded shields is acceptable once an initial linearity test using the decay method is successfully completed. Refer to the manufacturer's recommendations on the use of the graded shields. The graded source method requires accurate measurements of volume or mass, and is not generally recommended.

Table 1 is an example of ongoing performance checks for dose calibrators.

Table 1. List of Performance Tests

Parameter	Daily ^a	Annually (at a minimum)
Physical inspection	Yes	NA
System electronics	Yes	NA
Clock accuracy	Yes	NA
High voltage	Yes	NA
Zero adjust	Yes	NA
Background response/contamination check	Yes	NA
Radioactive calibration source (constancy and relative response)	Yes	NA
Accuracy	See footnote ^b	Yes
Reproducibility (precision)	See footnote ^b	Yes
System linearity	See footnote ^b	Yes
Supplier equivalence	See footnote ^b	Yes
^a At the beginning of each day of use. ^b This is required after repair.		

5. IDENTIFICATION OF RADIONUCLIDES

5.1 Half-Life Determination

The half-life is an identifying characteristic of a radionuclide. The half-life is determined by measuring the quantity of radioactivity in the test sample as a function of time. The approximate half-life may be used to confirm the radionuclidic identity. Perform the measurements using an appropriately calibrated instrument, such as a quantitative radioactivity detector, provided the quantity of radioactivity is within the linear range of the instrument throughout the measurements and the geometry of the test sample is not changed during the measurements.

The test sample may be used directly, or diluted and/or dried in a capsule as needed. The test sample is prepared in a manner that avoids losses during handling. If the test sample is a liquid or solution, the sample is held in a closed container. If the test sample is a residue from drying in a capsule, the sample is protected by a cover consisting of a sheet of adhesive cellulose acetate or other material. The quantity of radioactivity in the test sample should be sufficient to allow measurements over the time frame of the test. If necessary, correction for dead-time losses may be applied. The measured half-life should be within the range of the expected half-life as defined in each particular application of this test.

5.2 Gamma Ray Spectrometry

The gamma-ray emission spectrum may be used to identify and quantify gamma-emitting radionuclides. The detector should be calibrated with a radioactive calibration source traceable to a NMI. The calibration source used to determine the efficiency of the detector should have a

sufficient quantity of radioactivity to produce an adequate number of counts for each photopeak used in the calibration. The calibration source and the test sample should be measured with the same geometry and distance from the detector. It is critical that the geometry, including vial type and volume, be the same since these factors affect the amount of incident radiation on the detector. Photopeaks in the test sample should be comparable to those in the calibration source, both in terms of energy and intensity. The presence of unknown peaks or changes in peak abundance is indicative of impurities. Ensure that background radiation levels remain constant during the measurement, as changes may result in unknown peaks. Counting time or shielding should be optimized to achieve the necessary required minimum detectable activity to meet test specifications. It may be necessary to allow the radioactivity in the test sample to decay before it is possible to detect impurities at the required levels. There may also be short-lived impurities present, so an analysis should be completed to identify impurities and their half-lives to ensure the sample is assayed prior to their decay. Positron-emitting radionuclides typically cannot be differentiated since their emitted energy (511 keV) is the same for each radionuclide. Performance tests of the gamma-spectroscopy system should be completed on each day of use to ensure the functionality of the system.

5.3 Beta-Emitter Analysis and Identification

The purpose of the beta-emitter analysis and identification is to determine the maximum energy of the beta particle, which is unique and can be used for identification. Beta-emitting radionuclides can be identified either by evaluation of their spectrum or by measuring their mass attenuation coefficients in a series of absorption foils and constructing an attenuation curve. Performance tests of the beta counting system should be completed on each day of use to ensure the functionality of the system.

5.4 Alpha Spectrometry

The alpha emission spectrum may be used to identify and quantify alpha-emitting radionuclides. Pure alpha emitters present unique challenges due to their low penetration distance and their toxicity, especially if they are volatile compounds that could create airborne radioactivity and therefore internal radiation exposure to the experimenter or operator. Alpha particles can be detected either by the use of proportional counters, scintillation detection using a silver-activated zinc sulfide phosphor, or by the techniques of liquid scintillation counting (LSC). Performance tests of the alpha spectroscopy system should be completed on each day of use to ensure the functionality of the system.

6. ASSAY OF RADIONUCLIDES

6.1 Measurement of Radioactivity Using a Dose Calibrator

Using a holder, the test sample is placed in the chamber of the dose calibrator at a given position. Once the response is stable, the radioactivity reading is taken. Performing measurements on the test sample with the same geometry as the calibration source provides the most accurate results; however, geometry correction factors may be developed and used as necessary. Refer to the manufacturer's recommendations for the maximum radioactivity that can be determined in the dose calibrator.

6.2 Measurement of Radioactivity Using a Solid-State Detector

The test sample is positioned in front of the detector or into the well of a well-type detector. The detector must be adequately shielded and calibrated using a radioactive calibration source traceable to an NMI.

6.3 Measurement of Radioactivity Using Chromatographic Systems

A scintillation detector can be used for dynamic radioactivity measurements, e.g., the eluate of a liquid chromatograph is directed over or through a detector. The quantity of radioactivity in the test sample should provide a count rate that is within the linear range of the detector.

6.4 Measurement of Radioactivity Using a Liquid Scintillation Counter

LSC is the standard laboratory method to quantify radioactivity of particle-emitting radionuclides, mostly beta- and alpha-emitting radionuclides. The sample to be analyzed may require a liquid scintillation fluid (cocktail) depending on the energy of the beta particle. The LSC detection method uses liquid scintillation cocktails to transform emitted radiation into detectable light photons. The sample to be analyzed is placed into a liquid scintillation vial and the cocktail is added in the required amount. While a sample that can be dissolved into the fluid is preferred, other samples can be assayed if the impact on the light output is quantified. The detector should be calibrated using an appropriate radioactive calibration source traceable to an NMI. The absorption of scintillation photons is called quench, and it is necessary to develop a "quench curve" during PQ and additional quench curves may be necessary for different types of samples to correct the counting efficiency. Performance tests of the LSC should be completed on each day of use to ensure the functionality of the system.

7. GLOSSARY

The following definitions apply to commonly encountered words and phrases when dealing with radioactivity.

Alpha particles (α): Positively charged particles emitted from nuclei during radioactive decay. Alpha particles are essentially ^4He nuclei, consisting of two protons and two neutrons, but no electrons.

Beta particles (β^-): Negatively charged particles that are emitted from nuclei during radioactive decay. Beta particles are essentially electrons.

Beyond-use date: Date (and time, if appropriate) that establishes a limit for the use of a compounded preparation. The acceptable use period (i.e., the period of time between the date and time of compounding and the beyond-use date and time) is based on a knowledge of the radioactive properties of the preparation, the results of stability studies on the preparation, and, as appropriate, the assurance of sterility of the compounded sterile preparation.

Bremsstrahlung: Electromagnetic radiation produced by the deceleration of a charged particle through interaction with another charged particle, typically an electron and an atomic nucleus, respectively. The moving particle (e.g., beta particle) loses kinetic energy, which is converted into photons (X-rays). This electromagnetic radiation exhibits a continuous spectrum, with peak intensity a function of the energy of the incident particle.

Calibration factor: The coefficient used to convert the measured ionization chamber current to a nominal radioactivity. This term is often referred to as the "calibration coefficient".

Calibration time: Arbitrary time and date, if appropriate at which the specified amount of radioactivity is present.

Carrier-free: A preparation free from stable isotopes of the same element as the radionuclide.

Counting assembly: Consists of a sensing unit and an electronic scaling device. The sensing unit may be a Geiger-Müller tube, a proportional counter, a scintillation detector in which a photomultiplier tube is employed in conjunction with a scintillator, or a solid-state semiconductor.

Dose calibrator: A well-type ionization chamber commonly used to assay radiopharmaceuticals. Display units are typically in Curies ($\mu\text{Ci}/\text{mCi}/\text{Ci}$) or Becquerels ($\text{kBq}/\text{MBq}/\text{GBq}$).

Expiration date: Date (and time, if appropriate) that establishes a limit for the use of the manufactured product, which is based on the knowledge of the radioactive properties of the product and the results of stability studies on the finished dosage form.

Gamma rays (Γ rays): Electromagnetic radiation emitted from nuclei during radioactive decay. Gamma rays have a wide range of energies. The gamma rays emitted from a given radionuclide are always at the same energy(ies) providing a unique signature that enables the identification of a gamma-emitting radionuclide.

Geiger-Müller counter: Often referred to as G-M counter or Geiger counter. An instrument in which a high voltage potential is applied across a volume of inert gas for the purpose of collecting ions produced by a radiation field. The negative electrons are internally multiplied to produce a readily detectable current pulse. Display units are typically counts per min (cpm) or milliroentgen per h (mR/hr).

Geometry: The characteristics of a radioactive source (i.e., container type, container wall thickness, volume and position of the container in the well chamber) that, along with the physical characteristics of the ionization chamber, affect the magnitude of the calibration coefficient for a specific radionuclide. The principal geometry considerations that may affect the accuracy of a source measurement in a dose calibrator are container configuration, source volume, position of the source in the chamber well and the radionuclide itself. [Note—It is customary to compare a standardized preparation and radiopharmaceutical drug or preparation using identical geometry conditions for assay, identification, and other parameters. The validity of the result is critically dependent upon the reproducibility of the spatial relationships of the source to the detector and its surroundings and upon the accuracy of the standardized preparation.]

Ionization chamber: An instrument in which an electric field is applied across a volume of inert gas for the purpose of collecting ions produced by a radiation field. The positive ions and negative electrons drift along the lines of force of the electric field, and are collected on electrodes, producing an ionization current. The most commonly used form of ionization chambers for measurement of the activities of radiopharmaceuticals is a well-type ionization chamber known as a dose calibrator.

Isobars: Nuclides with the same mass number (protons + neutrons).

Isomers: Atoms with the same number of protons and neutrons, but a different nuclear energy configuration. Short-lived radioactive isomers are generally referred to as metastable. Different

isomers are different nuclides based on their nuclear energy configurations.

Isotones: Nuclides with the same number of neutrons and a different number of protons. Isotones are different elements with different atomic masses.

Isotopes: Nuclides with the same number of protons and a different number of neutrons. Isotopes are the same element with a different atomic mass.

Isotopic carrier: A stable isotope of the element concerned either present in or added to the radioactive preparation in the same chemical form as the radionuclide.

Liquid scintillation counter (LSC): An instrument which detects scintillation light from the absorption of radiation energy in a scintillation liquid. This instrument is used primarily for beta-emitting radionuclides that do not also emit gamma photons. For best results, the radioactive sample should be soluble in the scintillation liquid.

Minimum detectable activity: Smallest quantity of radioactivity that can be detected above the background with a specified level of confidence.

National Metrology Institute (NMI): A measurement standards body, which is a laboratory of metrology that establishes standards for a country or organization. For example, National Institute of Standards and Technology (NIST) is the NMI for the United States.

No-carrier-added: A preparation where no stable isotopes of the same element as the radionuclide being tested are intentionally added in the stated chemical form or at the position of the radionuclide in the molecule being tested.

Nuclide: An atom with a specific number of protons and neutrons in a given nuclear energy state.

Positrons (β^+): Positively charged particles emitted from a nucleus during radioactive decay. Positrons are anti-electrons.

Radioactivity: 1) The spontaneous transformation of nuclei by radioactive decay. Radioactivity is typically described as atoms undergoing radioactive decay per unit time (or disintegrations per unit time). 2) The quantity of radioactive material, as measured in units of Curies (US units) or Becquerels (SI units). The quantity of radioactive material may also be referred to as activity.

Radiochemical identity: The molecular structure of the intended active radiopharmaceutical ingredient, which is present in the radiopharmaceutical preparation.

Radiochemical purity: The ratio, expressed as a percentage, of the radioactivity of the intended active radiopharmaceutical ingredient to the total radioactivity of all radioactive ingredients and impurities present in the radiopharmaceutical preparation.

Radioisotope: A radioactive atom, generally used in the context of an isotope of an element.

Radionuclide: An unstable nuclide that undergoes radioactive decay; a radioactive nucleus. The terms radionuclide and radioisotope are commonly used interchangeably.

Radionuclidic identity: The intended radionuclide in the radiopharmaceutical preparation.

Radionuclidic purity: The ratio, expressed as a percentage, of the radioactivity of the intended radionuclide to the total radioactivity of all radionuclides in the radiopharmaceutical

preparation.

Radiopharmaceutical (Radiopharmaceutical preparation/Radioactive drug): A finished dosage form that contains a radioactive substance in association with one or more other ingredients and that is intended to diagnose, stage a disease, monitor treatment, or provide therapy. A radiopharmaceutical includes any nonradioactive reagent kit or radionuclide generator that is intended to be used in the preparation of any such substance. The terms radiopharmaceutical and radioactive drug are commonly used interchangeably.

Scintillation crystal counter: An instrument consisting of a crystal scintillator, such as NaI (TI), with attached photomultiplier tube and associated electronics. Scintillation light produced from the absorption of gamma and X-rays in the crystal is converted to electrons and amplified in the photomultiplier tube. The resultant current pulse may be further analyzed with regard to photon energy. A commonly used form of this instrument that has a hole in the crystal of sufficient size to allow placement of a test tube or similar container is known as a well counter.

Semiconductor detector: An instrument consisting of a semiconductor material, such as silicon or germanium crystals, that detects ionizing radiation through generation of charge carriers (passage of electrons through holes). The current pulse produced by migration of these charge carriers, under the influence of a voltage potential across the material, can be further amplified and analyzed to determine the quantity and energy of the incident radiation.

Solid-state detector: A crystal-based detector, in contrast to a gas-based detector. Often is used as a synonym for a semiconductor detector.

Specific activity: The radioactivity of a radionuclide per unit mass of the element or compound. The unit of specific activity is radioactivity per mass expressed on a gram or mole basis [e.g., mCi/ μ g (MBq/ μ g), Ci/mmol (GBq/mmol)].

Strength: The radioactivity concentration of the radiopharmaceutical at the calibration time. The unit of strength is the amount of radioactivity on a volume basis (e.g., mCi/mL or MBq/mL).

Total radioactivity: The radioactivity of the radionuclide, expressed per unit (e.g., vial, capsule, ampoule, generator, and others) at the calibration time.

Validation: Establishment of documented evidence that a method, process, or system meets its intended requirements.

Verification: Confirmation that an established method, process, or system meets predetermined acceptance criteria.

X-rays: A type of electromagnetic radiation emitted from the electron orbitals. While they do not arise from the nucleus they are often present immediately following a decay event if there are interactions between the emitted radiation and the orbital electrons.

8. REFERENCES

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2. American Association of Physicists in Medicine (AAPM). The selection, use, calibration, and quality assurance of radionuclide calibrators used in nuclear medicine. AAPM, College Park, MD; June 2012. Report No.: 181.

■ 2S (USP39)

[‡] ~~These certified reference standards are obtainable from the National Institute of Standards and Technology, Washington, DC 20234.~~ ■ 2S (USP39)

BRIEFING

⟨1004⟩ **Mucosal Drug Products—Performance Tests.** The General Chapters Dosage Forms Expert Committee proposes *Mucosal Drug Products—Performance Tests* ⟨1004⟩ to provide guidance on in vitro performance tests for drug products delivered by the mucosal route. The information on performance tests in this chapter represents guidance based on the information available.

Well-defined compendial apparatuses are not always available or adaptable to the in vitro performance testing of products administered by the mucosal route. The use of noncompendial apparatus is suggested throughout. Examples include the use of the small-volume apparatus included in this proposal for films, suspensions, sublingual tablets, and buccal tablets. Proper qualification standards for such apparatuses remain to be developed. Information is requested on qualification standards for small-volume dissolution apparatuses or other noncompendial apparatuses mentioned in this proposed general information chapter.

Other related information is available from *Mucosal Drug Products—Product Quality Tests* ⟨4⟩.

(GCDF: W. Brown.)

Correspondence Number—C158588

Comment deadline: November 30, 2015

Add the following:

■ ⟨1004⟩ MUCOSAL DRUG PRODUCTS—PERFORMANCE TESTS

INTRODUCTION

Mucosal drug products deliver drug substances to the body via the mucosal route. For the purposes of this chapter, the mucosal route of drug administration is divided into seven membrane surfaces characterized as otic, ophthalmic, nasal, oropharyngeal, urethral, vaginal, and rectal. Mucosal drug products include a wide variety of dosage forms such as solutions, suspensions, emulsions, creams, ointments, gels, inserts, strips, aerosols, sprays, films, medicated chewing gums, lozenges, tablets, and suppositories. Some of these dosage forms are also administered by other routes. For example, creams can be administered by the mucosal route (vaginal) and also by the topical route. Two categories of tests—product quality and product performance—are performed on these products. These tests provide assurances of batch-to-batch quality, reproducibility, reliability, and performance of a drug product. Product quality tests are performed to assess attributes such as assay, identification, and content uniformity and are part of the compendial monograph (see *Mucosal Drug Products—Product Quality Tests* ⟨4⟩). Product performance tests are conducted to assess the drug release from the dosage form. For certain mucosal drug products, determination of aerodynamic particle size or globule size may serve as a product performance test.

Where a compendial performance test exists for a dosage form administered by a nonmucosal route, such as a dissolution test for an oral tablet, that test may have application for the

dosage form administered by a mucosal route (e.g., buccal tablets or sublingual tablets).

PERFORMANCE TESTS FOR MUCOSAL DRUG PRODUCTS

The performance tests for the various mucosal drug products can be broadly divided into two categories: 1) test procedures that use or can adopt methodology in existing general chapters, and 2) tests that need additional developmental work before they can be recommended.

The Dissolution Procedure: Development and Validation (1092) should be a reference when developing a drug release test (e.g., selecting the drug release medium, apparatus/procedure, or analytical method). For several mucosal drug products, drug release procedures described in *Dissolution* (711), *Drug Release* (724), and *Semisolid Drug Products—Performance Tests* (1724) may be applicable.

In some instances, mini-basket or mini-paddle apparatuses may be suitable. These apparatuses resemble *Dissolution* (711), *Apparatus 1 (Basket Apparatus)* and *Dissolution* (711), *Apparatus 2 (Paddle Apparatus)*, with dimensions scaled down to accommodate medium volumes of <500 mL (1,2). Several designs are commercially available. However, as of now, these apparatuses are not standardized.

Because of the varied and specific environments characterizing the mucosal route of administration, researchers may be inclined to use “physiological medium” for the drug release of the specific dosage form. It may not be essential to use such a medium for the performance test of the product, and in many instances a simple buffer may suffice. A reference (3) for the composition of such media is provided for additional information.

AEROSOLS AND NASAL SPRAYS

Performance tests for aerosols and nasal sprays are largely concerned with droplet or particle size distribution and aerodynamic size distribution. The procedures in *Aerosols* (601) can be applied to products administered by mucosal routes. When the drug substance is present as a solid with a modified-release mechanism in the administered dose, attempts should be made to determine the dissolution of the particles (4).

CREAMS, GELS, AND OINTMENTS

Drug release tests for creams, gels, and ointments can be performed using a procedure described in (1724).

EMULSIONS

Performance tests for emulsions include globule size determination and dissolution/drug release testing. Globule size can be determined using a procedure described in *Globule Size Distribution in Lipid Injectable Emulsions* (729). The drug release test can be performed using *Dissolution* (711), *Apparatus 2 (Paddle Apparatus)* or a vertical diffusion cell as described in *Semisolid Drug Products—Performance Tests* (1724), *Drug Release Rate Determination Using Vertical Diffusion Cell Apparatus*.

FILMS

Drug Release (724), *Apparatus 5 (Paddle over Disk)* can be used to determine the drug release from film dosage forms. A mini-basket can be used for drug release testing of films.

GUMS

For gum products, the performance test includes drug release from the formulation. A device is described in the *European Pharmacopoeia* (5). The release of drug from the formulation requires masticatory activity that renews the surface exposed to the medium. The rate of release will be a function in part of the frequency of chewing that is simulated by the test apparatus. Gums can require conditioning at the temperature of the mouth to deform plastically under the action of the oscillating platens of the test apparatus.

Important parameters for the apparatus include: dissolution medium volume, distance between upper and lower chewing surfaces, recommended rotation angle, temperature, and chewing frequency. The dissolution medium chosen, the test time(s), and the volume sampled are also important considerations.

INSERTS

Drug release testing for inserts can be performed using *Dissolution* (711), *Apparatus 1 (Basket Apparatus)* or *Dissolution* (711), *Apparatus 2 (Paddle Apparatus)*.

LOZENGES

Drug release testing of lozenges can be performed using either *Dissolution* (711), *Apparatus 2 (Paddle Apparatus)* at high agitation (175 rpm) or *Dissolution* (711), *Apparatus 3 (Reciprocating Cylinder)*.

SUPPOSITORIES

There are two types of suppositories: 1) hydrophilic (water soluble), and 2) lipophilic (oil soluble or melting). Drug release (dissolution) for water-soluble suppositories can be performed using *Dissolution* (711), *Apparatus 1 (Basket Apparatus)*, *Dissolution* (711), *Apparatus 2 (Paddle Apparatus)*, or *Dissolution* (711), *Apparatus 4 (Flow-Through Cell)*. Drug release testing for lipophilic suppositories may need modification of the dissolution procedure to avoid analytical interference from the oil globules. Several alternative methods have been proposed (6–8). The flow-through cell apparatus using the cell for suppositories may be useful (see *Disintegration and Dissolution of Dietary Supplements* (2040), *Figure 2*). The selection of the method will be dependent on the nature of the formulation.

SUSPENSIONS

The dissolution test for suspensions can be performed using *Dissolution* (711), *Apparatus 2 (Paddle Apparatus)*. A small-volume, mini-paddle apparatus may be used.

SUBLINGUAL TABLETS AND BUCCAL TABLETS

Drug release for these dosage forms can be performed using *Dissolution* (711), *Apparatus 1 (Basket Apparatus)* or *Dissolution* (711), *Apparatus 2 (Paddle Apparatus)*. Mini-baskets or the mini-paddles can also be used for drug release testing of buccal and sublingual tablets.

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■ 2S (USP39)

BRIEFING

(1015) **Automated Radiochemical Synthesis Apparatus**, *USP 38* page 717. This chapter is primarily intended for use in providing the information regarding synthesis of Positron Emission Tomography (PET) drugs. A comprehensive informational chapter *Positron Emission Tomography (PET) Drugs—Information* (1823) is being proposed elsewhere in this issue of *Pharmacoepial Forum*. The proposed new chapter captures all the relevant information from

(1015) making the existing chapter redundant. Hence, the General Chapters—Physical Analysis Expert Committee proposes to omit this chapter from *USP-NF*. Interested parties are invited to submit comments to USP on or before January 31, 2016.

(CHM4: R. Ravichandran.)

Correspondence Number—C155320

Comment deadline: November 30, 2015

Delete the following:

■ ~~(1015) AUTOMATED RADIOCHEMICAL SYNTHESIS APPARATUS~~

~~The preparation and quality control of diagnostic radiopharmaceuticals labeled with the very short-lived positron-emitting nuclides (e.g., ^{15}O , ^{13}N , ^{11}C and ^{18}F having half-lives of 2, 10, 20, and 110 minutes, respectively), are subject to constraints different from those applicable to therapeutic drugs: (1) Synthesis must be rapid, yet must be arranged to protect the chemist or pharmacist from excessive radiation exposure. (2) Except to a limited extent for ^{18}F , synthesis must occur at the time of use. (3) With the exception of ^{18}F , each batch of radiopharmaceutical generally leads to only a single administration.~~

~~These factors raise the importance of quality control of the final drug product relative to validation of the synthesis process. Since with few exceptions every dose is individually manufactured, ideally every dose should be subjected to quality control tests for radiochemical purity and other key aspects of quality before administration. Because quality testing of every batch is not possible, batches are selected at regular intervals for examination to establish and completely characterize their radiopharmaceutical purity. This routine and thorough quality testing of selected batches forms the basis of process validation, which is absolutely essential for prospective assessment of batch quality and purity when dealing with such extremely short-lived radiopharmaceuticals. Since radiopharmaceuticals used in positron emission tomography (PET) are administered intravenously or (for radioactive gases) by inhalation, batch-to-batch variability in bioavailability is not an issue. Furthermore, the very small scale of radiopharmaceutical syntheses (almost always less than 1 milligram and often in the microgram range) and the fact that patients generally receive only a single dose of radioactive drug minimize the likelihood of administering harmful amounts of chemical impurities. These statements are not intended to contest the need for quality control in the operation of automated synthesis equipment, but to place the manufacture of positron-emitting radiopharmaceuticals in an appropriate perspective and to reemphasize the overwhelming importance of prospective process validation and finished product quality control.~~

~~The routine synthesis of radiopharmaceuticals can result in unnecessarily high radiation doses to personnel. Automated radiochemical synthesis devices have been developed, partly to comply with the concept of reducing personnel radiation exposures to "as low as reasonably achievable" (ALARA). These automated synthesis devices can be more efficient and precise than existing manual methods. Such automated methods are especially useful where a radiochemical synthesis requires repetitive, uniform manipulations on a daily basis.~~

~~The products from these automated radiosynthesis devices must meet the same quality assurance criteria as the products obtained by conventional manual syntheses. In the case of~~

positron-emitting radiopharmaceuticals, these criteria will include many of the same determinations used for conventional nuclear medicine radiopharmaceuticals, for example, tests for sterility and bacterial endotoxins. Many of the same limitations apply. Typical analytical procedures such as spectroscopy are not generally applicable because the small amount of product is below the minimum detection level of the method. In all cases, the applicable Pharmacopeial method is the conclusive arbiter (see *Procedures* under *Tests and Assays* in the *General Notices*).

Preparation of Fludeoxyglucose F-18 Injection and other positron-emitting radiopharmaceuticals can be adapted readily to automated synthesis. In general, the equipment required for the manual methods is simpler and less expensive than that used in automated methods but is more labor-intensive. Of special concern are the methods involved in validating the correct performance of an automated apparatus. For a manual procedure, human intervention and correction by inspection can nullify many procedural errors. In an automated system, effective feedback also can begin during the synthesis. For example, radiation detectors can monitor activity at various stages of radiosynthesis. Failure to obtain the appropriate activity could activate an alarm system that would lead to human intervention.

Radiochemicals versus Radiopharmaceuticals—It is appropriate to draw a distinction between a radiochemical and a corresponding radiopharmaceutical. In research PET centers, automated equipment is used to prepare labeled compounds for animal experiments. These radiochemicals are not regarded as radiopharmaceuticals if (1) they are not prepared according to a validated process that provides a high degree of assurance that the preparation meets all established requirements for quality and purity; and (2) have not been certified by qualified personnel (licensed pharmacists and approved physicians) in accordance with published Pharmacopeial methods for individual radiopharmaceuticals.

Automated Equipment—The considerations in this chapter apply to synthesis conducted by general purpose robots and by special purpose apparatus. Both are automated devices used in the synthesis of radiochemicals. The exact method of synthesis device control is variable. Both hard-wired and software-controlled synthesis devices fall under the general designation, and there is a spectrum ranging from traditional manual equipment through semi-automated devices to completely automatic devices.

Common Elements of Automated Synthesis Equipment—To manipulate a chemical apparatus to effect the synthesis of a radiochemical, control of parameters such as time, temperature, pressure, volume, and sequencing are needed. These parameters can be monitored and constrained to fall within certain bounds.

Equipment Quality Assurance—The goal of quality assurance is to help ensure that the subsequent radiopharmaceutical meets Pharmacopeial standards. Although the medical device good manufacturing practice regulations (21 CFR 820) are not applicable, they may be helpful in developing a quality assurance program. As a practical matter this involves documented measurement and control of all relevant physical parameters controlled by the synthesis apparatus.

Routine Quality Control Testing—Routine quality control testing of automated equipment implies periodic testing of all parameters initially certified during the quality assurance qualification. Depending on the criticality and the stability of the parameter setting, testing may be as often as daily. This process performance assessment must be augmented by regular end-product testing. For example, variations in the temperature of an oil bath may be

acceptable if the radiochemical (end product) can be shown to meet all relevant testing criteria.

Reagent Audit Trail—Materials and reagents used for the synthesis of radiopharmaceuticals should conform to established quality control specifications and tests. Procedures for the testing, storage, and use of these materials should be established. In this context, a reagent is defined as any chemical used in the procedure leading to the final radiochemical product, whereas materials are defined as ancillary supplies (tubing, glassware, vials, etc.). For example, in some processes compressed nitrogen is used to move liquid reagents. In this case, both the nitrogen and the tubing should meet established specifications.

Documentation of Apparatus Parameters—Key synthesis variables should be identified, monitored, and documented. These characteristics include meaningful physical, chemical, electrical, and performance attributes. A method for specifying, testing, and documenting computer software and hardware is especially important for microprocessor and computer-controlled devices. This program should include periodic generalized testing of the computer hardware. In addition, the software program code should be periodically examined to determine that it has not been modified and that it continues to result in the final product's meeting all specifications. In-process feedback is one means of confirming that the synthesis is under control. Changes to the software code should involve a formal authorization procedure, and changes should be documented.

Each type of radiochemical synthesis device requires a set of specific procedures for testing and monitoring the reliability and reproducibility of the various subsystems that make up the total synthesis system.

It is essential that calibration of each of the components be confirmed according to an established maintenance timetable and that measurements or monitoring be made under actual synthesis conditions.

Delivery times, reagent volumes, temperatures, gas pressures, and rates of flow need to be measured and shown to be stable and reproducible within established limits. Delivery of the reagents and solvents needs to be calibrated periodically. Other components to be routinely calibrated include the radiation detection system and process monitoring sensors and system.

For illustration, elements of system validation of several representative components of an automatic synthetic device are as follows:

Reaction vessels may be cleaned and inspected by an established documented method. The vessels themselves may be numbered and their performance tracked.

Heating and cooling systems (such as oil baths) may be monitored by thermometers or thermocouples. The temperatures may be recorded in a batch sheet, or they may be automatically printed out as part of a computerized log. Maintenance involves periodic calibration.

Gases and gas delivery system performance may be tracked by pressure gauges and flowmeters. Gas purity may be established via supplier certificates of analysis or may be verified by independent testing. Maintenance of gauges and flowmeters involves periodic calibration with standards.

Position-dependent motor performance may be verified by limit switches. Maintenance could involve actual measurement of distance traversed and elapsed time.

~~Solenoid valves may be checked electrically, by flow and pressure tests.~~

~~Heater output is evidenced by proper thermocouple readings. Additional tests could involve resistance measurements.~~

~~Reagents may be accepted on the basis of suppliers' certificates of analysis. Alternatively, the chemical could be tested in-house or sent to an independent testing laboratory. Periodic retesting may be necessary depending on stability.~~

~~Computer programs may be tested by documenting elapsed time of synthesis, with printouts verifying that all appropriate manipulations occurred, including printing of relevant parameters such as times, temperature, pressures, and activities.~~

~~Patterns of activity distribution such as absolute amount of product, percentage yield, and individual impurity activity levels afford the experienced user an opportunity to discern systems failures.~~

~~**Changes in the Synthesis Method**—Some changes in the synthesis apparatus can be considered to be trivial. This category would often include changes not affecting any of the monitored parameters. However, it is important that care be taken to ensure that seemingly innocuous changes do not have an unexpected impact. For example, changes in a comment line of a computer program may result in inadvertently changing or deleting a vital instruction. Any changes in monitored parameters have the potential for changing the process output. If the resultant radiochemical does not meet specifications or if the subsequent radiopharmaceutical does not meet Pharmacopeial criteria, the process change is unacceptable; the fault must be corrected and the process revalidated.~~

■ 2S (USP39)

BRIEFING

(1029) **Good Documentation Guidelines**, *PF* 40(3) [May–June 2014]. This revision is proposed on the basis of public comments received on its previous publication in *PF*. This proposed new general chapter provides guidelines on how to develop and maintain good documentation practices within Good Manufacturing Practice (GMP) operations. This chapter was created to address a need for descriptions of what constitutes good documentation, for example, records of all types that are clear, accurate, and complete. These records may include protocols, procedures, reports, and raw data. The overarching goal of this chapter is to provide useful information to the GMP-regulated industries, thereby assisting the user in designing procedures that will promote record integrity and control. In turn, record integrity and control are essential for the evaluation and reconstruction of GMP activities, and ultimately for ensuring the quality and safety of regulated ingredients and products.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCCA: A. Hernandez-Cardoso.)

Correspondence Number—C140448

Comment deadline: November 30, 2015

Add the following:

■ (1029) GOOD DOCUMENTATION GUIDELINES

INTRODUCTION

Purpose

Documentation can be viewed as the foundation of all quality systems because clear, complete, accurate records are essential to all operations and procedures. This general chapter provides guidelines on good documentation practices for the Good Manufacturing Practice (GMP)-regulated industries, to be used in the production and control of pharmaceutical products, active pharmaceutical ingredients (APIs), excipients, dietary supplements, food ingredients, and medical devices. This chapter describes the underlying principles of proper documentation for GMP operations to assist the user while working with GMP activities. These guidelines should be helpful for building the basic foundation of a quality system that will ensure proper documentation as well as record integrity and control.

Scope

This chapter covers different levels and types of documentation, including paper and electronic records that consist of raw data, reports, protocols, and procedures related to manufacturing controls and analytical data. The chapter also includes recommendations on information that should be recorded for various types of GMP documents. Electronic systems should be developed to meet guidelines described in this chapter.

This chapter does not provide information about all applicable current legal requirements, nor does it affect any applicable current requirements under GMP regulations.

PRINCIPLES OF GOOD DOCUMENTATION

All steps related to the manufacturing, testing, packaging, or holding of pharmaceutical products, APIs, excipients, dietary supplements, food ingredients, and medical devices should be documented.

Good documentation principles for manual or electronic records include the following, as applicable:

- Records should be clear, concise, accurate, and legible.
- Data entries should be recorded promptly when actions are performed.
- Backdating and postdating are not allowed.
- All corrections to the original entries should be initialed and dated, with an explanation included in cases where the reason for the change is not obvious.
- Data entries should be traceable to the person who made the entry.
- Uncommon abbreviations and acronyms should be defined.
- Controls should be in place to protect the integrity of the records.
- In the event that ink may have faded over time (e.g., thermal paper), a copy can be used with verification of its accuracy; the copy should be initialed and dated.
- Notebooks, data sheets, and worksheets should be traceable.
- An adequate documentation system is needed to ensure data integrity and availability

of current and archived records.

- Records should be retained per regulatory requirements and be readable during the retention period.
- All pages should be paginated. Attachments (supporting documents) should be paginated with a reference to the parent document.

DATA COLLECTION AND RECORDING

Formats for data collection and recording include, but are not limited to, the following:

- Paper forms, data sheets, and worksheets
- Notebooks and logbooks
- Instrument printouts
- Electronic data obtained with a system such as an electronic data system, laboratory information management system (LIMS), or electronic laboratory notebook (ELN)

All data should be permanently recorded directly and legibly when the activity is performed. If it is paper record, then it should be recorded in indelible ink. The use of transient records must not be used for documentation of GMP activities. All data entries should be traceable to who made the entry and when. Additionally, electronic records must meet the requirements of the *Code of Federal Regulations Title 21* (21 CFR), Part 11.

Any change to an entry should be made in a way that does not obscure the original entry, with an explanation in cases where the reason for the change is not obvious. Changes should be traceable to who made the change and when the change was made. For clarity, predefined correction codes may be used, for example, WD = wrong date.

Notebook pages and worksheets should be used consecutively, and information should be recorded chronologically. GMP records such as batch records, test methods, and specifications should be given unique identifiers and use version control for the documents.

All data entry fields should be completed. A single line and/or "N/A" should be drawn through portion(s) of a page that are not used. If the record is in an electronic system and the system provides traceability of who filled each field and when, the field can be left blank.

Decimals less than one should be preceded by a zero. Rounding rules and guidelines on significant figures are described in *USP–NF General Notices and Requirements*.

All dates should be expressed in a format that clearly indicates the day, month, and year.

All GMP records should undergo appropriate review and signature by a second person to confirm the accuracy, compliance, and completeness of the entries. Additional signatures may be required on the basis of local Standard Operating Procedures (SOPs) for different levels of review (e.g., performed by, verified by, checked by, reviewed by, approved by) as accountability steps.

An official record of signature and initials should exist for each employee or can be contained within the document. Controls should be in place for assigning signature approval requirements and delegation of signature authority, when needed.

In the event that verified copies of raw data have been prepared, the verified copy may be substituted for the original source as raw data.

All multiple-page data sheets or instrument printouts should be signed/initialed on the first or last page with a note indicating the total number of pages. The first page and all subsequent pages should be uniquely identified to the activity being performed, such as the notebook reference, study number, or worksheet reference.

DIFFERENT TYPES OF GMP DOCUMENTS

The following or similar documents should include the following information, but are not limited to these items.

Laboratory Records

Laboratory records should be organized to ensure that the records are concise, clear, legible, and accurate, and detail the following:

- Description of materials, such as reagents. This information typically includes the material name, manufacturer and lot number, titer or concentration, expiration date, grade (if known), and a reference to the lab notebook if prepared in the lab.
- Identification of equipment used. This information typically includes equipment name, unique control number, and calibration expiration date, as applicable.
- Procedures used.
- Measurements.
- Formulae and calculations.
- Results and conclusions.

Equipment-Related Documentation

All equipment used in manufacturing, testing, packing, or holding of a raw material, component, API, finished product, or other similar item should be maintained and qualified for its intended use. The documentation related to equipment includes:

- Policies and procedures for operation and maintenance
- Use and maintenance logs
- Calibration or qualification records
- Instrument labeling

Deviations and Investigations

All aberrations, anomalies, and exceptions related to manufacturing, testing, packing, or holding of a raw material, component, API, finished product, or other similar item should be documented. Once documented, the deviation should be evaluated and investigated, as appropriate. Procedures should be in place for documenting, evaluating, and investigating such events. Documentation of the investigation should at least include the following:

- Description of the event
- Root-cause investigation and trend data analysis
- Responsibilities of people involved in the investigation or deviations
- Corrective Action and Preventive Action (CAPA) with timelines
- Review and approval

Batch Records

A Master Batch Record (MBR) is created as a template for the manufacture of a specific product. An Executed Batch Record, based on the MBR, is used to document the steps and materials involved in the production of a specific batch of a raw material, component, API,

finished product, or other similar item. Typically, the following sections are included in a Batch Record, and should be approved by an appropriate representative from the manufacturing site or packaging site:

- Header information (e.g., product name, batch number, manufacturing site)
- Unit of operation (e.g., blending, coating, filling)
- Manufacturing process
 - Target weights (raw materials)
 - Conditions (time, temperature)
 - Deviations and investigations
- In-process sampling or testing
- Other critical information, as applicable
- Sampling plan for release, stability, and retention
- Review and approval with approval dates

Certificate of Analysis

The purpose of the Certificate of Analysis ("C of A" or CoA) is to report analytical results for a specific batch of a raw material, component, API, finished product, or other similar item. Typically, the following sections are included on a C of A and should be approved by an appropriate representative from the testing site:

- Vendor, supplier, or manufacturer information (as applicable)
- Product information (name and strength)
- Results for the specific batch, with name of test, acceptance criteria, and result for each test
- Conformance statement or equivalent
- Reference to procedure and specification document
- Reference of data source
- Approval and date
- Expiration date or retest information

Standard Operating Procedures

The purpose of an SOP is to provide directions to trained personnel regarding a given set of activities. SOPs should be clear and concise. The following sections are typically included in an SOP:

- Purpose and scope
- Instructions and procedure
- Responsibilities and roles
- Materials or equipment, as appropriate
- Definitions or references, as needed
- Review and approval
- Revision history

Protocols and Reports

Many tasks and activities are executed on the basis of a predefined, preapproved protocol. The results of these activities are then documented in a final report with conclusions. Examples of such activities are as follows:

- Equipment qualification
- Analytical method validation or verification
- Manufacturing process validation
- Analytical method or manufacturing technology transfer
- Cleaning validation
- Stability study or testing
- Comparability study

Both the protocol and the report should typically include the following sections:

- Purpose
- Plan or instructions
- Predetermined acceptance criteria
- Deviations or investigations, including impact assessment (or a reference to)
- Assessment or evaluation (for report only)
- Data reference (for report only)
- Review and approval
- Revision history

Analytical Procedures

Analytical procedures provide direction to an operator on how to perform a given analytical test. The following sections will typically be included in the analytical procedure:

- Purpose
 - Test information
 - Product information
- Safety information, if applicable
- Materials and equipment
- Procedure, as applicable
 - System suitability
 - Preparation of solutions and reagents
 - Preparation of standards and samples
 - Instrument parameters
 - Calculations and reporting
- Review and approval with approval dates
- Revision history

Training Documentation

Personnel should be trained to perform their assigned tasks. The training should be documented, and the training records should be retained and kept readily accessible. In general, training documentation should include:

- Training description including name of training, version, and mode (self-training or instructor led)
- Completion date
- Information on the trainer, as applicable

RETENTION OF DOCUMENTS

An adequate policy for record retention and archiving should be established for the above records. The required length of time depends on the regulatory requirements or company procedures; however, it should be at least 1 year after the batch expiration date. ■ 2S (USP39)

BRIEFING

⟨1063⟩ **Shear Cell Methodology for Powder Flow Testing.** This proposed new general chapter describes the use of shear cells for testing the powder flow properties of pharmaceutical ingredients and their mixtures. This chapter incorporates responses to a *Stimuli* article published previously in *Pharmacopeial Forum*, and thus it represents a consensus view on the appropriate use of shear cells for the testing of pharmaceutical powders. It is not intended as a prescriptive method, nor as a replacement for a given shear cell user manual or standard method. Instead, the chapter describes best practices for obtaining reliable and accurate powder flow data using a shear cell. A *Stimuli* article included in this *PF* provides responses to the comments received on the previously published *Stimuli* article in *PF* 39(3) [May–June 2013].

(GCPA: G. Holloway.)

Correspondence Number—C156317

Comment deadline: November 30, 2015

Add the following:

■ ⟨1063⟩ SHEAR CELL METHODOLOGY FOR POWDER FLOW TESTING

1. INTRODUCTION
2. THEORY AND PRINCIPLES
3. DESCRIPTION OF SHEAR CELL COMPONENTS AND DECISIONS
4. SHEAR CELL MEASUREMENTS
 - 4.1 Sample Preparation
 - 4.2 Instrument Preparation
5. SELECTION OF TEST CONDITIONS
6. TEST PROCEDURE
7. DATA ANALYSIS AND CALCULATIONS
8. REFERENCE MATERIALS AND REPRODUCIBILITY
9. APPENDIX

1. INTRODUCTION

A large number of pharmaceutical processes involve powder transfer and feeding, and the ability of powders to flow in a controlled manner during these operations is critical to product quality. For example, drug product attributes such as weight and content uniformity depend on powder flow characteristics. Shear cells are among the most important methods for measuring powder flow properties, and the data from shear cell testing can be used to predict a wide variety of powder flow behaviors during pharmaceutical manufacturing. Shear cells have many

advantages over simpler methods of measuring powder flow (see *Powder Flow* (1174)), but their operation is more complex and the procedures for their use must be carefully controlled to produce accurate and reproducible data. This general chapter describes best practices for obtaining reliable and accurate powder flow data using a shear cell.

This chapter focuses on the three most popular shear cell types used for measuring powder flow properties:

1. Translational (Jenike type)
2. Annular (Schulze type)
3. Rotational (Peschl type)

These three shear cell types are categorized as direct shear tests in which a region of the powder is sheared under a series of controlled normal stresses. From these data, a wide variety of parameters can be obtained, including the yield locus representing the shear-stress to normal-stress relationship at incipient flow, the angle of internal friction, the unconfined yield strength, powder cohesion, and a variety of related parameters such as the flow function. In addition, these three shear cells can be set up with wall coupons (see 9. *Appendix*) to measure the powder wall friction. When the shear cell data are combined with unconfined yield strength, wall friction data, and bulk density data, they can be used for bin and hopper evaluation and design. Other testing approaches, such as triaxial testers and indirect or hybrid testers (e.g., the Johanson Indicizer), are outside the scope of this chapter.

2. THEORY AND PRINCIPLES

The flow behavior of a powder is fundamentally different from the flow of a fluid. First, powder flow properties and shear behaviors are strongly dependent on the consolidation stresses applied to the powder and are minimally dependent on the strain or flow rate (under the assumption of quasi-static conditions such as flow in a bin). Fluid flow, in contrast, is strongly dependent on the strain rate (where viscosity describes the relationship between shear stresses and strain rates) and is minimally dependent on absolute pressure. Second, when shear stresses are applied to powders, they may not immediately fail (that is, they can avoid flow under a sustained shear stress), whereas Newtonian and viscoelastic fluids do not behave this way and always flow under an applied shear stress. Thus, powders have the potential for arching and ratholing, depending on the flow pattern (see 9. *Appendix*).

However, powders can sustain a shear stress without flowing only up to a certain point. The yield locus for a given powder is a function of many variables, including its composition, particle size and shape, moisture content, temperature, time stored at rest, and the state of consolidation. Once a powder is subjected to stresses (whether by gravity or some mechanical means) that reach or exceed the yield locus, the powder flows. Hence, determining the yield locus for a given powder under conditions representative of its manufacturing process is an essential step in evaluating the flow behaviors for that process. In some circumstances, this may involve testing under controlled environmental conditions, as well as holding the powder under load for an extended period before shearing (a "time test").

Because powder properties are highly dependent upon the degree of consolidation, the preparation of a uniform powder bed (consistent bulk density throughout the powder bed) is

the first critical step of shear cell testing. The next stage of testing is the application of a normal stress (Σ) and shear stress (τ) to the powder bed to achieve steady-state shear, resulting in a known state of consolidation. The shear stress then is removed, and a reduced normal stress is applied. A shear stress then is applied and is progressively increased until the powder bed yields and begins to flow. This procedure is repeated at several different normal stress conditions to create a "yield locus" plot. To complete a full flow function analysis, the operator must determine several yield loci, which requires that the unconfined yield strength be determined under several different levels of consolidation.

Although this chapter focuses on the powder (particle-particle) properties, the wall (particle-wall) properties and bulk density are also important. Such properties are used for bin design and also are essential when one compares different wall materials (e.g., different grades and finishes of stainless steel, or the effect of plastic coatings on powder flow behaviors). The most fundamental property of a wall material in this regard is ϕ' , the angle of friction between the bulk powder and the wall material, or, correspondingly, the coefficient of wall friction (μ_w):

$$\mu_w = \tan(\phi') = \tau_w / \Sigma_w$$

ϕ' and μ_w = function of the applied stress (Σ_w)

τ_w = wall shear stress

Σ_w = wall normal stress

3. DESCRIPTION OF SHEAR CELL COMPONENTS AND DESIGNS

Figures 1–3 provide schematics of the different types of shear cells considered in this general chapter. All have the same general operating principle of being able to measure the force required to shear a powder bed to which a normal load has been applied. The applied load or measured force can be expressed as a stress by dividing by the cross-sectional area of the shear plane under consideration.

The translational shear cell (*Figure 1*) has a fixed base with a movable ring above it, and both hold the bulk powder. A cover that fits within the ring is used to contain the powder and to provide a uniform application of the normal load (N). The ring and cover are pushed as a unit while a load cell records the shear forces (F) that are generated. The shear plane forms between the powder that is contained in the base and the powder contained in the ring.

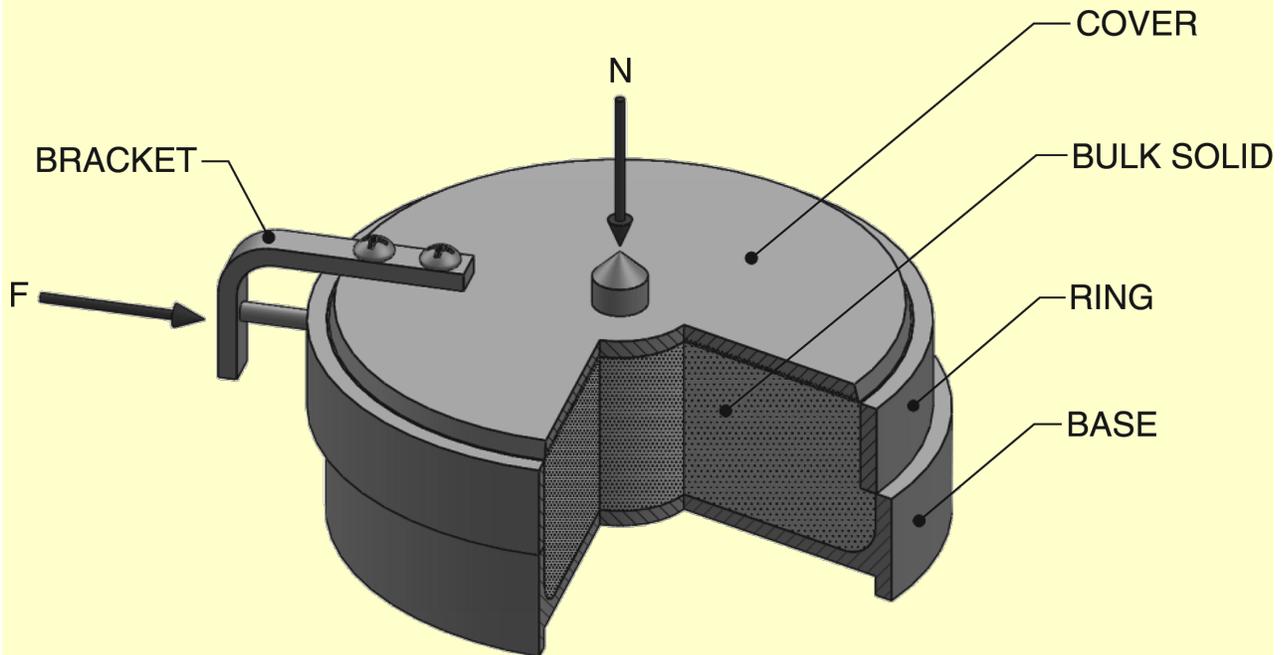


Figure 1. Descriptive schematic of the translational shear cell.

The annular shear cell (*Figure 2*) consists of a shear cell or base that holds the powder. A cover that fits within the cell is used to contain the powder and to provide a uniform application of the normal load (N). The cover is free to move up and down, but otherwise remains fixed in place by a load cell that measures the shear forces (F) that are generated. The shear cell is rotated at a constant angular velocity (Ω) to create a shear plane that forms in the powder bed somewhere between the cell bottom and the cover. The cover and shear cell usually have baffles or other surface features that prevent the powder from sliding or shearing at the interface between the powder and the cover or shear cell base.

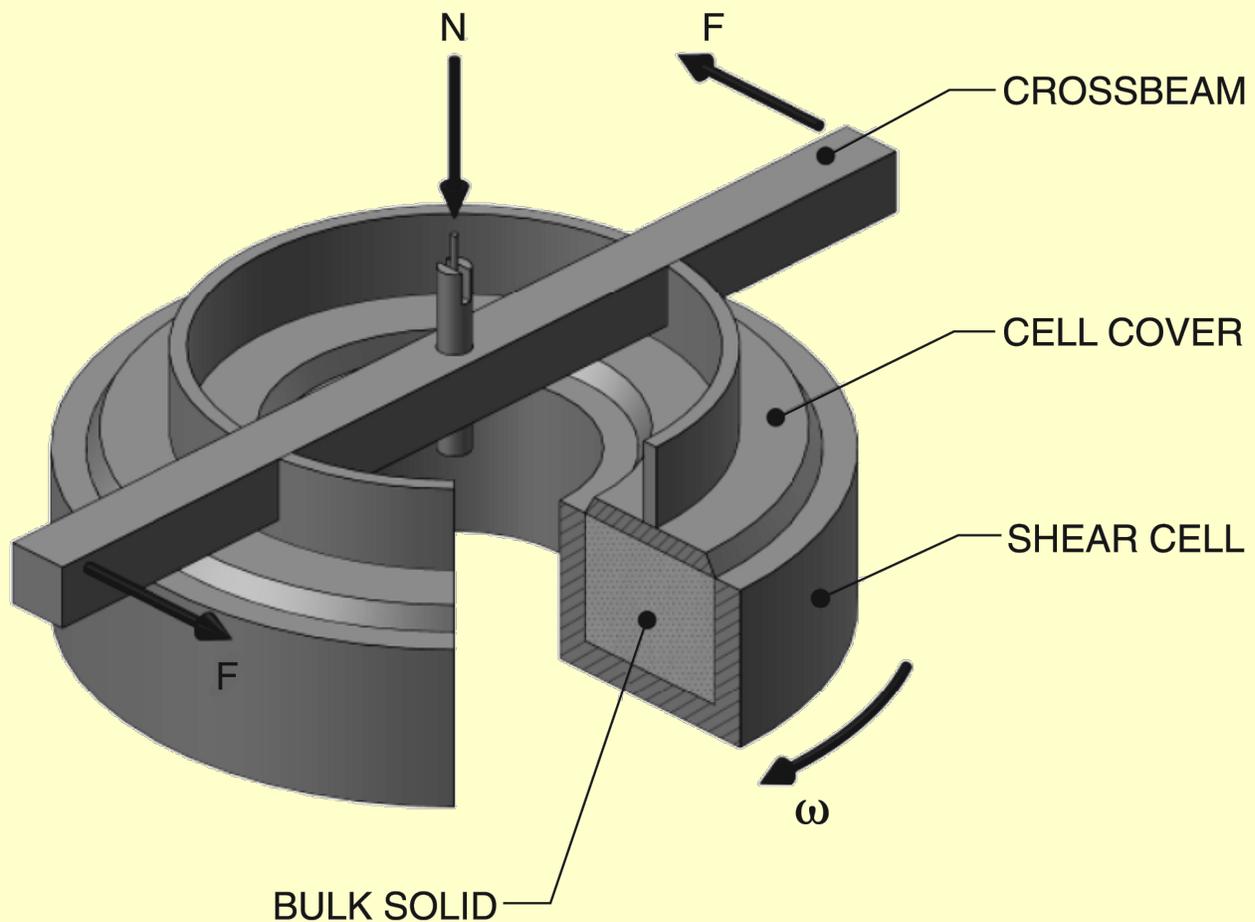


Figure 2. Descriptive schematic of the annular shear cell.

The rotational shear cell (*Figure 3*) has a base and ring that hold the powder. A loading lid that fits within the ring is used to contain the material and to provide a uniform application of the normal load (N). The loading lid is free to move up and down, but otherwise remains fixed in place, connected to a load cell that measures the shear forces (F) that are generated. The shear cell base then is rotated at a constant angular velocity (Ω) to create a shear plane that forms in the powder bed somewhere between the ring and the base. Alternatively, the base can be fixed, and the lid can be rotated to create the shear plane in the powder specimen. The ring and base usually have surface features that prevent the powder from sliding at the powder-surface interface.

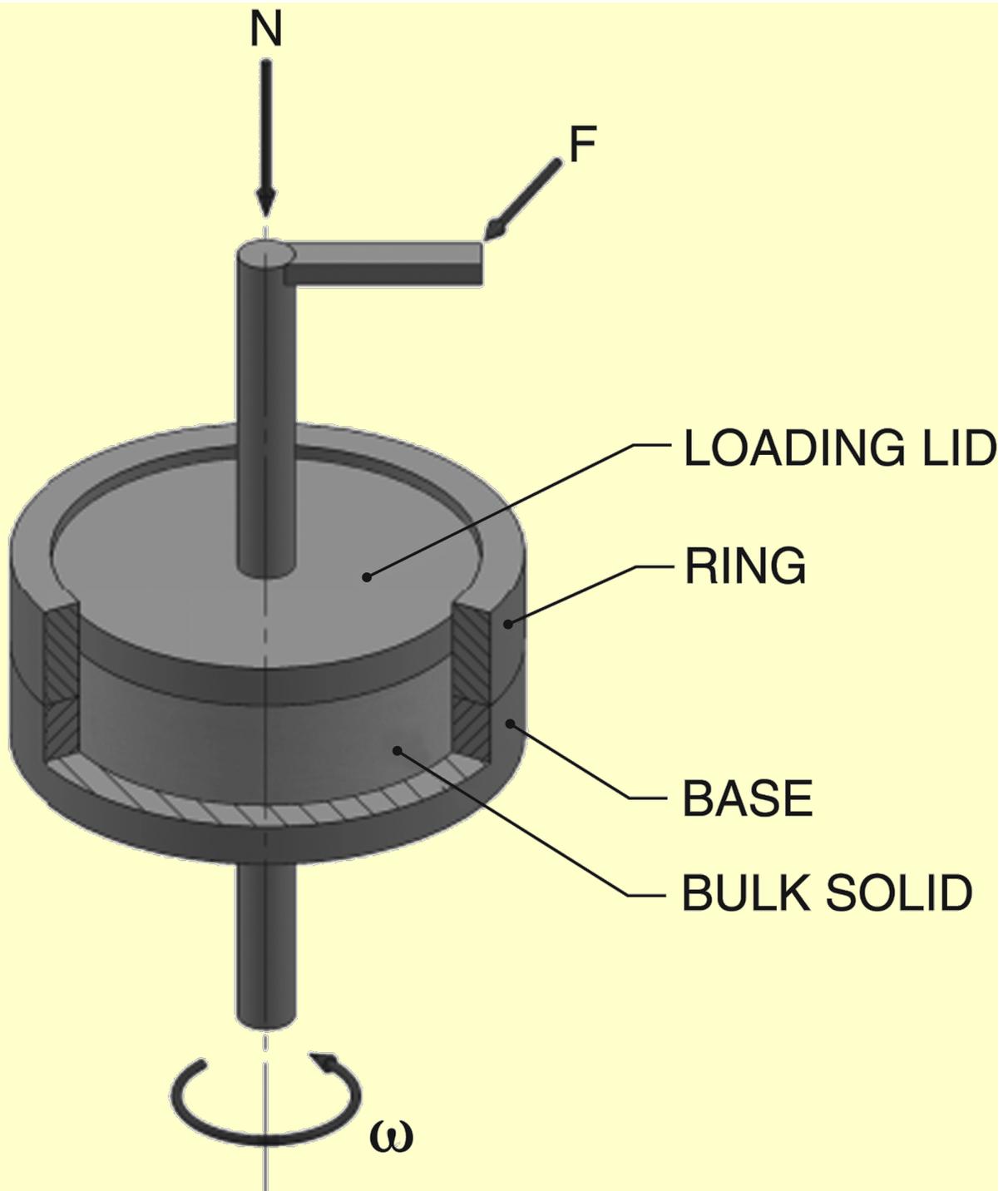


Figure 3. Descriptive schematic of the rotational shear cell.

The choice of one shear cell type over another depends on factors such as the availability of equipment and ease of use for a particular technician. Because of the infinite travel permitted with the annular and rotational shear cell geometry, these types of cells are more suited for powders for which reaching steady state requires large strains. Another advantage of the infinite travel of rotational testers is that a preconsolidation step may not always be required. Wall friction tests can be carried out using all of the shear cells referenced in this chapter with only minor modifications, namely by creating a shear plane in which the powder slides over a suitable wall coupon rather than having the shear plane within the powder bed. This usually is

achieved by replacing the base of the shear cell with a coupon of the wall material and adjusting the amount of powder used to create a shear plane at the powder-coupon interface.

4. SHEAR CELL MEASUREMENTS

4.1 Sample Preparation

The applicability of shear cell data depends strongly on the use of a representative powder sample. Detailed discussion of appropriate sampling procedures can be found in *Bulk Powder Sampling Procedures* (1097).

The cell size (volume and diameter) usually is selected based on the maximum particle size of the powder sample, and the proportion of large particles in the sample. The particle size limit is usually reported by the supplier of commercially available testing equipment. For a translational cell, the maximum particle size is approximately 5% of the diameter of the cell, and for an annular cell the maximum particle size is approximately 10% of the width of the annulus, i.e., the difference between the outer and inner radius of the shear cell trough. Fresh powder is desired for each test, if possible, so the use of a smaller cell may be preferable to reusing the powder. Under some circumstances, a smaller cell may be needed to reach higher consolidation stresses. Smaller cell sizes may have reduced precision and increased bias, depending on the specific tester and the cell size under consideration, because of the larger contribution of non-idealities caused by wall effects.

The shear strength of some powders (such as fibrous or flaky solids) is often caused by interlocking of particles; therefore, such materials may prove to be unsuitable for testing with a conventional shear cell. Likewise, powders with high springback (rubberlike or highly elastic properties) sometimes can fall outside of the practical limits of a shear tester. Poor reproducibility and unexpected results may indicate a problem with testing these types of powders.

Powder samples must be handled and tested under conditions that are relevant from a practical standpoint. For example, many pharmaceutical powders will sorb/desorb moisture from their surroundings according to the ambient relative humidity and temperature. This sorbed moisture can significantly affect the measured powder flow properties. Controlling the environmental conditions during sample handling and testing so that sample behavior is representative of the processing conditions of interest is essential for most powders.

Some pharmaceutical powders "age" quickly after they are produced, and such powders may need to be tested immediately after they are manufactured. The testing of some powders may result in caking or particle attrition that can render a powder unsuitable for retesting. In these circumstances, fresh powder samples should be used for each test.

4.2 Instrument Preparation

The shear cell should be situated in an area that is free of vibrations. Vibrations can affect the instrument readings and also can densify or dilate the powder while it is being tested.

Because shear testers measure forces, their load cells must be calibrated for the forces being measured. Some testers also measure the displacement (linear or angular) of the cell (indicating travel) and/or the vertical displacement of the cover or lid (used to calculate the volume of the cell and hence an average bulk density). In these cases, the displacement transducers must

also be calibrated. The rate of any changes in force and displacement will need to be confirmed as part of this calibration. In addition, the shear cell is operated under the assumption that all of the parts are precisely aligned, and there is a minimal degree of wobble when the cell rotates. For example, if the cover and shear cell trough are not parallel during testing, it will be difficult to get reproducible results.

Thus, the alignment and mechanical operation of the shear cell must be evaluated at regular intervals. No other adjustments with respect to calibration are performed, although the correct performance of the apparatus may be confirmed by the measurement of the yield locus of a reference powder under standardized test conditions.

Wall coupons used for testing must be representative of the surfaces upon which the powder will slide. Directional surfaces can be either oriented in the direction of flow in the application (e.g., grain oriented down the length of a hopper), oriented in the worst-case condition (often, grain oriented perpendicular to the direction of flow in the hopper), or evaluated in both directions.

All shear cell components (including wall coupons) should be carefully cleaned before use with a method that does not abrade the surface or leave any chemical residue behind. In addition, the cleaning method should be capable of removing all of the components of the test sample, including lubricants and other additives.

5. SELECTION OF TEST CONDITIONS

Preshear normal stresses usually are selected based on the powder's density values. Standards, such as ASTM Standard 6128, provide tables of specific initial preshear normal stresses as a function of the sample bulk density.¹ Subsequent preshear normal stresses are given in multiples of the initial preshear value (such as two, four, and eight times the initial level). The number of preshear normal stress levels should be at least four. It is often valuable to match preshear stresses in the test to the stresses expected in the processing situation of interest. Hold times for time tests are selected based on matching the hold time in the practical application. If long hold times are unrealistic, operators may consider several intermediate time points and extrapolation of the data.

Normal stress levels for shear are selected to provide a range of data points on the yield locus. Typically, a range between 25% and 80% of the preshear normal stress is valid, although powders with high unconfined yield strength or internal friction may require a narrower range. The range of normal shear stress levels should be sufficient to allow meaningful fitting and extrapolation of the data to determine the unconfined yield stress and other related parameters.

6. TEST PROCEDURE

Shear cell testing involves a sequence of steps that consolidates the powder to a known extent and then shears it under carefully controlled conditions while recording the applied normal stresses and measuring the shear stresses. In most cases, the essential steps are as follows:

1. Fill the test cell with an appropriate and representative sample of the powder in a manner that provides a uniform bulk density and composition. This sample in the test cell is referred to as the specimen. Detailed procedures vary according to the type of

tester being used, but in all cases it is essential to evenly distribute the powder and avoid pockets of air that can be difficult to remove later.

2. For translational shear cells, perform a preconsolidation step to create the desired density in the cell. This is accomplished by twisting the cover while under a compressive normal load. This step reduces the travel needed to achieve a steady-state preshear value and can limit the total vertical displacement of the cover during the test. A preconsolidation step may be conducted in other cell types, but this is often not conducted, because the unlimited rotation of the annular and rotational cells can generally provide a sufficient state of consolidation. The appropriate level of preconsolidation is critical, and care must be taken not to over- or under-consolidate the specimen.
3. Consolidate the specimen by applying a known normal stress to the powder specimen via the cell cover/lid.
4. Preshear the specimen until a steady-state shear value is reached. Care should be taken to avoid over-consolidating the specimen. The applied shear stress is then reduced to zero.
5. An instantaneous shear test is run by shearing the specimen under a reduced normal stress (with respect to the applied preshear stress) until the shear stress goes through a maximum value and then begins to decrease.

Steps 1–4 are repeated at a series of different reduced normal stress conditions to create a complete set of data (yield locus) and then at a series of different preshear normal stress conditions to create a “flow function”. The test cell is preferably emptied and refilled before generating each point on the yield locus. For annular or rotational shear cell types, it is common that steps 3–5 are repeated a number of times for the same specimen without emptying and refilling the cell. If the same specimen is used for multiple test points, caution must be taken to ensure that the specimen has not changed from test to test. A wall friction test is run in an analogous manner by sliding the specimen over a coupon of wall material and measuring the shear stress as a function of the applied normal stress. In a time test, a normal stress is applied to the specimen for a predetermined period of time before shearing. Both wall-friction and time-consolidation tests can be conducted with the three types of shear cells described in this chapter, as long as directionality (if any) of the wall surface, relative to the cell movement, is taken into account.

7. DATA ANALYSIS AND CALCULATIONS

Illustrative results from the shear cell test procedure are presented in *Figure 4*. For analysis, the applied preshear normal stress (Σ_p) and all valid applied normal stress points (Σ_{si}) for $i = 1, 2, 3, \dots, n$ and their corresponding measured shear stress points $\tau_p, \tau_{s1}, \tau_{s1}, \dots, \tau_{sn}$ are plotted in (Σ, τ) -coordinates. These data are then used to generate a smooth line through all the valid shear points to obtain the yield locus. Typically, the yield locus passes above or through the preshear point. If not, the test results should be analyzed in more detail. In some circumstances, the yield locus can be forced to pass through the preshear point and can be

fitted to all yield points.

The “unconfined yield strength” of the powder is obtained by drawing a Mohr circle through the origin and tangential to the yield locus. The higher point of intersection of this Mohr circle and the Σ -axis is the unconfined yield strength f_c . The “major consolidation stress”, Σ_1 , is found by drawing a second Mohr circle through the preshear point and tangential to the yield locus. The higher point of intersection of this Mohr circle (consolidation locus) and the Σ -axis is the major consolidation stress.

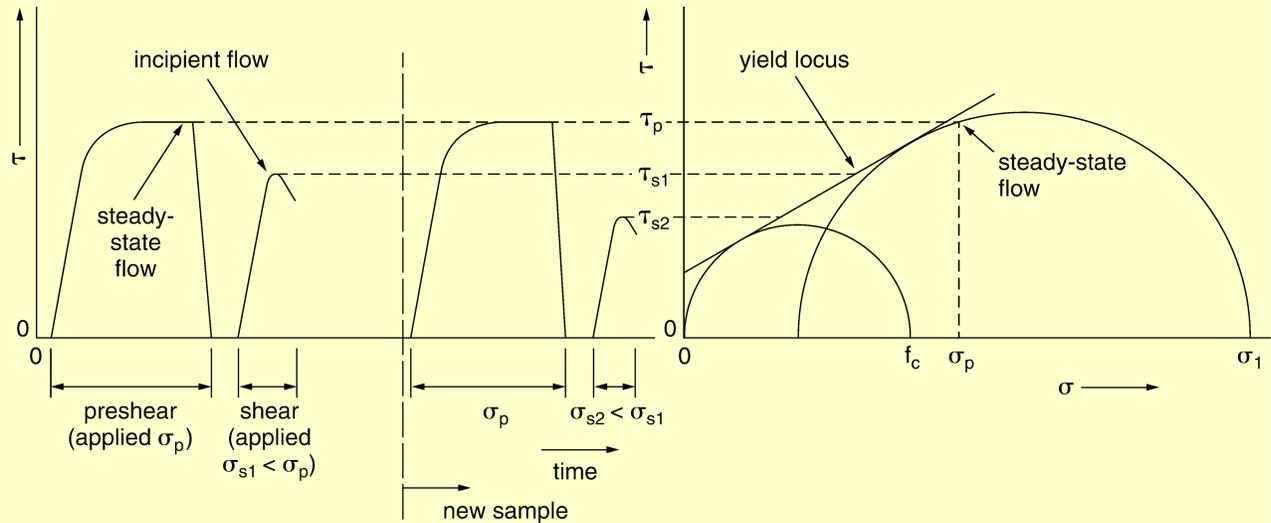


Figure 4. Shear cell testing and data analysis: shear cell raw data (left) and yield locus calculated from raw data (right).

The “angle of internal friction”, ϕ , is defined as the slope of the yield locus, as shown in Figure 5. A line drawn through the origin and tangential to the steady-state Mohr circle has an angle, Δ , that is defined as the “effective angle of friction”. The “cohesion,” C , is the intersection of the yield locus and the τ -axis.

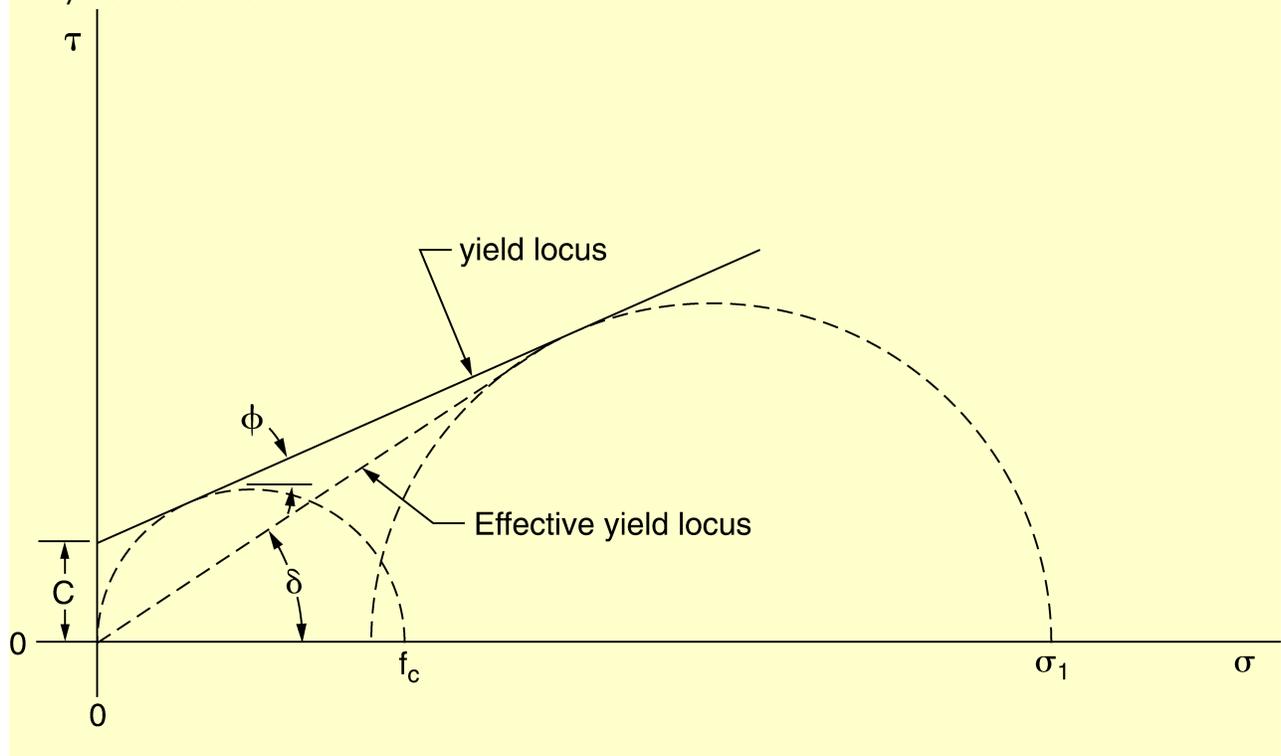


Figure 5. Graphical representation of the angle of internal friction (ϕ), effective angle of friction (Δ), and cohesion (C).

From a family of yield loci generated at different preshear normal stresses, it is possible to plot the unconfined yield strength, f_c , as a function of the major consolidation stress, Σ_1 , as shown in Figure 6. The best-fit curve to these points is called the “flow function” and can be used to calculate the potential for arching and ratholing in a storage bin.

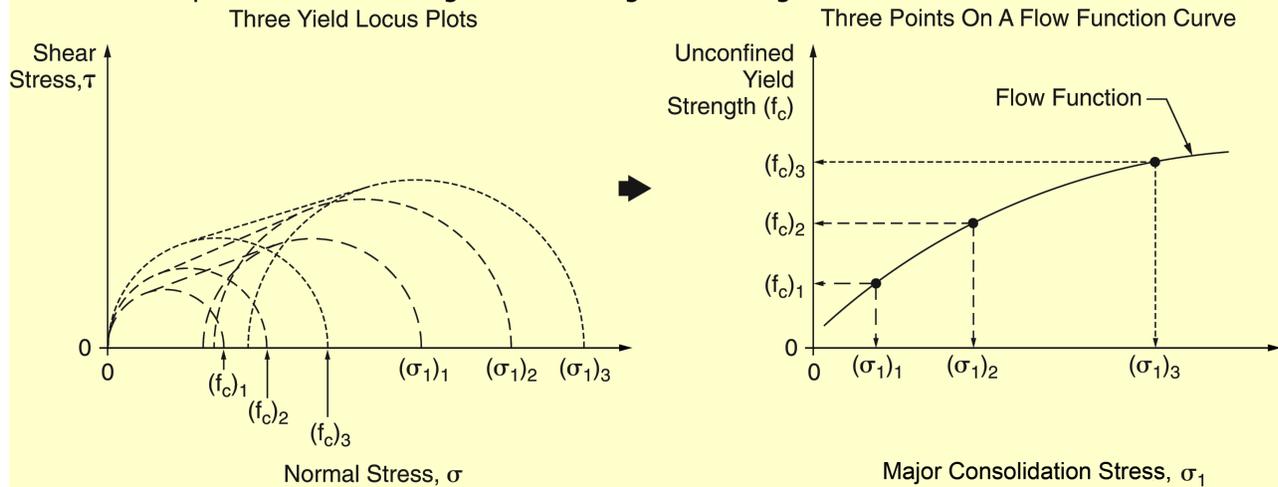


Figure 6. Calculation of the flow function from multiple yield locus plots.

The “kinematic wall yield locus” is developed in a manner similar to the calculation of the yield locus. This is depicted in Figure 7. The “wall friction angle”, ϕ' , is defined as the inclination of a line from the origin to a point on the wall yield locus. In general, low wall friction angles are desirable.

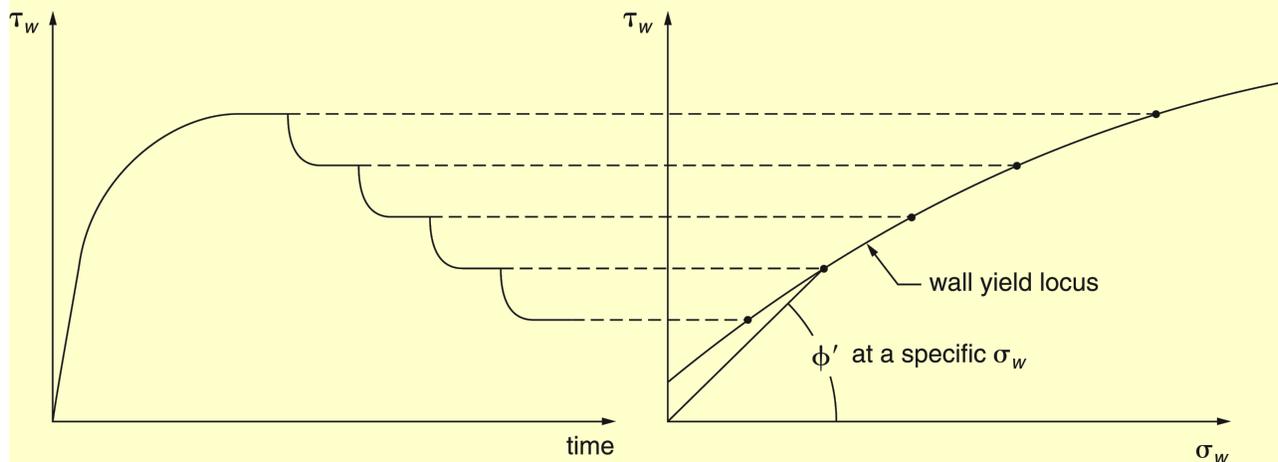


Figure 7. Calculation of the wall yield locus.

Test conditions should be carefully documented for every shear cell analysis to allow for accurate data interpretation and meaningful data comparisons. When analysts compare multiple powders—matching the tester type, specific tester model, test cell size, sample preparation, test procedures, applied normal stresses, and environmental conditions—increased confidence is provided that any differences in the results are attributable to the powder and not to the test. Changes in material sourcing or manufacturing may require the repetition of the initial studies.

Shear cell tests allow the analyst to characterize consolidated powders under quasi-static conditions. The tests are unable to directly measure powder flow properties at very low stress conditions or high shear rates, which may occur in some powder handling situations. Thus, no single parameter can describe powder flowability. Instead, the parameters described in this general chapter should be interpreted together and in the context of the conditions under which the powder will be stored and handled (e.g., equipment dimensions, environmental conditions, and others).

The ratio of the major consolidation stress Σ_1 to the unconfined yield strength f_c at a particular value of the major consolidation stress provides a general, simplified way of assessing powder flowability. As a general case, the flow of powdered materials is classified in *Table 1*:

Table 1. General Classification of Flow Character³

Σ_1/f_c	Flow Character
<2	Very cohesive, nonflowing
2-4	Cohesive
4-10	Easy-flowing
>10	Free-flowing

This approach can be useful for rank ordering the flow character of pharmaceutical powders as part of formulation and process development activities. However, it should be noted that this simplified view may lead to significant errors in interpretation, because this approach does not take into account the flow pattern, or the size or geometry, of the manufacturing equipment being used.

8. REFERENCE MATERIALS AND REPRODUCIBILITY

For a tester that is installed and operated correctly, the performance of the instrument and operator can be assessed by testing a standard material. There is currently no specific pharmaceutical reference material for shear cell test method verification. Limestone powder has been used as a certified reference material.⁴

Figures 8, 9, 10, 11, 12, and 13 show representative yield loci and flow function plots for several common pharmaceutical excipient powders determined at a range of conditions. The plots are provided to illustrate the type of data that can be generated for pharmaceutical powders using a shear cell and the typical reproducibility of the data. The data in *Figures 8, 9, 10, and 11* were collected using a procedure consistent with that described for *Figure 4*. These plots are not intended for use as reference data, and they should not be assumed to be representative of all test conditions and material types.

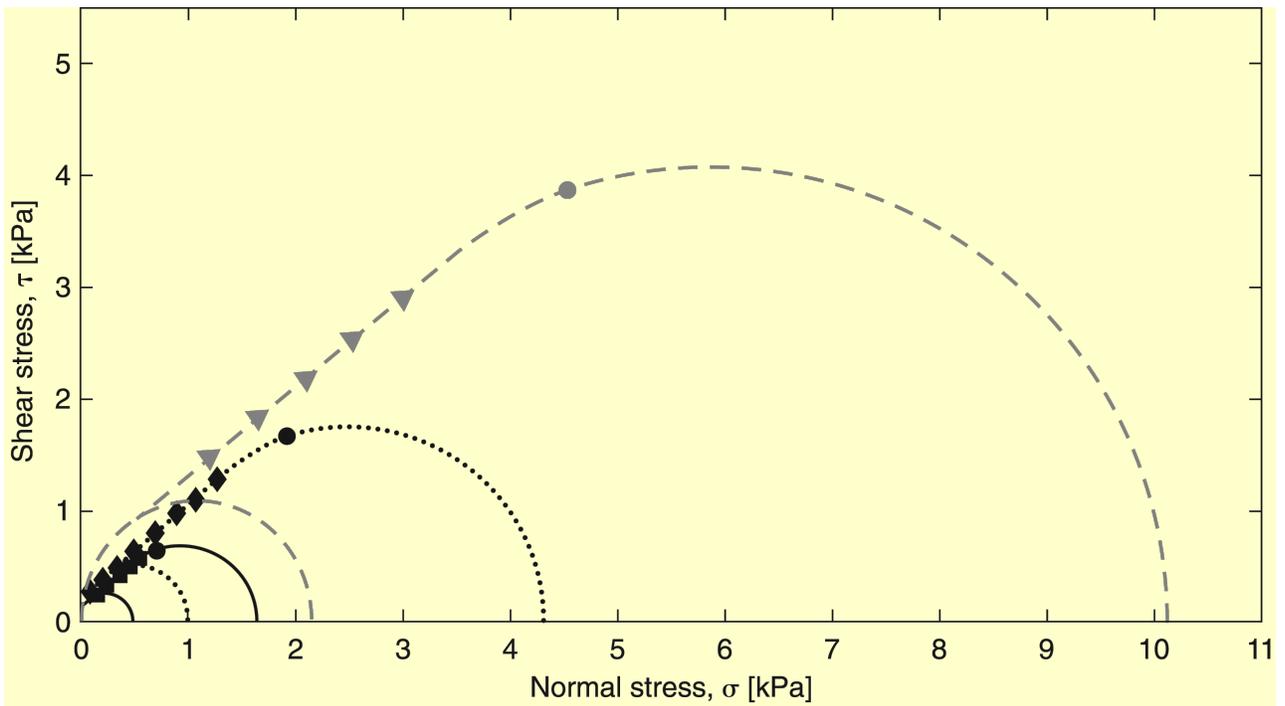


Figure 8. Representative yield loci for microcrystalline cellulose NF (Avicel PH-101) at preshear stresses of 730 Pa (solid), 1.9 kPa (dot), and 4.6 kPa (dash).

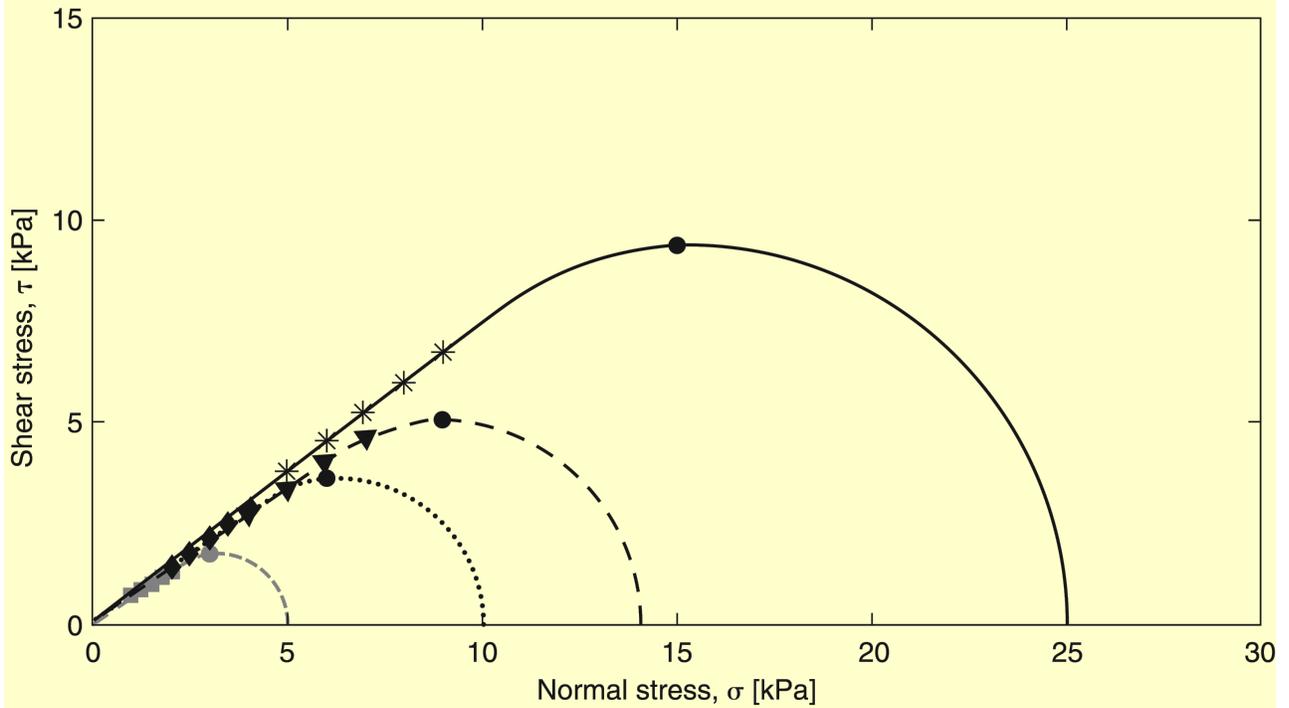


Figure 9. Representative yield loci for sorbitol at preshear stresses of 3 kPa (gray, dash), 6 kPa (dot), 9 kPa (black, dash), and 15 kPa (solid).

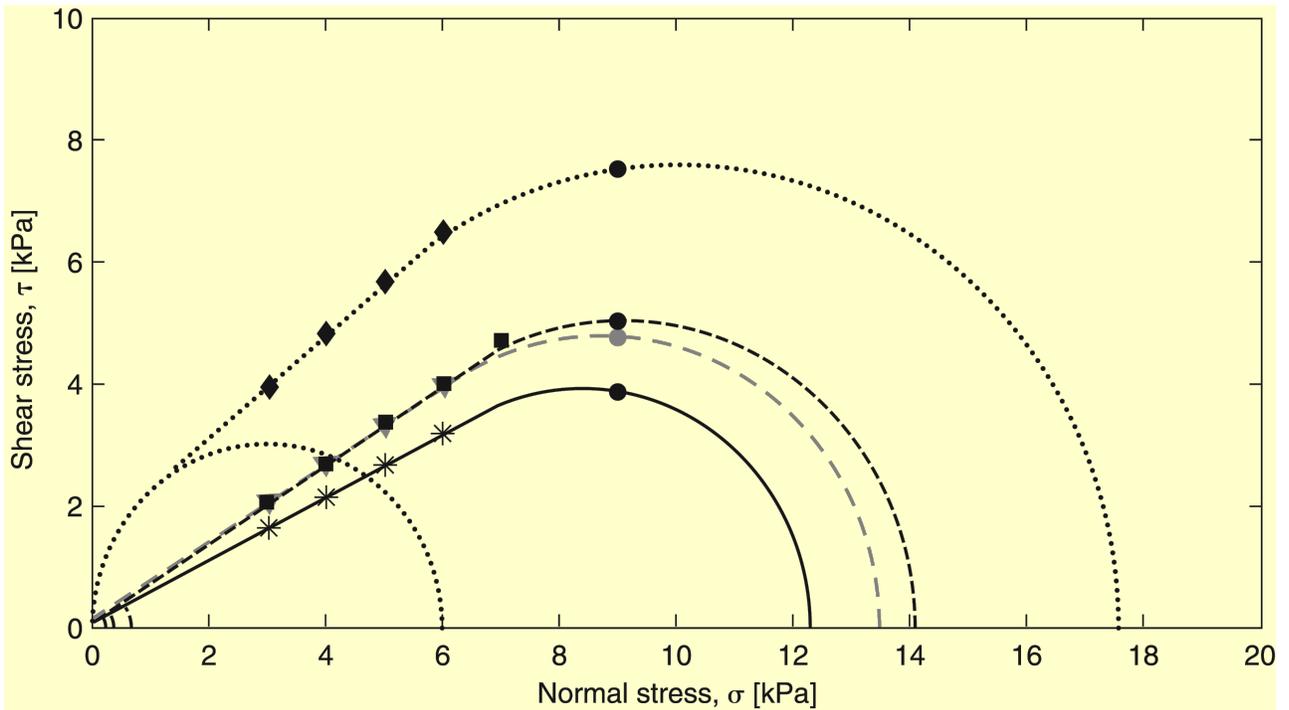


Figure 10. Representative yield loci for mannitol (*dot*), sorbitol (*black, dash*), sieved lactose (*gray, dash*), and spray-dried lactose (*solid*) at a preshear stress of 9 kPa.

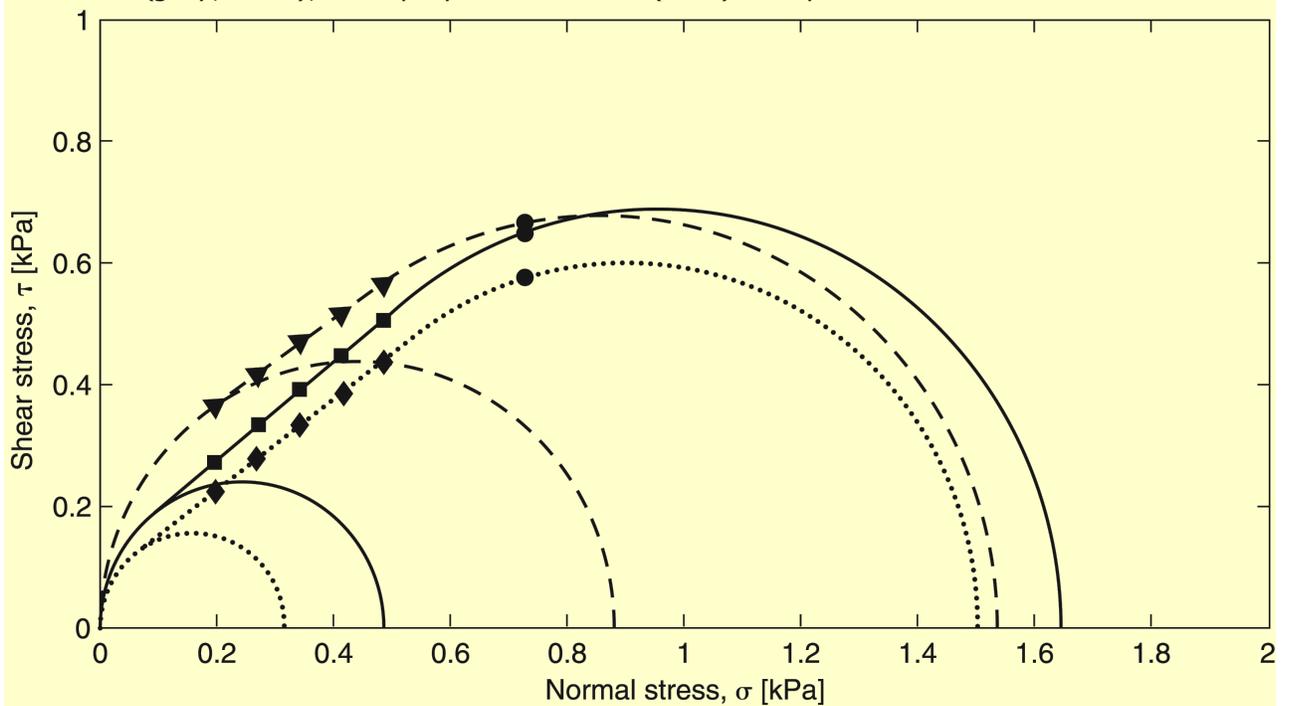


Figure 11. Representative yield loci for several different grades of microcrystalline cellulose NF. Avicel PH-101 (*solid*), Avicel PH-102 (*dot*), and Avicel PH-105 (*dash*) at a preshear stress of 730 Pa.

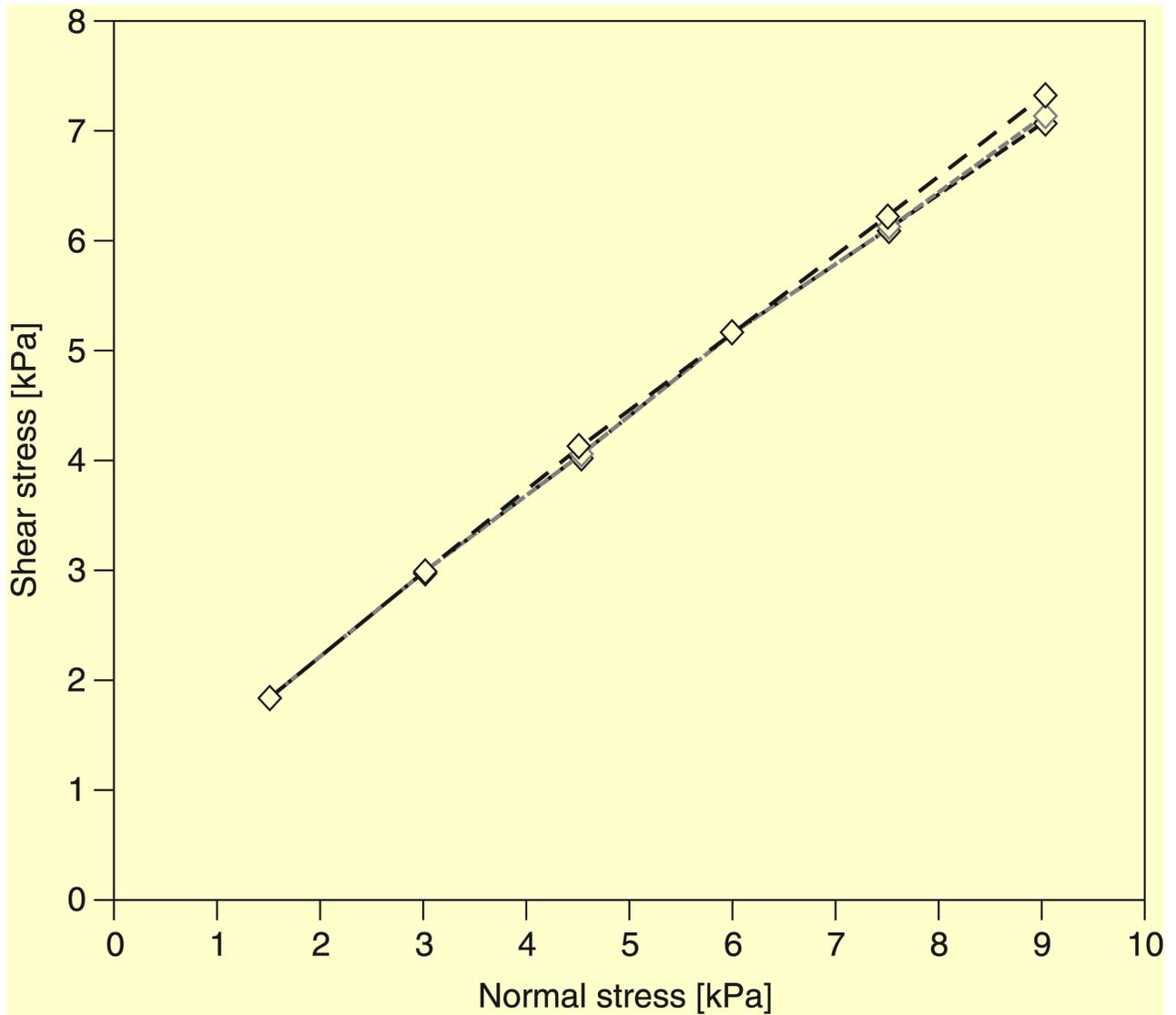


Figure 12. Representative yield loci for microcrystalline cellulose (Avicel PH102) from three independent determinations conducted at a preshear stress of 9 kPa. [Figure adapted from Sun CC. Setting the bar for powder flow properties in successful high speed tableting. *Powder Technol.* 2010;201(1):106-108.]

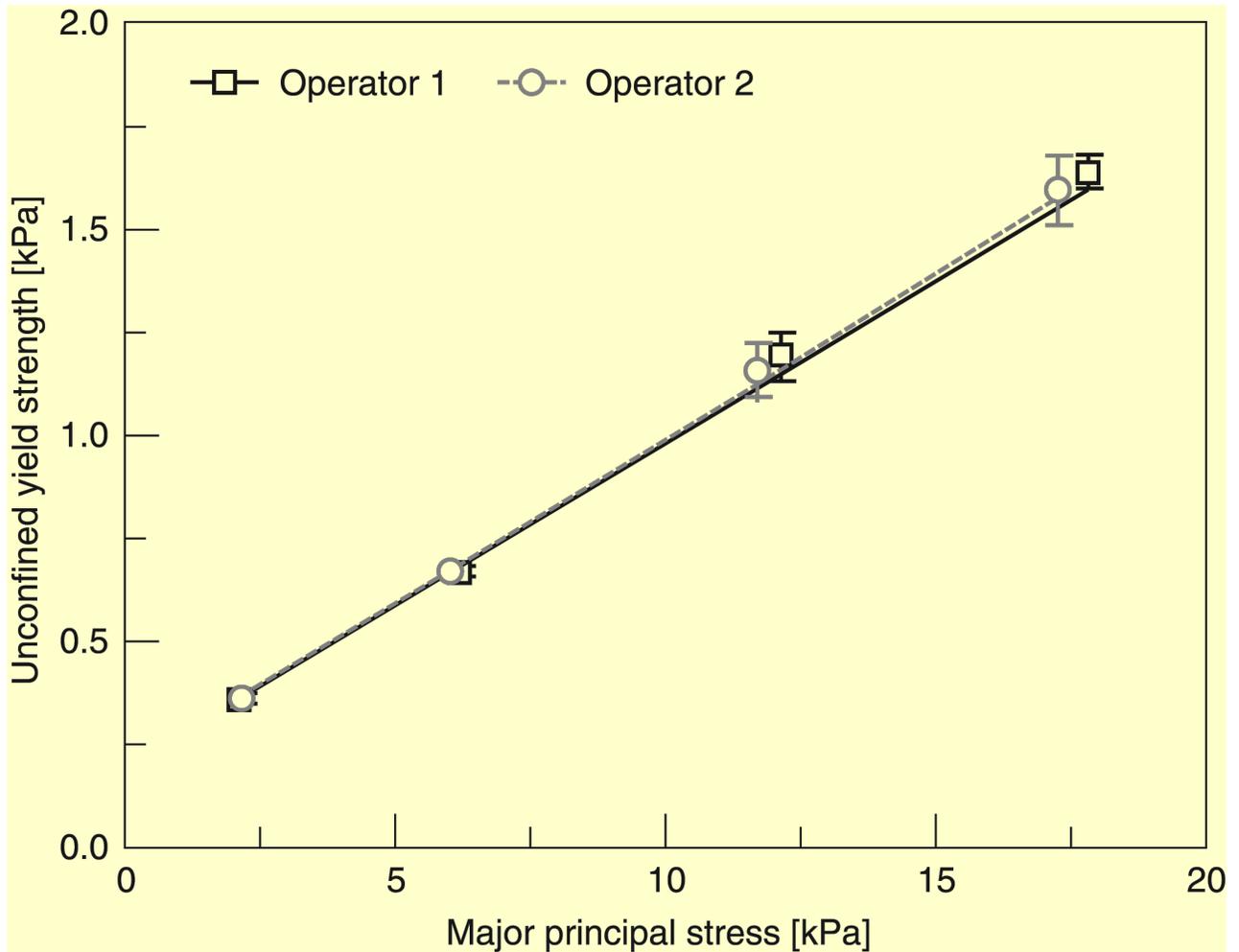


Figure 13. Representative flow function plots for microcrystalline cellulose (Avicel PH102) from two independent operators [Figure adopted from Shi L, Chatteraj S, Sun CC. Reproducibility of flow properties of microcrystalline cellulose—Avicel PH102. *Powder Technol.* 2011;212(1):253–257.]

9. APPENDIX

Notation

Term	Symbol and SI Units	Definition	Comments
Angle of internal friction	ϕ , degrees	The inclination of the yield locus (<i>Figure 5</i>).	
Angular velocity	Ω , degrees per second	The angular velocity at which the shear cell base rotates.	Is usually held constant.
Annular shear cell	NA	A shear cell based on a rotating annulus design (<i>Figure 2</i>).	The Schulze Ring Shear Tester is a common annular shear cell.

Term	Symbol and SI Units	Definition	Comments
Arching	NA	The formation of a bridge of powder across an opening caused by attractive interactions between particles.	Also known as "bridging".
Bulk density	ρ , kg/m ³	The mass of a quantity of a powder divided by its total volume.	Varies with the applied normal stress and history of a sample.
Cohesion	C , Pa, kPa	The failure shear stress at zero normal stress, normally obtained by extrapolation of the yield locus (<i>Figure 5</i>).	An indication of the intrinsic strength of an unconsolidated powder.
Consolidation	NA	The process of increasing the density of a powder, which usually results in increasing its unconfined yield strength. Achieved in a shear cell by applying a preshear normal load and shear load to the specimen.	
Coupon	NA	Flat surface that is in contact with powder specimen during wall friction testing.	The coupon surface finish must be representative of the wall surface of interest.
Effective angle of friction	Σ , degrees	The inclination of the effective yield locus (<i>Figure 5</i>).	Sometimes used as a measure of relative flowability.
Effective yield locus	NA	The straight line passing through the origin of the Σ - τ plot and tangential to the steady-state Mohr's circle, corresponding to steady-state flow conditions of a powder of given bulk density. (<i>Figure 5</i>).	
Flowability	NA	A qualitative estimate of the relative flow properties of a powder.	Often based on measured values of the flow function or effective angle of friction.

Term	Symbol and SI Units	Definition	Comments
Flow function	NA	The plot of unconfined yield strength versus major consolidation stress for one specific powder (<i>Figure 6</i>). Quantified as the ratio of the major consolidation stress to the unconfined yield strength at a particular value of major consolidation stress.	
Funnel flow	NA	A flow pattern where an active flow channel forms through stagnant material.	
Lid	NA	Cover of the cell containing the specimen within a shear cell.	The lid position can be measured to determine displacement, bulk density, and powder dilation.
Major consolidation stress	Σ_1, Pa	The major principal stress given by the steady-state Mohr circle. This circle is tangential to the yield locus and effective yield locus and passes through the preshear point. (<i>Figure 4</i>).	
Mass flow	NA	A flow pattern in a converging hopper where all material is in motion, including material sliding along the hopper walls.	
Mohr's circle	NA	A graphical representation of a state of stress in coordinates of normal and shear stresses (<i>Figure 4</i>).	
Normal stress	Σ, Pa	The stress acting normally (perpendicularly) to the considered plane.	Σ_p is the preshear normal stress. Σ_{Si} for $i = 1, 2, 3, \dots$ the test normal stresses.
Preconsolidation	NA	Normal stress applied to consolidate a powder before testing in some shear cells (<i>Figure 4</i>).	
Preshear	NA	Application of normal stress while shearing to attain steady-state shear conditions (<i>Figure 4</i>).	
Ratholing	NA	Tendency of powder to flow only in the region above an opening, thus forming a narrow rathole in the powder.	Also known as "core flow".

Term	Symbol and SI Units	Definition	Comments
Rotational shear cell	NA	A shear cell operating under rotational shear conditions (that is, rotational shearing perpendicular to the applied normal stress). (<i>Figure 3</i>).	The Peschl and Freeman shear cells are common rotational shear cells.
Shear stress	τ , Pa	The stress required to shear the powder in a direction perpendicular to the normal stress.	
Time consolidated strength	f_{ct} , Pa	The unconfined yield strength of a powder after being held at fixed consolidation conditions for a certain time.	
Time yield locus	NA	The yield locus of a powder that has remained at rest under a given normal stress for a certain time.	
Translational shear cell	NA	A shear cell operating under translational shear conditions (that is, linear shearing perpendicular to the applied normal stress). (<i>Figure 1</i>).	The Jenike shear cell is a common translational shear cell.
Unconfined yield strength	f_c , Pa	The major principal stress of the Mohr stress circle that is tangential to the yield locus when the minor principal stress is zero (<i>Figure 4</i>).	
Wall friction angle	ϕ' , degrees	The arctan of the wall friction coefficient (<i>Figure 7</i>).	
Wall friction coefficient	μ_w	The ratio of the wall shear stress to the wall normal stress at a given normal stress condition.	
Wall yield locus	NA	A plot of the wall shear stress versus wall normal stress. Used to determine the wall friction angle for a powder-wall combination (<i>Figure 7</i>).	
Yield locus	NA	A plot of shear stress versus normal stress at failure. The yield locus sometimes is called the instantaneous yield locus to differentiate it from the time yield locus (<i>Figure 4</i>).	A powder exhibits a series of yield loci for each different level of consolidation (density).

■ 2S (USP39)

¹ ASTM. D6128-06. Standard test method for shear testing of bulk solids using the Jenike shear cell. West Conshohocken, PA: ASTM; 2006.

² For a given normal stress applied, the measured steady-state shear stress values should be consistent from test to test. To account for normal experimental variations, a given measured shear stress value may be prorated in an appropriate fashion, relative to the steady-state value associated with that point.

³ Jenike A. Storage and flow of solids: bulletin no. 123 of the Utah Engineering Experiment Station. Salt Lake

City, UT: University of Utah; 1964.

⁴ Limestone powder certified reference material (BCR-116) is available from the Institute for Reference Materials and Measurements of the European Commission and instrument suppliers.

BRIEFING

(1228.3) **Depyrogenation by Filtration.** The General Chapters—Microbiology Expert Committee proposes this new general chapter as an addition to the *Depyrogenation* (1228) family of chapters. Production of parenteral products requires not only sterile manufacturing but also processes that prevent harmful levels of pyrogens. Effective destruction or removal of pyrogens, or depyrogenation, depends on the product that has been manufactured and the proposed method of removal and/or destruction. Liquid products can be depyrogenated through filtration. This chapter provides an overview of that process and the various approaches and technologies used to complete it.

(GCM: R. Tirumalai.)

Correspondence Number—C161851

Comment deadline: November 30, 2015

Add the following:

■ (1228.3) DEPYROGENATION BY FILTRATION

1. INTRODUCTION
2. TECHNOLOGIES USED FOR DEPYROGENATION BY FILTRATION
 - 2.1 Microporous Membrane Filtration
 - 2.2 Reverse Osmosis
 - 2.3 Ultrafiltration
 - 2.4 Charge-Modified Depth Filters
 - 2.5 Activated-Carbon Depth Filters
 - 2.6 Membrane Adsorbers
3. VALIDATION
4. REFERENCES
5. APPENDIX
 - 5.1 Additional References

1. INTRODUCTION

Endotoxins are lipopolysaccharides from the cell walls of Gram-negative bacteria. Endotoxins are responsible for making up the majority of pyrogens, which must be removed from pharmaceutical products including injectable biologics. There are many factors to be considered when designing a depyrogenation filtration process for solutions containing proteins and peptides: type of target protein and its concentration; electrolyte concentration; pH and buffer system; protein molecular weight and isoelectric point (pI); filtration parameters (e.g., flow rate); and interactions with other components causing aggregation. In general, a combination of these factors determines the most effective depyrogenation method.

Depyrogenation of liquids may be accomplished by means of filtration through various types of

filter media including microporous membranes, reverse osmosis (RO) membranes, ultrafilters, charge-modified depth filters, activated carbon, and membrane absorbers. Depyrogenation filtration processes are not intended to remove microorganisms from a process stream; however, by their nature, filters selected for use in depyrogenation processes may also be capable of retaining many types of microorganisms.

2. TECHNOLOGIES USED FOR DEPYROGENATION BY FILTRATION

2.1 Microporous Membrane Filtration

Microporous membranes (typically with pore size or retention ratings between 1.0 and 0.1 μm) can be very effective in removing intact bacteria via size exclusion and adsorption within flow pathways. The use of microporous membranes on a freshly prepared solution to be filtered can effectively prevent bacterial proliferation in the solution, along with any potential subsequent endotoxin formation. Endotoxin, however, is composed of fragments of bacterial cell wall, often $<0.025 \mu\text{m}$ (1) that may easily penetrate most bacteria-retentive membrane filters. These negatively charged particles with endotoxin activity can be removed via adsorption by positively charged membranes (2). Adsorption of endotoxin has also been shown by hydrophobic membranes, where it is thought that a hydrophobic interaction occurs between the Lipid A core and hydrophobic sites on the membrane flow path surfaces (3). Reduction or removal of endotoxin activity by adsorption to microporous membranes can be dependent on flow rate, pH, concentration, and fluid and membrane surface properties. Once the effective binding capacity of the membrane approaches saturation under applied conditions, remaining endotoxin will pass through the membrane.

2.2 Reverse Osmosis

RO membranes are the tightest membranes in size separation. They can separate dissolved salts and sugars from water. Pyrogens, and essentially everything else, are removed from water via size exclusion. RO systems are operated most efficiently at high pressure (200–1000 psi) to overcome osmotic pressure. RO membrane rating or tightness is measured and expressed with retention or rejection of marker salts such as sodium chloride or magnesium sulfate.

RO membranes may be composites (thin film coated on top of ultrafiltration membranes) or cast as a single layer (cellulose acetate type). Configuration of RO membrane modules can be flat sheet, tubular, or hollow fiber. All commercially available RO membranes are polymeric, and most are of a spiral-wound, flat-sheet format.

RO systems are not intended to remove all bacteria, and because they are run at ambient temperatures, microbiological contamination is a concern. Ultraviolet (UV) light may be used in the system downstream from the RO units to control microbiological contamination.

2.3 Ultrafiltration

Ultrafiltration (UF) is a process whereby a fluid is passed through membranes with pore sizes nominally between about 1 and 100 nm under pressure. The filters are usually not rated by the pore size but by the molecular weight cut-off (MWCO). The methods to determine the MWCO vary by the manufacturer and usually involve measuring passage of molecules of a certain size, such as a solution of mixed dextrans, polyethylene glycol, or proteins to assign a numerical rating (4).

UF membranes are usually polymeric porous structures, manufactured from a range of materials, most commonly regenerated cellulose or polyether sulfone, but also ceramics. UF membranes may be produced as flat sheet, hollow fibers, or ceramic tubes.

UF is generally operated in tangential/cross flow mode, which separates the starting (feed) solution into two components: permeate (the portion of solution going through the membrane) and retentate (the concentrated solution that is passed over the membrane). UF membranes need to be encased in a suitable integral device to enable practical operation. Heat sealing, over-molding, and resin-potting are all used to assemble membrane devices and ensure integral flow paths. Ceramic tubes are sealed by gaskets within tubular cylinders.

It is generally assumed that the basic subunit of lipopolysaccharide (LPS) is about 10–20 kDa (5). Membranes of 6–10 kDa cut-off are often used for depyrogenation by size exclusion. However, monomeric LPSs are rarely found in solution because of their poor solubility in water. LPS is usually present in aggregated forms, such as vesicles ranging in molecular weight from 300 to 1000 kDa. Thus, endotoxin can be successfully removed by higher flux membranes, with MWCOs of 30–100 kDa (6).

Adsorption, in addition to size exclusion, also can be a mechanism of endotoxin removal by UF. Several hollow-fiber membrane materials have been evaluated, and the best removal was obtained with more hydrophobic membranes. Endotoxin removal was correlated to the degree of endotoxin adsorption on the membranes in an equilibrium experiment (7).

UF has been used successfully to depyrogenate small molecule drugs, buffers, electrolytes, antibiotics, and antifungal agents (8). UF is generally not recommended for endotoxin removal from solutions containing larger molecules such as proteins.

2.4 Charge-Modified Depth Filters

Depth filters exhibit two primary clarification mechanisms because of their structural and chemical composition: size exclusion, either through sieving or entrapment; and adsorption, either through electrokinetic (positive zeta potential) or hydrophobic interactions.

Size exclusion of particles is a function of the tortuous flow path through the media as well as the depth or length of the flow path in relation to the size distribution of the contaminate loading, e.g., cellular debris, including LPS from cell walls and hard particles. Depth filtration efficiency depends on many factors, including the filter media characteristics, materials of construction, e.g., cellulose, filter aids, binding resins—the fluid characteristics, e.g., viscosity, dirt load, cell debris, temperature—as well as the particle characteristics: solid/hard, pleomorphic, proteinaceous, colloidal. Electrokinetic adsorption is attributed to the resin binders and filter aids that impart a net-positive charge, positive zeta potential, to the filter medium. Adsorption is a complex mechanism that will vary based on a combination of parameters including positive zeta potential, hydrophobic adsorption, particle surface charge, pH, and ionic strength of process fluids. This positive zeta potential can remove negatively charged particles smaller than the nominal rating of the depth filter medium. The adsorptive mechanism results in high removal efficiencies for fine particles, colloidal and cellular materials, e.g., bacterial endotoxins, nucleic acids, and removal of negatively charged trace contaminants, whereas the depth medium porosity influences operating parameters such as pressure differentials, flow rates, dirt load capacity, and throughput.

Most cellulose-based depth filters contain a filter aid to enhance particle retention and flow characteristics. Filter aids are available in various particle sizes and levels of purity. Common filter aids include diatomaceous earth, perlite (volcanic origin), carbon (natural sources), and silica- and/or metallic-based materials.

The cartridges and capsule configurations are constructed of primarily polypropylene and other common elastomers and polymers, e.g., nylon, polycarbonate, polysulfone. Depth filters are available in standard filter cartridge/capsule configurations; lab-scale discs (47 mm/90 mm), flat stock sheets, lenticular cartridges (stacked discs), and capsules.

In general, the charge-modified depth filters showed lower endotoxin breakthrough levels at charge exhaustion as compared to charge-modified membrane filters.

Membranes demonstrated total endotoxin breakthrough once the charge capacity of the membrane is saturated. Generally, charge-modified depth filter media demonstrate lower endotoxin unit (EU) levels that increase slowly at the point of first endotoxin detection as compared to membranes that exhibit complete breakthrough.

Benefits of charge-modified depth filters include removal of bacterial endotoxin [4–5 log reduction value (LRV)] (9–11), DNA fragments, host cell protein, reduction of viruses, and economical throughput with low extractable levels. System flow rate determinations are necessary to optimize residence time to maximize adsorptive capture. This parameter is especially important for the removal of colloids and endotoxins.

Cellulosic depth filters commonly contain extractable *Limulus* amoebocyte lysate (LAL)-reactive materials that are often determined to be β -1,3-glucans. β -1,3-Glucans activate an alternative LAL pathway, Factor G. The activation of Factor G by β -1,3-glucans will induce the proclotting enzyme, causing a non-endotoxin-positive LAL result (or enhanced result). To reduce the risk of β -1,3-glucan extractables from cellulosic depth filters, it is important to follow the recommended rinse conditions of the specific depth filter. An alternative to reduce the effects of β -1,3-glucans is to select LAL reagents tolerant of β -glucans or to add a β -glucan blocking buffer to LAL samples.

To some users, the most important attribute of charge-modified depth filters is their effectiveness as prefilters. In more difficult filtrations, such as those containing colloids, bacteria, or endotoxins, the user can realize substantial cost savings.

2.5 Activated Carbon Depth Filters

Depth filtration, using activated carbon as a filter aid adsorbent, removes color, odor, and bacterial endotoxins and nucleic acids. Activated carbon is derived from organic materials, e.g., peat, wood, coconut, bone, lignite coal. The microstructure of the carbon contains millions of pores that create a highly adsorptive material with a vast internal effective surface area as compared to polymeric microporous structures. These carbon filter aids are typically activated by steam or chemical treatment such as acid. Although highly effective in reducing endotoxin (4–5 log reduction) and other undesirable contaminants, active carbon may, because of this highly adsorptive characteristic, remove other process components and target molecules due to this nonspecific adsorption property. The high loading capacity and strong adsorptive attributes make activated carbon depth filtration an attractive alternative to conventional filtration methods or addition of bulk carbon, where care must be taken to remove fine carbon particulates in the effluent.

2.6 Membrane Adsorbers

When the target protein in the solution is in the same molecular weight range as that of the endotoxins (10–20 kDa for endotoxin monomers), the target proteins cannot be separated by UF. Ion exchange chromatography is the most common depyrogenation method for proteins; however, it has some drawbacks, which limit its usefulness as a depyrogenation step. This

includes handling and usage problems such as packing, channeling, low flow rates, long regeneration times, compressibility, and limited chemical stability. Charge-modified membrane adsorbers with ion exchange ligands functionalized on the membrane surface can provide the required performance needed for depyrogenation from the laboratory up to process scale. Generally, two strategies can be used for removal of endotoxin from solutions with such membrane adsorber devices. Using the strong basic anion exchanger of quaternary amine (Q) type in a buffer with pH lower than the pI of the protein, endotoxin will bind to the charged membrane substrate, and protein will pass through the membrane (negative chromatography). Alternatively, a strong acidic ion exchanger type S also can be used with a buffer pH lower than the pI of the protein. In this case, the endotoxin will pass through, and the protein will be bound to the charged membrane substrate, which can be subsequently eluted using appropriate buffers in the next step.

Such membrane adsorbers have been used as validated endotoxin clearance steps in downstream processing of monoclonal antibody (mAb) or recombinant protein manufacturing. Typical log reduction values (LRVs) reported are >4 (12) based on lab scale testing.

Another solution is to use mixed-mode membrane adsorbers exhibiting both anionic and hydrophobic chemistries. Endotoxins (hydrophobic and negatively charged) tightly bind onto the membrane surfaces. By adjusting the concentration of salt or pH appropriately, proteins flow through the mixed-mode membrane adsorber by charge repulsion, while endotoxins remain bound. Mixed-mode membrane adsorbers allow the depyrogenation of protein solution or buffers with higher concentrations of salt (e.g., 100–500 mM) than with the Q adsorber. Such membrane adsorbers have been used as validated endotoxin clearance steps in downstream processing of mAb or recombinant protein manufacturing. Typical LRVs reported are in the 3–4 range based on lab scale testing.

3. VALIDATION

See *Depyrogenation* (1228) for a comprehensive discussion of depyrogenation process validation and the use of endotoxin standards.

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5. APPENDIX

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BRIEFING

{1228.5} **Endotoxin Indicators for Depyrogenation.** The General Chapters—Microbiology Expert Committee proposes this new general chapter as an addition to the *Depyrogenation* {1228} family of chapters. The effectiveness of the depyrogenation process can be measured biologically by conducting a challenge study using a known quantity of endotoxin that is attached to, or contained in a carrier, in a manner that is representative of the articles intended to be depyrogenated. In this chapter the biological tool used to challenge the depyrogenating capabilities of a process will be defined as an endotoxin indicator. The following topics will be discussed in this chapter: endotoxin and lipopolysaccharide, applications of endotoxin indicators, preparation and use of endotoxin indicators, and analysis of test results.

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Comment deadline: November 30, 2015

Add the following:

■ {1228.5} ENDOTOXIN INDICATORS FOR DEPYROGENATION

1. INTRODUCTION
2. ENDOTOXIN AND LPS
3. APPLICATION OF ENDOTOXIN INDICATORS
4. PREPARATION AND USE OF ENDOTOXIN INDICATORS

- 4.1 Methodology to Create a Laboratory-Prepared Endotoxin: Principles to Consider
- 4.2 Inoculation of EIs
- 4.3 Recovery of Endotoxin from EIs
- 4.4 Choice of Test Methodology for the Analysis of EIs
- 5. ANALYSIS OF RESULTS OF DEPYROGENATION STUDIES
- 6. REFERENCES

1. INTRODUCTION

Depyrogenation is defined as destruction or removal of pyrogens (see *Depyrogenation* (1228)). For the purposes of this chapter, the terms "bacterial endotoxin or endotoxin" refer to a component of the outer cell membrane of Gram-negative bacteria, which is known to induce a febrile response in humans and other mammals. The endotoxin complex contains many cell wall components including, but not limited to, phospholipids, lipoproteins, and lipopolysaccharides. Lipopolysaccharide (LPS) is the biologically active portion of both naturally occurring and laboratory-prepared endotoxin complexes. The USP Endotoxin Reference Standard (which, by convention, is abbreviated as RSE) and Control Standard Endotoxin (CSE) preparations purchased from lysate manufacturers and other third-party vendors are not endotoxins but rather are preparations of purified LPS.

"Endotoxin indicators" (EIs) are tools that are used (where required) in conjunction with physical measurements to analyze the effectiveness of a depyrogenation process. EIs used to determine the effectiveness of dry heat depyrogenation processes are commonly purchased as glass vials that are inoculated with a known level of LPS activity. This chapter expands the definition of EI to include any carrier, including glass vials, inoculated with endotoxin or LPS that is used to challenge a depyrogenation process. EIs can be used to analyze the effectiveness of endotoxin removal by washing, rinsing, cleaning, or by using separation technologies, such as filtration or chromatography. Carriers can be a variety of materials, including rubber stoppers to assess stopper washing processes, bulk product to assess and validate processing steps, and stainless steel coupons to assess the cleaning of production vessels.

Purified LPS, such as CSE obtained from lysate manufacturers or other third-party vendors, has historically been a convenient choice for use as the analyte used in the preparation of EIs. However, EIs prepared in-house using laboratory-derived endotoxin more closely mimic product contamination, and as a result can provide a more realistic assessment of the depyrogenating capability of various production processes than does highly purified LPS. This chapter provides information on the preparation and use of these more specialized indicators to assure both consistency and comparability of data among method development and validation studies.

2. ENDOTOXIN AND LPS

A bacterial endotoxin is defined in the *Introduction*. LPS is the biologically active portion of the naturally occurring and laboratory-prepared endotoxin complex. Highly purified LPS, extracted from the natural endotoxin complex, is used to prepare the primary compendial endotoxin Reference Standard (RSE) or secondary Control Standard Endotoxin (CSE) preparations, such as those purchased from *Limulus* amoebocyte lysate (LAL) reagent manufacturers. LPS consists of three distinct regions:

1. The structure of the hydrophobic lipid A portion of the molecule is the most highly conserved among Gram-negative species and is responsible for most, if not all, of the biological activity of endotoxins.
2. A core oligosaccharide links the lipid A to the hydrophilic O-specific side chain or O-antigen.
3. The hydrophilic O-antigen is a highly variable region that confers serological specificity to the organism and is often used to distinguish strains of Gram-negative bacteria.

When drug products and devices are contaminated with endotoxin, the contaminant is not purified LPS but rather whole Gram-negative cells and/or cell wall fragments containing LPS. LPS and endotoxin are therefore dissimilar in many respects.

The amphipathic nature of the LPS molecule [i.e., having both a polar (hydrophilic) end and a nonpolar (hydrophobic) end] enables it to form complicated, three-dimensional, aggregated structures in solution. The aggregated forms of LPS have the capacity to adsorb, or "stick", to surfaces, and depending on the LPS formulation and the surface, extraction and detection may prove difficult using conventional extraction methods (see below). The degree of aggregation of the purified molecule is also affected by the conditions to which the LPS is exposed. Factors such as temperature, pH, salt concentration, divalent cation concentration, chelating agents, and detergents can have a profound effect on the biological activity and stability of LPS in solution. Purified LPS preparations used for depyrogenation studies should not contain any "fillers" or excipients. The excipients that are commonly used in the formulation of CSE have been shown to reduce the heat resistance of LPS (1) and may interfere in the recovery of LPS because of a caramelized excipient that has been post-processed by dry heat.

Endotoxins contaminating parenteral products may exhibit greater stability of activity in solution and less surface adsorption than purified LPS. As well, the detection of endotoxin may be less influenced than LPS by aggregation, disaggregation, or other conformations induced by some product matrices. Information on principles to consider when preparing endotoxin in the laboratory can be found below.

3. APPLICATION OF ENDOTOXIN INDICATORS

The choice of an EI should be relevant to the process being validated. For physical depyrogenation, such as dry heat, the carrier material for the EI may be a surface such as a glass vial or appropriate coupon material onto which a known quantity of LPS or of endotoxin has been inoculated. For stopper washing/depyrogenation studies, stopper carriers are inoculated with known levels of LPS or endotoxin. For raw materials or process intermediates that are inherently contaminated with assayable levels of endotoxin activity, there may not be a need to add LPS or endotoxin to validate endotoxin reduction in the manufacturing process, as the level of contamination may be sufficient to accurately measure activity upstream and downstream of the depyrogenating step(s).

For processes using raw materials or for upstream intermediates that are not contaminated with endotoxins, the use of either the USP RSE or CSE, which are both highly purified preparations, may not reflect the actual removal or reduction potential of the product stream depyrogenation step(s) under challenge. For these purposes, endotoxins harvested from Gram-negative cultures may be more suitable for depyrogenation processes typically found in

biopharmaceutical product streams. The cell wall fragments and outer membrane constituents associated with these endotoxins represent realistic challenges to process operations such as ultrafiltration, affinity chromatography, and the use of charged media membranes or columns. Challenge studies for LPS or endotoxin removal in process streams should be conducted at the laboratory or pilot scale so as not to introduce high levels of endotoxin or LPS into the actual production environment.

4. PREPARATION AND USE OF ENDOTOXIN INDICATORS

4.1 Methodology to Create a Laboratory-Prepared Endotoxin: Principles to Consider

Glass vial EIs purchased from third-party vendors do not need further preparation before use. These indicators are labeled with a nominal value of inoculated LPS, and the label claim should be confirmed upon receipt to assure that there is sufficient activity (endotoxin unit, or EU) available for the study.

- There is not one “best” or “standard” method for preparing endotoxin in the laboratory, but one example of a published method for the preparation of laboratory-prepared endotoxin may be found in Bowers and Tran (2). Regardless of the methodology for preparation, the following recommendations should be considered to properly and consistently produce, identify, and maintain laboratory-prepared endotoxin for use as a tool for depyrogenation studies. An appropriate Gram-negative bacterial strain from a recognized culture collection is a good choice for preparing a laboratory-derived endotoxin. Alternatively, a Gram-negative organism isolated from a facility, water system, raw material, or product that is identified to the species level, that has been shown to be genetically stable and that is properly maintained, may also be considered. Establishing the identity and baseline genetic fingerprint of an environmental organism will assure that subsequent preparations are consistent.
- The laboratory should create detailed procedures or laboratory work instructions for culture maintenance and endotoxin preparation to assure consistency between batches of endotoxin. For example, endotoxin may be isolated from a live culture or a culture that has been subjected to autoclaving by filtration through a filter of 0.22- μm pore size into a sterile container. Whatever the methodology for growth and endotoxin isolation that is developed by the laboratory, the methodology should be documented and used consistently.
- Consistent with good microbiological practice, the culture and maintenance of the cells used to produce a laboratory-prepared endotoxin should be consistent with *Microbiological Best Laboratory Practices* (1117). Instructions on: 1) the proper maintenance of the organism; 2) growth conditions, including any requirements to prepare media, nutrient requirements, and time/temperature of incubation; 3) methods for cryopreservation or lyophilization for master cell banks and working cell banks; 4) storage of the endotoxin, once prepared including concentration, vessel type, and volume; and 5) master batch production records to assure consistency in subsequent studies should be written, managed via change control, and followed.
 - Once isolated, the relative activity of the endotoxin preparation should be established by comparing its activity to a known LPS standard such as RSE, or a

CSE that has been standardized against the RSE. Determination of activity involves diluting the endotoxin preparation and assaying the dilutions against an LPS standard curve such that the result of the dilution falls within the range of the referenced standard curve. As with the CSE standard used in the bacterial endotoxins test (BET) assay from *Bacterial Endotoxins Test* (85), the activity of the endotoxin may vary, depending on the lot of lysate and lot of LPS used for the analysis. It is recommended, consistent with the assignment of potency for the CSE, that activity of an endotoxin preparation be evaluated for each lysate manufacturer, lysate lot, and test method (gel, kinetic turbidimetric, or kinetic chromogenic) in use in the laboratory.

The activity of the stock endotoxin preparation in EU/mL is reported as:

$$(\text{Test result in EU/mL}) \times (\text{dilution factor}) = \text{EU/mL of the starting endotoxin preparation}$$

- Once activity has been determined, and if applicable to the study design, a standard series of dilutions of the newly prepared endotoxin should demonstrate onset times that result in slope and y-intercept values that are consistent with the standard curve parameters of the RSE/CSE standard using the same lot of lysate. This demonstrates that the activity of endotoxin preparation dilutes and reacts with the lysate in a manner that is similar to LPS.
- Characterization of the endotoxin preparation should also include data on the stability of the preparation, because stability is critical to the comparison of data from one study to the next. If the endotoxin preparation is stored, storage parameters including the concentration of the preparation in EU/mL, the composition of the vessel, the temperature of storage, and the length of storage, should be defined. An expiration date should be assigned based on determined stability.

4.2 Inoculation of EIs

To prepare an EI in house, inoculate endotoxin or LPS onto an article (carrier) that will serve as the substrate for the EI. Carriers for EIs can be anything that is subject to depyrogenation such as: vials (for dry heat depyrogenation), stoppers (for stopper washing), stainless steel coupons (for vessel cleaning), or product (for depyrogenation of process streams).

The simplest way to inoculate these indicators is to add a small volume of a highly concentrated solution of endotoxin or LPS to the carrier. The volume and concentration of added endotoxin or LPS should be calculated to add at least 1000 EU to the carrier, although higher or lower concentrations may be justified. For nonliquid carriers, the endotoxin is "fixed" or dried onto the carrier substrate. This fixing step is most easily accomplished by drying in a unidirectional air flow cabinet or hood, although other drying methods including vacuum drying, lyophilization, and other fixation methods could be used. In depyrogenation challenge studies, once a fixing method is chosen, it should not change in subsequent studies to assure comparability of results. Before using the EIs, a recovery procedure, consisting of a reconstitution or extraction method, should be developed and verified for consistency (3). For liquid carriers such as bulk product, the level of inoculation in EU/mL should be justified based on "worst case" challenge for the depyrogenation step under study, meaning that the

highest concentration of endotoxin that could be in the upstream product, based on process knowledge and historical endotoxin values, should be used. Such justification should take all contributing factors into account, including but not limited to: Gram-negative bioburden in raw materials and bulk; endotoxin content in raw materials including water, contributions by product contact surfaces; and the effect of hold times, particularly for nonsterile bulk.

4.3 Recovery of Endotoxin from EIs

To use EIs, it is necessary to recover and quantify the activity of the endotoxin or LPS from both unprocessed indicators (controls) and from processed indicators (i.e., those that have been through the depyrogenation process). LPS tends to adsorb to surfaces and may aggregate or disaggregate in some product matrices; therefore, recovery of activity from EIs made with LPS is often not 100% of the nominal or measured spike value. This section addresses the methodology for recovery and possible strategies for addressing recoveries that may be observed in challenge testing.

In the case of commercially available EIs prepared with LPS, the manufacturer's directions for extraction and recovery should be followed. With such products, there should be little difficulty in achieving recovery within a factor of 2 of the labelled LPS concentration. If recoveries within the specified range cannot be achieved, the manufacturer should be contacted for technical assistance.

For EIs made in-house using LPS, the composition of carriers, such as plastics, can affect recovery or result in inconsistent recovery because of adsorption. For these carriers, there is no prelabelled concentration to verify. In this case, the expected recovery should be based on the measure of the activity of endotoxin or LPS added to the article and the volume of extraction fluid used to recover it. The actual (measured) activity in the extract should then be compared to the measured activity of the endotoxin or LPS added to determine the percentage of recovery.

For example, consider a stock endotoxin or LPS preparation containing a measured activity of 100,000 EU/mL that is used to prepare in-house EIs. If a volume of 50 μ L of this preparation is dried on the surface of each of a number of 10-mL vials, the known amount of activity added is 5000 EU. If the recovery/extraction is performed in 5 mL of water for BET, and the recovery is 100%, the expected activity in the extract solution is 1000 EU/mL.

$$\frac{5,000 \text{ EU/vial}}{5 \text{ mL extraction solution/vial}} = 1000 \text{ EU/mL}$$

If, however, the measured activity after extraction is 200 EU/mL as opposed to the expected 1000 EU/mL, the efficiency of the extraction method is 20%.

Recovery of endotoxin or LPS from nonliquid EIs prepared in-house can follow recommendations for the extraction of medical devices in preparation for LAL testing. USP general chapter *Medical Devices—Bacterial Endotoxin and Pyrogen Tests* (161) states, "The standard extraction method is to soak or immerse the device or flush the fluid pathway with extracting fluid that has been heated to $37 \pm 1.0^\circ$, keeping the extracting fluid in contact with the relevant surface(s) for NLT 1 h." The volume used for reconstitution or extraction should be appropriate for the material, size, and shape of the EI, recognizing that a volume too low may not efficiently recover the endotoxin or LPS and that excessive volumes will unnecessarily dilute the endotoxin or LPS that has been extracted.

If the recovery of added endotoxin or LPS is variable, an alternate extraction method may be developed and validated. This may include agitation or mixing, sonication or alternative extraction solutions. A combination of extraction in 0.01% sodium laurel sulfate, sonication, and vortex mixing is one such approach that has been reported to be more effective than extraction in water for medical devices (4–6). Other extraction methods are summarized by Bryans et al. (7) and in ANSI/AAMI standard ST72:2011 (8).

Another situation concerns liquid endotoxin or LPS preparations that are used either to validate a depyrogenation process in a process stream or to investigate the destruction or removal of endotoxin or LPS in a manufacturing process. In these cases, the initial concentration of the stock liquid endotoxin or LPS solution should be measured before it is added to the system or process. If some of this preparation is added (“spiked”) to a bulk process solution that is then subject to a particular process or treatment, the activity of endotoxin or LPS in this bulk solution should be measured and recorded as the starting activity. It is important to determine whether changes in the endotoxin or LPS activity of the processed solution are due to effects of the process and not to instability of the LPS or endotoxin in the solution. The stability of the activity of the LPS or endotoxin in these preparations should be verified over a period appropriate to the proposed use of the preparation.

As with the spiking method (choice of endotoxin/LPS and “fixing” process), whichever reconstitution/extraction procedure is chosen should be verified for consistency and should be used for all subsequent studies to assure comparability of results.

4.4 Choice of Test Methodology for the Analysis of EIs

Any of the test methods described in (85) can be used for the analysis of processed and unprocessed EIs. As with the rest of the methodology, it is highly recommended that an assay (kinetic turbidimetric, kinetic chromogenic, or gel clot assay) be chosen during method development and used consistently throughout the initial study and in subsequent studies to assure that data are comparable. The use of alternate assays is permissible, provided that they are validated to assure that they are equivalent to or non-inferior to the standard compendial assays.

5. ANALYSIS OF RESULTS OF DEPYROGENATION STUDIES

To evaluate the effectiveness of a depyrogenation process, the residual activity that is recovered from processed indicators is compared to the endotoxin or LPS activity of unprocessed controls. Typically, the \log_{10} of the endotoxin or LPS activity measured for the processed EI (or solution) is subtracted from the \log_{10} of the measured endotoxin or LPS activity of unprocessed control indicator. The result of the subtraction is the log reduction that is attributable to the depyrogenation process. If there are multiple controls and/or samples of processed material (and there usually are), the most conservative approach is to subtract the highest \log_{10} concentration recovered from the processed EIs (or solution samples) from the lowest \log_{10} unprocessed control endotoxin activity. For example:

- The activities in three unprocessed EIs are 1286, 1000, and 1532 EU/mL.
- The activities in three processed EIs are 0.634, 0.512, and 0.496 EU/mL.

The log reduction is calculated as:

$$\log_{10} (1000) - \log_{10} 0.634 = 3 - (-0.198) = 3.198 \text{ log reduction}$$

Historically, a ≥ 3 -log reduction has been required by regulatory/compliance guidance. However, depending on the process and historical data, a 3-log reduction may be either excessive or inadequate. For example, for glass vials with a low or nonmeasurable endotoxin content upon receipt, the requirement to continually and repeatedly revalidate with an acceptance criterion of a 3-log reduction of the endotoxin spike of >1000 EU is excessive. Alternatively, a fermentation process with an endotoxin content of $>10^7$ EU/mL in the clarified culture supernatant will require more than a 3-log reduction to achieve safe levels of endotoxin in the drug substance or drug product. In any event, an appropriate specification for the log reduction of processed indicators should be established and justified in a preapproved protocol for the study. The total reduction, of course, may be achieved over several steps in a purification process. Thus, the necessary reduction is often achieved additively over the course of multiple purification steps.

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- 2S (USP39)

(1229.13) **Sterilization-in-Place.** The General Chapters—Microbiology Expert Committee proposes this new general chapter as an addition to the *Sterilization of Compendial Articles* (1229) family of chapters. This chapter discusses sterilization of a system or piece of process equipment in situ (sterilization-in-place; SIP) to reduce the need for post-sterilization handling. This chapter provides information on the common elements of the SIP process, different physical methods used for performing SIP, and routine process control.

(GCM: R. Tirumalai.)

Correspondence Number—C161848

Comment deadline: November 30, 2015

Add the following:

■ **(1229.13) STERILIZATION-IN-PLACE**

INTRODUCTION

Sterilization-in-place (SIP) can be defined as the sterilization of a system or piece of process equipment in situ. The purpose of SIP is to eliminate, or greatly reduce, the need for post-sterilization handling, including that necessary to make aseptic connections. Mobile process equipment (e.g., portable tanks, storage vessels, and other equipment), once sterilized in this manner, may be relocated. The SIP process can be carried out by using any of the following physical methods: moist heat, dry heat, gas, liquid, or vapor (described below) according to the approaches described in *Sterilization of Compendial Articles* (1229), as adapted for use with the specific equipment or system.

COMMON ELEMENTS OF STERILIZATION-IN-PLACE PROCESSES

There are a number of considerations appropriate for the design and use of SIP procedures that apply to all of the sterilization methods:

- The use of an SIP method is normally associated with a “closed system.” Closed systems are almost always sterilized in situ, and the design elements of the typical closed system are consistent with many SIP process needs.
- For large systems, it may be necessary to sterilize in portions. The individual sterilization processes should overlap to ensure treatment of all internal surfaces.
- The focus of the SIP procedure is sterilization of the product contact surfaces (the interior of the system). Demonstration of process lethality relies upon physical measurements and biological indicators. This confirmation should extend to the “sterile boundary” of the system, including vessel headspace, connections to other vessels/equipment, and other parts of the system. The interior surfaces of the process equipment, irrespective of their materials of construction, should be exposed to lethal conditions sufficient to sterilize the system and confirmed as lethal with an appropriate biological challenge.

- SIP is accomplished almost exclusively using the overkill approach to sterilization. The components of the physical equipment should be chosen based upon their ability to withstand the sterilizing conditions to be used. Filters, whether membrane or high-efficiency particulate air (HEPA), in the process system are typically the most susceptible to damage during SIP, and care must be taken to preserve the integrity of the filters. Filter manufacturers can provide guidance on acceptable sterilization methods and parameters.
- The absence of specifically designed equipment in which the sterilization process is performed places the bulk of responsibility for design onto the user. SIP systems ordinarily cannot be purchased directly in the way one purchases a steam sterilizer or a dry heat oven. Instead, the system, which was designed for the operating process, may require modification to accommodate the SIP process to be used. The user must assume the role of designer for the process, equipment, and control system.
- The system design and operating procedures must provide for an efficient means of introducing and removing the sterilization agent. Establishment of a reliable process sequence is a critical part of the cycle development exercise. The sterilizing agent is normally introduced through a filter on the system that may also serve as a process, purge gas, or vent filter.
- At the conclusion of the sterilization process sequence and until ready for use, the system should be pressurized with a purge gas (sterile air or nitrogen are the most common) to prevent the introduction of contaminants to the sterilized system.
- The critical process parameters for the SIP process should be recorded as the process is executed. The important parameters may include temperature, pressure, concentration, flow rate, humidity, and time, among others.

CLOSED SYSTEMS

In pharmaceutical manufacturing operations, closed systems are used for various applications including maintenance of large quantities of materials (liquids or powders) in a sterile state; manufacture of biological and synthetic organic active ingredients (especially where microbial absence is essential); and preparation of process equipment for use in sterile drug product manufacturing and filling. The use of closed systems provides superior separation of sterile materials from the surrounding environment. Typically, closed systems are maintained under positive pressure at all times. The characteristics of a closed system that establish its designation as "closed" include the following:¹

- It maintains integrity during all operating periods and under all conditions.
- It is sterilized-in-place or sterilized while closed before use.
- It can be adapted for materials transfer in and/or out while maintaining its sterile state.
- It can be connected to other closed systems while maintaining the integrity of all systems.
- It is subject to scheduled preventive maintenance.

- It uses sterilizing-grade filters for sterilization of liquid and gas process streams.

STERILIZATION-IN-PLACE METHODS

Moist Heat

The use of saturated steam is the most prevalent method for SIP of large systems. The majority of installations use gravity displacement cycles adapted from those originally used in steam sterilizers (the size and complexity of many systems preclude the use of pre-vacuum cycles). Important considerations include the provision for air removal, condensate discharge, and steam removal post-dwell.^{2,3} This method is commonly used for bioreactors, sterile bulk production, holding tank and delivery lines, and other large systems.

Superheated Water

Systems used for *Water for Injection* and *Purified Water* can be sterilized by using superheated water (water that is heated above its boiling point and pressurized to maintain it as a liquid) circulating through the system. This method has the ability to sterilize vessels, filters, and other wetted components at the same time.⁴

Dry Heat

Dry heat has been used for SIP of spray dryers and their associated material collection systems. The air supply for sterilization in these systems is provided through HEPA filters.

Gas

Gas-phase SIP has been used for non- and low-pressure-rated process equipment, such as freeze dryers, pre-freezers, process vessels, and other equipment.

Liquid

Liquid chemical sterilization is best suited for liquid-handling systems and can be used only for fully wetted surfaces.

Vapor

Sterilizing vapors have been used for the in situ sterilization of the same types of process equipment as those treated with sterilizing gases. The precautions associated with vapor sterilization described in *Vapor Phase Sterilization* (1229.11) are required.

ROUTINE PROCESS CONTROL

SIP processes are subject to formal controls that maintain a validated state over time. The practices outlined in (1229) include the general requirements appropriate for all sterilization systems as well as those specific to an individual sterilization method. Sterilization is accomplished by a number of related practices that are essential for continued use of the

process over an extended period of time. The essential practices to maintain validated status include calibration, physical measurements, physical integrators or indicators, ongoing process control, change control, preventive maintenance, periodic re-assessment, and training.

■ 2S (USP39)

¹ Parenteral Drug Association, Technical Report No. 28, Revised. Supplement Volume 60, No. S-2. Process simulation testing for sterile bulk pharmaceutical chemicals. PDA, Bethesda, MD; 2005.

² Agalloco J. Steam sterilization-in-place technology and validation. In: Agalloco J, Carleton FJ, editors. *Validation of Pharmaceutical Processes*. 3rd ed. Informa USA, New York; 2007.

³ Parenteral Drug Association, Technical Report No. 61. Steam in place. PDA, Bethesda, MD; 2013.

⁴ Haggstrom M. Sterilization-in-place using steam or superheated water. In: *Proceedings of the PDA Basel Conference*. PDA, Bethesda, MD; 2002.

BRIEFING

{1231} **Water for Pharmaceutical Purposes**, *USP 38* page 1492. On the basis of the conclusions of the former Pharmaceutical Waters Expert Committee (from the 2005–2010 volunteer cycle) and public comments received suggesting that the chapter be revised to make it more user-friendly, the Pharmaceutical Waters Expert Panel of the General Chapters Chemical Analysis Expert Committee was tasked to revise chapter {1231} *Water for Pharmaceutical Purposes*.

The overall intent and purpose of the chapter are unchanged. The following list includes a summary of the changes:

1. Updating the chapter to improve the organization and clarity of the information and remove redundant discussion text. This includes the organization into nine specific sections listed below.
2. Removing wording redundant to referenced monograph wording.
3. Adding a detailed *Outline/Table of Contents* to improve user's topic discussion findability (topics will be hyperlinked in future electronic *USP* versions).

Significant revisions have been made or discussion added in several reorganized sections: 1. *Introduction*; 2. *Source Water Considerations*; 3. *Waters Used for Pharmaceutical Manufacturing and Testing Purposes*; 4. *Validation and Qualification of Water Purification, Storage, and Distribution Systems*; 5. *Design and Operation of Purified Water and Water for Injection Systems*; 6. *Sampling*; 8. *Microbial Considerations*; and 9. *Alert and Action Levels and Specifications*. The remaining section 7. *Chemical Evaluations* is not being revised at this time.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCCA: A. Hernandez-Cardoso.)

Correspondence Number—C153849

Comment deadline: November 30, 2015

{1231} WATER FOR PHARMACEUTICAL PURPOSES

Change to read:

INTRODUCTION

Water is widely used as a raw material, ingredient, and solvent in the processing, formulation, and manufacture of pharmaceutical products, active pharmaceutical ingredients (APIs) and intermediates, compendial articles, and analytical reagents. This general information chapter provides additional information about water, its quality attributes that are not included within a water monograph, processing techniques that can be used to improve water quality, and a description of minimum water quality standards that should be considered when selecting a water source.

This information chapter is not intended to replace existing regulations or guides that already exist to cover USA and international (ICH or WHO) GMP issues, engineering guides, or other regulatory (FDA, EPA, or WHO) guidances for water. The contents will help users to better understand pharmaceutical water issues and some of the microbiological and chemical concerns unique to water. This chapter is not an all-inclusive writing on pharmaceutical waters. It contains points that are basic information to be considered, when appropriate, for the processing, holding, and use of water. It is the user's responsibility to assure that pharmaceutical water and its production meet applicable governmental regulations, guidances, and the compendial specifications for the types of water used in compendial articles.

Control of the chemical purity of these waters is important and is the main purpose of the monographs in this compendium. Unlike other official articles, the bulk water monographs (*Purified Water* and *Water for Injection*) also limit how the article can be produced because of the belief that the nature and robustness of the purification process is directly related to the resulting purity. The chemical attributes listed in these monographs should be considered as a set of minimum specifications. More stringent specifications may be needed for some applications to ensure suitability for particular uses. Basic guidance on the appropriate applications of these waters is found in the monographs and is further explained in this chapter.

Control of the microbiological quality of water is important for many of its uses. Most packaged forms of water that have monograph standards are required to be sterile because some of their intended uses require this attribute for health and safety reasons. USP has determined that a microbial specification for the bulk monographed waters is inappropriate, and it has not been included within the monographs for these waters. These waters can be used in a variety of applications, some requiring extreme microbiological control and others requiring none. The needed microbial specification for a given bulk water depends upon its use. A single specification for this difficult-to-control attribute would unnecessarily burden some water users with irrelevant specifications and testing. However, some applications may require even more careful microbial control to avoid the proliferation of microorganisms ubiquitous to water during the purification, storage, and distribution of this substance. A microbial specification would also be inappropriate when related to the "utility" or continuous supply nature of this raw material. Microbial specifications are typically assessed by test methods that take at least 48–72 h to generate results. Because pharmaceutical waters are generally produced by continuous processes and used in products and manufacturing processes soon after generation, the water is likely to have been used well before definitive test results are available. Failure to meet a compendial specification would require investigating the impact and making a pass/fail decision on all product lots between the previous sampling's acceptable test result and a subsequent sampling's acceptable test result. The technical and logistical problems created by a delay in the result of such an analysis do not eliminate the user's need for microbial specifications. Therefore, such water systems need to be operated and maintained in a controlled manner that

requires that the system be validated to provide assurance of operational stability and that its microbial attributes be quantitatively monitored against established alert and action levels that would provide an early indication of system control. The issues of water system validation and alert/action levels and specifications are included in this chapter.

SOURCE OR FEED WATER CONSIDERATIONS

To ensure adherence to certain minimal chemical and microbiological quality standards, water used in the production of drug substances or as source or feed water for the preparation of the various types of purified waters must meet the requirements of the National Primary Drinking Water Regulations (NPDWR) (40 CFR 141) issued by the U.S. Environmental Protection Agency (EPA) or the drinking water regulations of the European Union or Japan, or the WHO drinking water guidelines. Limits on the types and quantities of certain organic and inorganic contaminants ensure that the water will contain only small, safe quantities of potentially objectionable chemical species. Therefore, water pretreatment systems will only be challenged to remove small quantities of these potentially difficult to remove chemicals. Also, control of objectionable chemical contaminants at the source water stage eliminates the need to specifically test for some of them (e.g., trihalomethanes and heavy metals) after the water has been further purified.

Microbiological requirements of drinking water ensure the absence of coliforms, which, if determined to be of fecal origin, may indicate the potential presence of other potentially pathogenic microorganisms and viruses of fecal origin. Meeting these microbiological requirements does not rule out the presence of other microorganisms, which could be considered undesirable if found in a drug substance or formulated product.

To accomplish microbial control, municipal water authorities add disinfectants to drinking water. Chlorine-containing and other oxidizing substances have been used for many decades for this purpose and have generally been considered to be relatively innocuous to humans. However, these oxidants can interact with naturally occurring organic matter to produce disinfection by-products (DBPs), such as trihalomethanes (THMs, including chloroform, bromodichloromethane, and dibromochloromethane) and haloacetic acids (HAAs, including dichloroacetic acid and trichloroacetic acid). The levels of DBPs produced vary with the level and type of disinfectant used and the levels and types of organic materials found in the water, which can vary seasonally.

Because high levels of DBPs are considered a health hazard in drinking water, drinking water regulations mandate their control to generally accepted nonhazardous levels. However, depending on the unit operations used for further water purification, a small fraction of the DBPs in the starting water may carry over to the finished water. Therefore, the importance of having minimal levels of DBPs in the starting water, while achieving effective disinfection, is important.

DBP levels in drinking water can be minimized by using disinfectants such as ozone, chloramines, or chlorine dioxide. Like chlorine, their oxidative properties are sufficient to damage some pretreatment unit operations and must be removed early in the pretreatment process. The complete removal of some of these disinfectants can be problematic. For example, chloramines may degrade during the disinfection process or during pretreatment removal, thereby releasing ammonia, which in turn can carry over to the finished water. Pretreatment

~~unit operations must be designed and operated to adequately remove the disinfectant, drinking water DBPs, and objectionable disinfectant degradants. A serious problem can occur if unit operations designed to remove chlorine were, without warning, challenged with chloramine-containing drinking water from a municipality that had been mandated to cease use of chlorine disinfection to comply with ever-tightening EPA Drinking Water THM specifications. The dechlorination process might incompletely remove the chloramine, which could irreparably damage downstream unit operations, but also the release of ammonia during this process might carry through pretreatment and prevent the finished water from passing compendial conductivity specifications. The purification process must be reassessed if the drinking water disinfectant is changed, emphasizing the need for a good working relationship between the pharmaceutical water manufacturer and the drinking water provider.~~

TYPES OF WATER

~~There are many different grades of water used for pharmaceutical purposes. Several are described in *USP* monographs that specify uses, acceptable methods of preparation, and quality attributes. These waters can be divided into two general types: bulk waters, which are typically produced on-site where they are used; and sterile waters, which are produced, packaged, and sterilized to preserve microbial quality throughout their packaged shelf life. There are several specialized types of sterile waters, differing in their designated applications, packaging limitations, and other quality attributes.~~

~~There are also other types of water for which there are no monographs. These are all bulk waters, with names given for descriptive purposes only. Many of these waters are used in specific analytical methods. The associated text may not specify or imply certain quality attributes or modes of preparation. These nonmonographed waters may not necessarily adhere strictly to the stated or implied modes of preparation or attributes. Waters produced by other means or controlled by other test attributes may equally satisfy the intended uses for these waters. It is the user's responsibility to ensure that such waters, even if produced and controlled exactly as stated, be suitable for their intended use. Wherever the term "water" is used within these compendia without other descriptive adjectives or clauses, the intent is that water of no less purity than *Purified Water* be used.~~

~~What follows is a brief description of the various types of pharmaceutical waters and their significant uses or attributes. *Figure 1* may also be helpful in understanding some of the various types of waters.~~

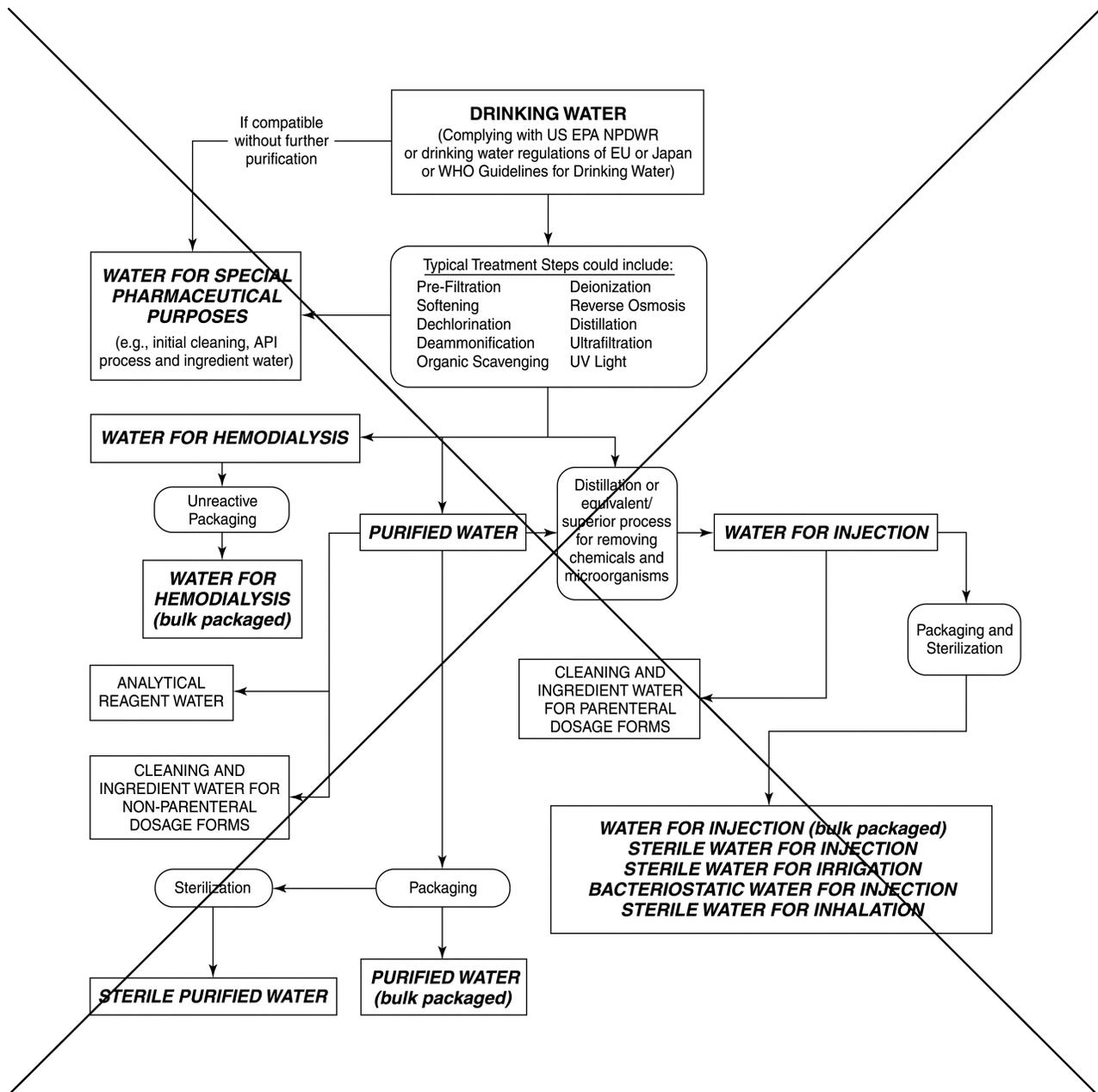


Figure 1. Water for pharmaceutical purposes.

Bulk Monographed Waters and Steam

The following waters are typically produced in large volume by a multiple-unit operation water system and distributed by a piping system for use at the same site. These particular pharmaceutical waters must meet the quality attributes as specified in the related monographs.

Purified Water—*Purified Water* (see the *USP* monograph) is used as an excipient in the production of nonparenteral preparations and in other pharmaceutical applications, such as cleaning of certain equipment and nonparenteral product contact components. Unless otherwise specified, *Purified Water* is also to be used for all tests and assays for which water is indicated (see *General Notices and Requirements*). *Purified Water* is also referenced throughout the *USP-NF*. Regardless of the font and letter case used in its spelling, water complying with the *Purified Water* monograph is intended. *Purified Water* must meet the requirements for ionic and organic chemical purity and must be protected from microbial contamination. The minimal

quality of source or feed water for the production of *Purified Water is Drinking Water*. This source water may be purified using unit operations that include deionization, distillation, ion exchange, reverse osmosis, filtration, or other suitable purification procedures. Purified water systems must be validated to reliably and consistently produce and distribute water of acceptable chemical and microbiological quality. Purified water systems that function under ambient conditions are particularly susceptible to the establishment of tenacious biofilms of microorganisms, which can be the source of undesirable levels of viable microorganisms or endotoxins in the effluent water. These systems require frequent sanitization and microbiological monitoring to ensure water of appropriate microbiological quality at the points of use.

The *Purified Water* monograph also allows bulk packaging for commercial use elsewhere. In contrast to *Sterile Purified Water*, bulk packaged *Purified Water* is not required to be sterile. Because there is potential for microbial contamination and other quality changes in this bulk packaged nonsterile water, this form of *Purified Water* should be prepared and stored in a fashion that limits microbial growth and/or is simply used in a timely fashion before microbial proliferation renders it unsuitable for its intended use. Also depending on the material used for packaging, there could be extractable compounds leaching into the water from the packaging. Although this article is required to meet the same chemical purity limits as the bulk water, packaging extractables will render the packaged water less pure than the bulk water. The nature of these impurities may even render the water an inappropriate choice for some applications. It is the user's responsibility to ensure fitness for use of this packaged article when used in manufacturing, clinical, or analytical applications where the pure bulk form of the water is indicated.

Water for Injection—*Water for Injection* (see the *USP* monograph) is used as an excipient in the production of parenteral and other preparations where product endotoxin content must be controlled, and in other pharmaceutical applications, such as cleaning of certain equipment and parenteral product contact components. The minimum quality of source or feed water for the generation of *Water for Injection* is *Drinking Water* as defined by the U.S. Environmental Protection Agency (EPA), EU, Japan, or WHO. This source water may be pretreated to render it suitable for subsequent distillation (or whatever other validated process is used according to the monograph). The finished water must meet all of the chemical requirements for *Purified Water* as well as an additional bacterial endotoxin specification. Because endotoxins are produced by the kinds of microorganisms that are prone to inhabit water, the equipment and procedures used by the system to purify, store, and distribute *Water for Injection* must be designed to minimize or prevent microbial contamination as well as remove incoming endotoxins from the starting water. *Water for Injection* systems must be validated to reliably and consistently produce and distribute this quality of water.

The *Water for Injection* monograph also allows bulk packaging for commercial use. In contrast to *Sterile Water for Injection*, bulk packaged *Water for Injection* is not required to be sterile. However, to preclude significant changes in its microbial and endotoxins content during storage, this form of *Water for Injection* should be prepared and stored in a fashion that limits microbial growth and/or is simply used in a timely fashion before microbial proliferation renders it unsuitable for its intended use. Also depending on the material used for packaging, there could be extractable compounds leaching into the water from the packaging. Although this article is required to meet the same chemical purity limits as the bulk water, packaging extractables will render the packaged water less pure than the bulk water. The nature of these impurities may

even render the water an inappropriate choice for some applications. It is the user's responsibility to ensure fitness for use of this packaged article when used in manufacturing, clinical, or analytical applications where the purer bulk form of the water is indicated.

Water for Hemodialysis—*Water for Hemodialysis* (see the *USP* monograph) is used for hemodialysis applications, primarily the dilution of hemodialysis concentrate solutions. It is produced and used on site and is made from EPA Drinking Water that has been further purified to reduce chemical and microbiological components. It may be packaged and stored in unreactive containers that preclude bacterial entry. The term "unreactive containers" implies that the container, especially its water contact surfaces, is not changed in any way by the water, such as by leaching of container-related compounds into the water or by any chemical reaction or corrosion caused by the water. The water contains no added antimicrobials and is not intended for injection. Its attributes include specifications for water conductivity, total organic carbon (or oxidizable substances), microbial limits, and bacterial endotoxins. The water conductivity and total organic carbon attributes are identical to those established for *Purified Water* and *Water for Injection*; however, instead of total organic carbon (TOC), the organic content may alternatively be measured by the test for *Oxidizable Substances*. The microbial limits attribute for this water is unique among the "bulk" water monographs, but is justified on the basis of this water's specific application that has microbial content requirements related to its safe use. The bacterial endotoxins attribute is likewise established at a level related to its safe use.

Pure Steam—*Pure Steam* (see the *USP* monograph) is also sometimes referred to as "clean steam". It is used where the steam or its condensate would directly contact official articles or article contact surfaces, such as during their preparation, sterilization, or cleaning where no subsequent processing step is used to remove any codeposited impurity residues. These *Pure Steam* applications include but are not limited to porous load sterilization processes, product or cleaning solutions heated by direct steam injection, or humidification of processes where steam injection is used to control the humidity inside processing vessels where the official articles or their in-process forms are exposed. The primary intent of using this quality of steam is to ensure that official articles or article contact surfaces exposed to it are not contaminated by residues within the steam.

Pure Steam is prepared from suitably pretreated source water analogously to either the pretreatment used for *Purified Water* or *Water for Injection*. The water is vaporized with suitable mist elimination, and distributed under pressure. The sources of undesirable contaminants within *Pure Steam* could arise from entrained source water droplets, anticorrosion steam additives, or residues from the steam production and distribution system itself. The attributes in the *Pure Steam* monograph should detect most of the contaminants that could arise from these sources. If the official article exposed to potential *Pure Steam* residues is intended for parenteral use or other applications where the pyrogenic content must be controlled, the *Pure Steam* must additionally meet the specification for the *Bacterial Endotoxins Test* (85):

These purity attributes are measured on the condensate of the article, rather than the article itself. This, of course, imparts great importance to the cleanliness of the *Pure Steam* condensate generation and collection process because it must not adversely impact the quality of the resulting condensed fluid.

Other steam attributes not detailed in the monograph, in particular, the presence of even small

quantities of noncondensable gases or the existence of a superheated or dry state, may also be important for applications such as sterilization. The large release of energy (latent heat of condensation) as water changes from the gaseous to the liquid state is the key to steam's sterilization efficacy and its efficiency, in general, as a heat transfer agent. If this phase change (condensation) is not allowed to happen because the steam is extremely hot and in a persistent superheated, dry state, then its usefulness could be seriously compromised. Noncondensable gases in steam tend to stratify or collect in certain areas of a steam sterilization chamber or its load. These surfaces would thereby be at least partially insulated from the steam condensation phenomenon, preventing them from experiencing the full energy of the sterilizing conditions. Therefore, control of these kinds of steam attributes, in addition to its chemical purity, may also be important for certain *Pure Steam* applications. However, because these additional attributes are use-specific, they are not mentioned in the *Pure Steam* monograph.

Note that less pure "plant steam" may be used for steam sterilization of nonproduct contact nonporous loads, for general cleaning of nonproduct contact equipment, as a nonproduct contact heat exchange medium, and in all compatible applications involved in bulk pharmaceutical chemical and API manufacture.

Finally, owing to the lethal properties of *Pure Steam*, monitoring of microbial control within a steam system is unnecessary. Therefore, microbial analysis of the steam condensate is unnecessary.

Sterile Monographed Waters

The following monographed waters are packaged forms of either *Purified Water* or *Water for Injection* that have been sterilized to preserve their microbiological properties. These waters may have specific intended uses as indicated by their names and may also have restrictions on packaging configurations related to those uses. In general, these sterile packaged waters may be used in a variety of applications in lieu of the bulk forms of water from which they were derived. However, there is a marked contrast between the quality tests and purities for these bulk versus sterile packaged waters. These quality tests and specifications for sterile packaged waters have diverged from those of bulk waters to accommodate a wide variety of packaging types, properties, volumes, and uses. As a result, the inorganic and organic impurity specifications and levels of the bulk and sterile packaged forms of water are not equivalent as their name similarities imply. The packaging materials and elastomeric closures are the primary sources of these impurities, which tend to increase over these packaged articles' shelf lives. Therefore, due consideration must be given to the chemical purity suitability at the time of use of the sterile packaged forms of water when used in manufacturing, analytical, and cleaning applications in lieu of the bulk waters from which these waters were derived. It is the user's responsibility to ensure fitness for use of these sterile packaged waters in these applications. Nevertheless, for the applications discussed below for each sterile packaged water, their respective purities and packaging restrictions generally render them suitable by definition.

Sterile Purified Water—*Sterile Purified Water* (see the *USP* monograph) is *Purified Water*, packaged and rendered sterile. It is used in the preparation of nonparenteral compendial dosage forms or in analytical applications requiring *Purified Water* where access to a validated *Purified Water* system is not practical, where only a relatively small quantity is needed, where *Sterile Purified Water* is required, or where bulk packaged *Purified Water* is not suitably microbiologically controlled.

Sterile Water for Injection—~~*Sterile Water for Injection* (see the USP monograph) is *Water for Injection* packaged and rendered sterile. It is used for extemporaneous prescription compounding and as a sterile diluent for parenteral products. It may also be used for other applications where bulk *Water for Injection* or *Purified Water* is indicated but where access to a validated water system is either not practical or where only a relatively small quantity is needed. *Sterile Water for Injection* is packaged in single-dose containers not larger than 1 L in size.~~

Bacteriostatic Water for Injection—~~*Bacteriostatic Water for Injection* (see the USP monograph) is sterile *Water for Injection* to which has been added one or more suitable antimicrobial preservatives. It is intended to be used as a diluent in the preparation of parenteral products, most typically for multi-dose products that require repeated content withdrawals. It may be packaged in single-dose or multiple-dose containers not larger than 30 mL.~~

Sterile Water for Irrigation—~~*Sterile Water for Irrigation* (see the USP monograph) is *Water for Injection* packaged and sterilized in single-dose containers of larger than 1 L in size that allows rapid delivery of its contents. It need not meet the requirement under small-volume injections in the general test chapter *Particulate Matter in Injections* (788). It may also be used in other applications that do not have particulate matter specifications, where bulk *Water for Injection* or *Purified Water* is indicated but where access to a validated water system is not practical, or where somewhat larger quantities than are provided as *Sterile Water for Injection* are needed.~~

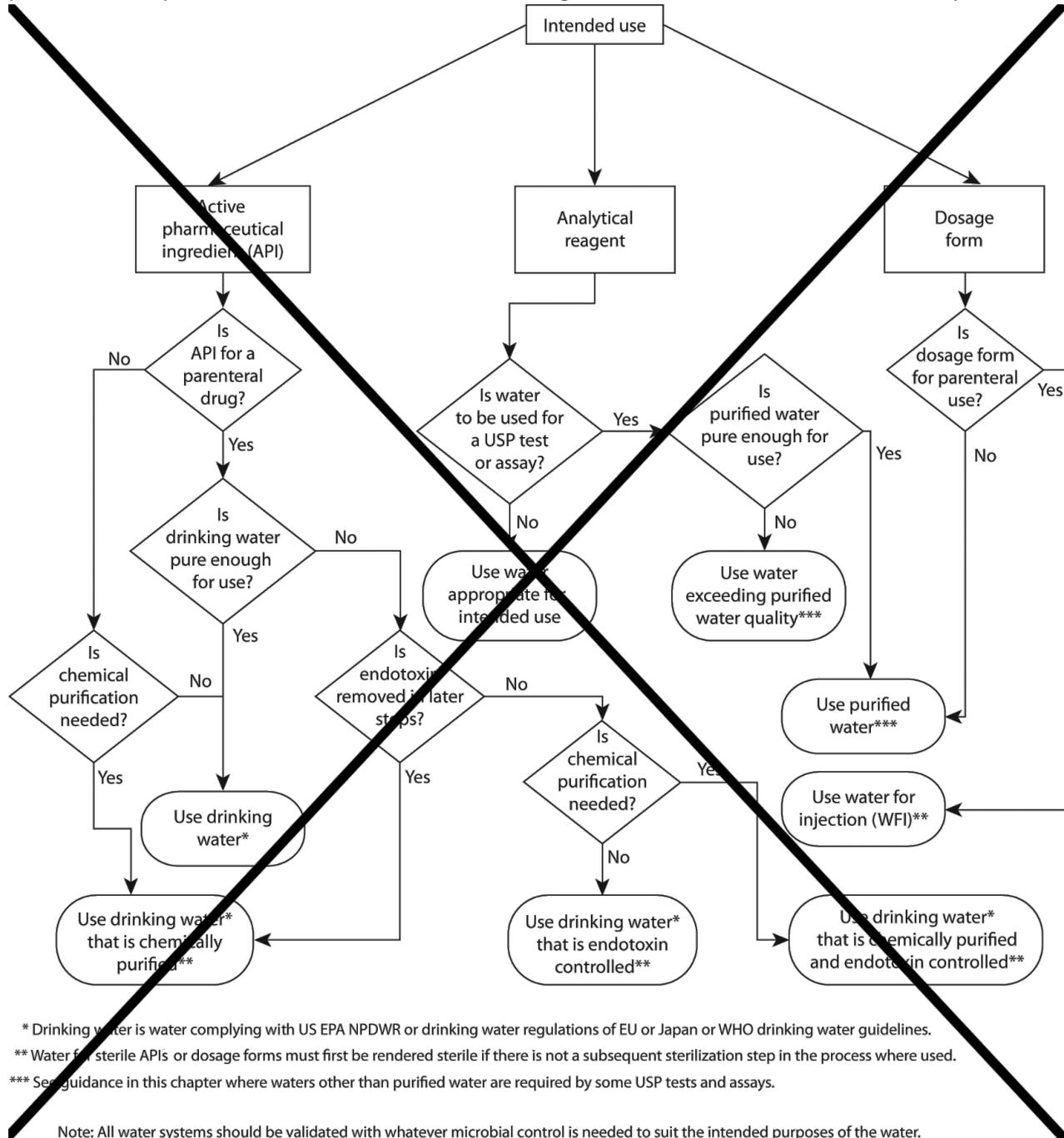
Sterile Water for Inhalation—~~*Sterile Water for Inhalation* (see the USP monograph) is *Water for Injection* that is packaged and rendered sterile and is intended for use in inhalators and in the preparation of inhalation solutions. It carries a less stringent specification for bacterial endotoxins than *Sterile Water for Injection* and therefore is not suitable for parenteral applications.~~

Nonmonographed Manufacturing Waters

In addition to the bulk monographed waters described above, nonmonographed waters can also be used in pharmaceutical processing steps such as cleaning, synthetic steps, or a starting material for further purification. The following is a description of several of these nonmonographed waters as cited in various locations within these compendia:

Drinking Water—This type of water can be referred to as Potable Water (meaning drinkable or fit to drink), National Primary Drinking Water, Primary Drinking Water, or National Drinking Water. Except where a singular drinking water specification is stated (such as the NPDWR [U.S. Environmental Protection Agency's National Primary Drinking Water Regulations as cited in 40 CFR Part 141]), this water must comply with the quality attributes of either the NPDWR, or the drinking water regulations of the European Union or Japan, or the WHO Drinking Water Guidelines. It may be derived from a variety of sources including a public water utility, a private water supply (e.g., a well), or a combination of these sources. *Drinking Water* may be used in the early stages of cleaning pharmaceutical manufacturing equipment and product-contact components. *Drinking Water* is also the minimum quality of water that should be used for the preparation of official substances and other bulk pharmaceutical ingredients. Where compatible with the processes, the allowed contaminant levels in *Drinking Water* are generally considered safe for use for official substances and other drug substances. Where required by the processing of the materials to achieve their required final purity, higher qualities of water may

be needed for these manufacturing steps, perhaps even as pure as *Water for Injection* or *Purified Water*. Such higher purity waters, however, might require only selected attributes to be of higher purity than *Drinking Water* (see Figure 2). *Drinking Water* is the prescribed source or feed water for the production of bulk monographed pharmaceutical waters. The use of *Drinking Water* specifications establishes a reasonable set of maximum allowable levels of chemical and microbiological contaminants with which a water purification system will be challenged. As seasonal variations in the quality attributes of the *Drinking Water* supply can occur, due consideration to its synthetic and cleaning uses must be given. The processing steps in the production of pharmaceutical waters must be designed to accommodate this variability.



* Drinking water is water complying with US EPA NPDWR or drinking water regulations of EU or Japan or WHO drinking water guidelines.
 ** Water for sterile APIs or dosage forms must first be rendered sterile if there is not a subsequent sterilization step in the process where used.
 *** See guidance in this chapter where waters other than purified water are required by some USP tests and assays.

Note: All water systems should be validated with whatever microbial control is needed to suit the intended purposes of the water.

Figure 2. Selection of water for pharmaceutical purposes.

Hot Purified Water—This water is used in the preparation instructions for *USP-NF* articles and

is clearly intended to be *Purified Water* that has been heated to an unspecified temperature in order to enhance solubilization of other ingredients. There is no upper temperature limit for the water (other than being less than 100°), but for each monograph there is an implied lower limit below which the desired solubilization effect would not occur.

Nonmonographed Analytical Waters

Both *General Notices and Requirements* and the introductory section to *Reagents, Indicators, and Solutions* clearly state that where the term “water”, without qualification or other specification, is indicated for use in analyses, the quality of water shall be *Purified Water*. However, numerous such qualifications do exist. Some of these qualifications involve methods of preparation, ranging from specifying the primary purification step to specifying additional purification. Other qualifications call for specific attributes to be met that might otherwise interfere with analytical processes. In most of these latter cases, the required attribute is not specifically tested. Rather, a further “purification process” is specified that ostensibly allows the water to adequately meet this required attribute.

However, preparation instructions for many reagents were carried forward from the innovator’s laboratories to the originally introduced monograph for a particular *USP-NF* article or general test chapter. The quality of the reagent water described in these tests may reflect the water quality designation of the innovator’s laboratory. These specific water designations may have originated without the innovator’s awareness of the requirement for *Purified Water* in *USP-NF* tests. Regardless of the original reason for the creation of these numerous special analytical waters, it is possible that the attributes of these special waters could now be met by the basic preparation steps and current specifications of *Purified Water*. In some cases, however, some of the cited post-processing steps are still necessary to reliably achieve the required attributes.

Users are not obligated to employ specific and perhaps archaically generated forms of analytical water where alternatives with equal or better quality, availability, or analytical performance may exist. The consistency and reliability for producing these alternative analytical waters should be verified as producing the desired attributes. In addition, any alternative analytical water must be evaluated on an application-by-application basis by the user to ensure its suitability. Following is a summary of the various types of nonmonographed analytical waters that are cited in the *USP-NF*.

Distilled Water—This water is produced by vaporizing liquid water and condensing it in a purer state. It is used primarily as a solvent for reagent preparation, but it is also specified in the execution of other aspects of tests, such as for rinsing an analyte, transferring a test material as a slurry, as a calibration standard or analytical blank, and for test apparatus cleaning. It is also cited as the starting water to be used for making *High Purity Water*. Because none of the cited uses of this water imply a need for a particular purity attribute that can only be derived by distillation, water meeting the requirements for *Purified Water* derived by other means of purification could be equally suitable where *Distilled Water* is specified.

Freshly Distilled Water—Also called “recently distilled water”, it is produced in a similar fashion to *Distilled Water* and should be used shortly after its generation. This implies the need to avoid endotoxin contamination as well as any other adventitious forms of contamination from the air or containers that could arise with prolonged storage. It is used for preparing solutions for subcutaneous test animal injections as well as for a reagent solvent in tests for which there appears to be no particularly high water purity needed that could be ascribable to being

"freshly distilled". In the test animal use, the term "freshly distilled" and its testing use imply a chemical, endotoxin, and microbiological purity that could be equally satisfied by *Water for Injection* (although no reference is made to these chemical, endotoxin, or microbial attributes or specific protection from recontamination). For nonanimal uses, water meeting the requirements for *Purified Water* derived by other means of purification and/or storage periods could be equally suitable where "recently distilled water" or *Freshly Distilled Water* is specified.

Deionized Water—This water is produced by an ion-exchange process in which the contaminating ions are replaced with either H^+ or OH^- ions. Similarly to *Distilled Water*, *Deionized Water* is used primarily as a solvent for reagent preparation, but it is also specified in the execution of other aspects of tests, such as for transferring an analyte within a test procedure, as a calibration standard or analytical blank, and for test apparatus cleaning. Also, none of the cited uses of this water imply any needed purity attribute that can only be achieved by deionization. Therefore, water meeting the requirements for *Purified Water* that is derived by other means of purification could be equally suitable where *Deionized Water* is specified.

Freshly Deionized Water—This water is prepared in a similar fashion to *Deionized Water*, although as the name suggests, it is to be used shortly after its production. This implies the need to avoid any adventitious contamination that could occur upon storage. This water is indicated for use as a reagent solvent as well as for cleaning. Due to the nature of the testing, *Purified Water* could be a reasonable alternative for these applications.

Deionized Distilled Water—This water is produced by deionizing (see *Deionized Water*) *Distilled Water*. This water is used as a reagent in a liquid chromatography test that requires a high purity. Because of the importance of this high purity, water that barely meets the requirements for *Purified Water* may not be acceptable. *High Purity Water* (see below) could be a reasonable alternative for this water.

Filtered Water—This water is *Purified Water* that has been filtered to remove particles that could interfere with the analysis where this water is specified. It is sometimes used synonymously with *Particle-Free Water* and *Ultra-Filtered Water* and is cited in some monographs and general chapters as well as in *Reagents*. Depending on its location, it is variously defined as water that has been passed through filters rated as 1.2 μm , 0.22 μm , or 0.2 μm ; or unspecified pore size. Although the water names and the filter pore sizes used to produce these waters are inconsistently defined, the use of 0.2 μm pore size filtered *Purified Water* should be universally acceptable for all applications where *Particle-Free Water*, *Filtered Water*, or *Ultra-Filtered Water* are specified.

High-Purity Water—This water may be prepared by deionizing previously distilled water, and then filtering it through a 0.45 μm rated membrane. This water must have an in-line conductivity of not greater than 0.15 $\mu S/cm$ (not less than 6.67 Megohm-cm) at 25°. If the water of this purity contacts the atmosphere even briefly as it is being used or drawn from its purification system, its conductivity will immediately increase, by as much as about 1.0 $\mu S/cm$, as atmospheric carbon dioxide dissolves in the water and equilibrates to hydrogen and bicarbonate ions. Therefore, if the analytical use requires that water conductivity remains as low as possible or the bicarbonate/carbon dioxide levels be as low as possible, its use should be protected from atmospheric exposure. This water is used as a reagent, as a solvent for reagent preparation, and for test apparatus cleaning where less pure waters would not perform acceptably. However, if a user's routinely available *Purified Water* is filtered and meets or

exceeds the conductivity specifications of *High-Purity Water*, it could be used in lieu of *High-Purity Water*.

Ammonia-Free Water—Functionally, this water must have a negligible ammonia concentration to avoid interference in tests sensitive to ammonia. It has been equated with *High-Purity Water* that has a significantly tighter *Stage 1* (see *Water Conductivity* (645)) conductivity specification than *Purified Water* because of the latter's allowance for a minimal level of ammonium among other ions. However, if the user's *Purified Water* were filtered and met or exceeded the conductivity specifications of *High-Purity Water*, it would contain negligible ammonia or other ions and could be used in lieu of *High-Purity Water*.

Carbon Dioxide-Free Water—The introductory portion of the *Reagents, Indicators, and Solutions* section defines this water as *Purified Water* that has been vigorously boiled for at least 5 minutes, then cooled and protected from absorption of atmospheric carbon dioxide. Because the absorption of carbon dioxide tends to drive down the water pH, most of the uses of *Carbon Dioxide-Free Water* are either associated as a solvent in pH-related or pH-sensitive determinations or as a solvent in carbonate-sensitive reagents or determinations. Another use of this water is for certain optical rotation and color and clarity of solution tests. Although it is possible that this water is indicated for these tests simply because of its purity, it is also possible that the pH effects of carbon dioxide-containing water could interfere with the results of these tests. A third plausible reason that this water is indicated is that outgassing air bubbles might interfere with these photometric-type tests. The boiled water preparation approach will also greatly reduce the concentrations of many other dissolved gases along with carbon dioxide. Therefore, in some of the applications for *Carbon Dioxide-Free Water*, it could be the inadvertent deaeration effect that actually renders this water suitable. In addition to boiling, deionization is perhaps an even more efficient process for removing dissolved carbon dioxide (by drawing the dissolved gas equilibrium toward the ionized state with subsequent removal by the ion-exchange resins). If the starting *Purified Water* is prepared by an efficient deionization process and protected after deionization from exposure to atmospheric air, water that is carbon dioxide free can be effectively made without the application of heat. However, this deionization process does not deaerate the water, so if *Purified Water* prepared by deionization is considered as a substitute water in a test requiring *Carbon Dioxide-Free Water*, the user must verify that it is not actually water akin to *Deaerated Water* (discussed below) that is needed for the test. As indicated in *High-Purity Water*, even brief contact with the atmosphere can allow small amounts of carbon dioxide to dissolve, ionize, and significantly degrade the conductivity and lower the pH. If the analytical use requires the water to remain as pH-neutral and as carbon dioxide free as possible, even the analysis should be protected from atmospheric exposure. However, in most applications, atmospheric exposure during testing does not significantly affect its suitability in the test.

Ammonia and Carbon Dioxide-Free Water—As implied by the name, this water should be prepared by approaches compatible with those mentioned for both *Ammonia-Free Water* and *Carbon Dioxide-Free Water*. Because the carbon dioxide-free attribute requires post-production protection from the atmosphere, it is appropriate to first render the water ammonia-free using the *High-Purity Water* process followed by the boiling and carbon dioxide-protected cooling process. The *High-Purity Water* deionization process for creating *Ammonia-Free Water* will also remove the ions generated from dissolved carbon dioxide and ultimately, by forced equilibration to the ionized state, all the dissolved carbon dioxide. Therefore, depending on its use, an acceptable procedure for making *Ammonia and Carbon Dioxide-Free Water* could be to transfer

and collect *High-Purity Water* in a carbon dioxide intrusion-protected container.

Deaerated Water—This water is *Purified Water* that has been treated to reduce the content of dissolved air by “suitable means”. In the *Reagents* section, approaches for boiling, cooling (similar to *Carbon Dioxide-Free Water* but without the atmospheric carbon dioxide protection), and sonication are given as applicable for test uses other than dissolution and drug release testing. Although *Deaerated Water* is not mentioned by name in *Dissolution* (711), suggested methods for deaerating dissolution media (which may be water) include warming to 41°, vacuum filtering through a 0.45- μm rated membrane, and vigorously stirring the filtrate while maintaining the vacuum. This chapter specifically indicates that other validated approaches may be used. In other monographs that also do not mention *Deaerated Water* by name, degassing of water and other reagents is accomplished by sparging with helium. *Deaerated Water* is used in both dissolution testing as well as liquid chromatography applications where outgassing could either interfere with the analysis itself or cause erroneous results due to inaccurate volumetric withdrawals. Applications where ambient temperature water is used for reagent preparation, but the tests are performed at elevated temperatures, are candidates for outgassing effects. If outgassing could interfere with test performance, including chromatographic flow, colorimetric or photometric measurements, or volumetric accuracy, then *Deaerated Water* should probably be used, whether called for in the analysis or not. The above deaeration approaches might not render the water “gas-free”. At best, they reduce the dissolved gas concentrations so that outgassing caused by temperature changes is not likely.

Recently Boiled Water—This water may include recently or freshly boiled water (with or without mention of cooling in the title), but cooling prior to use is clearly intended. Occasionally it is necessary to use when hot. *Recently Boiled Water* is specified because it is used in a pH-related test or carbonate-sensitive reagent, in an oxygen-sensitive test or reagent, or in a test where outgassing could interfere with the analysis, such as specific gravity or an appearance test.

Oxygen-Free Water—The preparation of this water is not specifically described in the compendia. Neither is there an oxygen specification or analysis mentioned. However, all uses involve analyses of materials that could be sensitive to oxidation by atmospheric oxygen. Procedures for the removal of dissolved oxygen from solvents, although not necessarily water, are mentioned in *Polarography* (801) and *Spectrophotometry and Light Scattering* (851). These procedures involve simple sparging of the liquid with an inert gas such as nitrogen or helium, followed by inert gas blanketing to prevent oxygen reabsorption. The sparging times cited range from 5 to 15 minutes to an unspecified period. Some *Purified Water* and *Water for Injection* systems produce water that is maintained in a hot state and that is inert gas blanketed during its preparation and storage and distribution. Although oxygen is poorly soluble in hot water, such water may not be oxygen-free. Whatever procedure is used for removing oxygen should be verified as reliably producing water that is fit for use.

Water for BET—This water is also referred to as LAL reagent water. This is often *Water for Injection*, which may have been sterilized. It is free from a level of endotoxin that would yield any detectable reaction or interference with the *Limulus Amoebocyte Lysate* reagent used in the *Bacterial Endotoxins Test* (85).

Organic-Free Water—This water is defined by *Residual Solvents* (467) as producing no significantly interfering gas chromatography peaks. Referenced monographs specify using this water as the solvent for the preparation of standard and test solutions for the *Residual*

solvents test.

Lead-Free Water—This water is used as a transferring diluent for an analyte in a *Lead* (251) test. Although no specific instructions are given for its preparation, it must not contain any detectable lead. *Purified Water* should be a suitable substitute for this water.

Chloride-Free Water—This water is specified as the solvent for use in an assay that contains a reactant that precipitates in the presence of chloride. Although no specific preparation instructions are given for this water, its rather obvious attribute is having a very low chloride level in order to be unreactive with this chloride sensitive reactant. *Purified Water* could be used for this water but should be tested to ensure that it is unreactive.

Hot Water—The uses of this water include solvents for achieving or enhancing reagent solubilization, restoring the original volume of boiled or hot solutions, rinsing insoluble analytes free of hot water soluble impurities, solvents for reagent recrystallization, apparatus cleaning, and as a solubility attribute for various *USP-NF* articles. In only one monograph is the temperature of "hot" water specified; so in all the other cases, the water temperature is less important, but should be high enough to achieve the desirable effect. In all cases, the chemical quality of the water is implied to be that of *Purified Water*.

VALIDATION AND QUALIFICATION OF WATER PURIFICATION, STORAGE, AND DISTRIBUTION SYSTEMS

Establishing the dependability of pharmaceutical water purification, storage, and distribution systems requires an appropriate period of monitoring and observation. Ordinarily, few problems are encountered in maintaining the chemical purity of *Purified Water* and *Water for Injection*. Nevertheless, the advent of using conductivity and TOC to define chemical purity has allowed the user to more quantitatively assess the water's chemical purity and its variability as a function of routine pretreatment system maintenance and regeneration. Even the presence of such unit operations as heat exchangers and use point hoses can compromise the chemical quality of water within and delivered from an otherwise well-controlled water system. Therefore, an assessment of the consistency of the water's chemical purity over time must be part of the validation program. However, even with the most well controlled chemical quality, it is often more difficult to consistently meet established microbiological quality criteria owing to phenomena occurring during and after chemical purification. A typical program involves intensive daily sampling and testing of major process points for at least one month after operational criteria have been established for each unit operation, point of use, and sampling point.

An overlooked aspect of water system validation is the delivery of the water to its actual location of use. If this transfer process from the distribution system outlets to the water use locations (usually with hoses) is defined as outside the water system, then this transfer process still needs to be validated to not adversely affect the quality of the water to the extent it becomes unfit for use. Because routine microbial monitoring is performed for the same transfer process and components (e.g., hoses and heat exchangers) as that of routine water use (see *Sampling Considerations*), there is some logic to including this water transfer process within the distribution system validation.

Validation is the process whereby substantiation to a high level of assurance that a specific process will consistently produce a product conforming to an established set of quality

attributes is acquired and documented. Prior to and during the very early stages of validation, the critical process parameters and their operating ranges are established. A validation program qualifies and documents the design, installation, operation, and performance of equipment. It begins when the system is defined and moves through several stages: installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ). A graphical representation of a typical water system validation life cycle is shown in *Figure 3*.

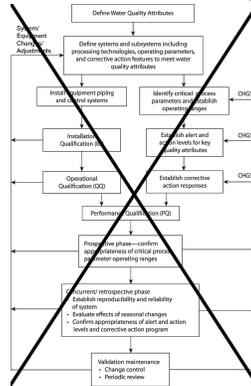


Figure 3. Water system validation life cycle.

A validation plan for a water system typically includes the following steps: (1) establishing standards for quality attributes of the finished water and the source water; (2) defining suitable unit operations and their operating parameters for achieving the desired finished water quality attributes from the available source water; (3) selecting piping, equipment, controls, and monitoring technologies; (4) developing an IQ stage consisting of instrument calibrations, inspections to verify that the drawings accurately depict the final configuration of the water system and, where necessary, special tests to verify that the installation meets the design requirements; (5) developing an OQ stage consisting of tests and inspections to verify that the equipment, system alerts, and controls are operating reliably and that appropriate alert and action levels are established (this phase of qualification may overlap with aspects of the next step); and (6) developing a prospective PQ stage to confirm the appropriateness of critical process parameter operating ranges (during this phase of validation, alert and action levels for key quality attributes and operating parameters are verified); (7) assuring the adequacy of ongoing control procedures, e.g., sanitization frequency; (8) supplementing a validation maintenance program (also called continuous validation life cycle) that includes a mechanism to control changes to the water system and establishes and carries out scheduled preventive maintenance including recalibration of instruments (in addition, validation maintenance includes a monitoring program for critical process parameters and a corrective action program); (9) instituting a schedule for periodic review of the system performance and requalification; and (10) completing protocols and documenting Steps 1 through 9.

PURIFIED WATER AND WATER FOR INJECTION SYSTEMS

The design, installation, and operation of systems to produce *Purified Water* and *Water for Injection* include similar components, control techniques, and procedures. The quality attributes of both waters differ only in the presence of a bacterial endotoxin requirement for *Water for Injection* and in their methods of preparation, at least at the last stage of preparation. The similarities in the quality attributes provide considerable common ground in the design of water systems to meet either requirement. The critical difference is the degree of control of the system and the final purification steps needed to ensure bacterial and bacterial endotoxin

removal.

Production of pharmaceutical water employs sequential unit operations (processing steps) that address specific water quality attributes and protect the operation of subsequent treatment steps. A typical evaluation process to select an appropriate water quality for a particular pharmaceutical purpose is shown in the decision tree in *Figure 2*. This diagram may be used to assist in defining requirements for specific water uses and in the selection of unit operations. The final unit operation used to produce *Water for Injection* is limited to distillation or other processes equivalent or superior to distillation in the removal of chemical impurities as well as microorganisms and their components. Distillation has a long history of reliable performance and can be validated as a unit operation for the production of *Water for Injection*, but other technologies or combinations of technologies can be validated as being equivalently effective. Other technologies, such as ultrafiltration following another chemical purification process, may be suitable in the production of *Water for Injection* if they can be shown through validation to be as effective and reliable as distillation. The advent of new materials for older technologies, such as reverse osmosis and ultrafiltration, that allow intermittent or continuous operation at elevated, microbial temperatures, show promise for a valid use in producing *Water for Injection*.

The validation plan should be designed to establish the suitability of the system and to provide a thorough understanding of the purification mechanism, range of operating conditions, required pretreatment, and the most likely modes of failure. It is also necessary to demonstrate the effectiveness of the monitoring scheme and to establish the documentation and qualification requirements for the system's validation maintenance. Trials conducted in a pilot installation can be valuable in defining the operating parameters and the expected water quality and in identifying failure modes. However, qualification of the specific unit operation can only be performed as part of the validation of the installed operational system. The selection of specific unit operations and design characteristics for a water system should take into account the quality of the feed water, the technology chosen for subsequent processing steps, the extent and complexity of the water distribution system, and the appropriate compendial requirements. For example, in the design of a system for *Water for Injection*, the final process (distillation or whatever other validated process is used according to the monograph) must have effective bacterial endotoxin reduction capability and must be validated.

UNIT OPERATIONS CONCERNS

The following is a brief description of selected unit operations and the operation and validation concerns associated with them. Not all unit operations are discussed, nor are all potential problems addressed. The purpose is to highlight issues that focus on the design, installation, operation, maintenance, and monitoring parameters that facilitate water system validation:

Prefiltration

The purpose of prefiltration—also referred to as initial, coarse, or depth filtration—is to remove solid contaminants down to a size of 7–10 μm from the incoming source water supply and protect downstream system components from particulates that can inhibit equipment performance and shorten its effective life. This coarse filtration technology utilizes primarily sieving effects for particle capture and a depth of filtration medium that has a high “dirt load” capacity. Such filtration units are available in a wide range of designs and for various applications. Removal efficiencies and capacities differ significantly, from granular bed filters such as multimedia or sand for larger water systems, to depth cartridges for smaller water

systems. Unit and system configurations vary widely in type of filtering media and location in the process. Granular or cartridge prefilters are often situated at or near the head of the water pretreatment system prior to unit operations designed to remove the source water disinfectants. This location, however, does not preclude the need for periodic microbial control because biofilm can still proliferate, although at a slower rate in the presence of source water disinfectants. Design and operational issues that may impact performance of depth filters include channeling of the filtering media, blockage from silt, microbial growth, and filtering-media loss during improper backwashing. Control measures involve pressure and flow monitoring during use and backwashing, sanitizing, and replacing filtering media. An important design concern is sizing of the filter to prevent channeling or media loss resulting from inappropriate water flow rates as well as proper sizing to minimize excessively frequent or infrequent backwashing or cartridge filter replacement.

Activated Carbon

Granular activated carbon beds adsorb low molecular weight organic material and oxidizing additives, such as chlorine and chloramine compounds, removing them from the water. They are used to achieve certain quality attributes and to protect against reaction with downstream stainless steel surfaces, resins, and membranes. The chief operating concerns regarding activated carbon beds include the propensity to support bacteria growth, the potential for hydraulic channeling, the organic adsorption capacity, appropriate water flow rates and contact time, the inability to be regenerated in situ, and the shedding of bacteria, endotoxins, organic chemicals, and fine carbon particles. Control measures may involve monitoring water flow rates and differential pressures, sanitizing with hot water or steam, backwashing, testing for adsorption capacity, and frequent replacement of the carbon bed. If the activated carbon bed is intended for organic reduction, it may also be appropriate to monitor influent and effluent TOC. It is important to note that the use of steam for carbon bed sanitization is often incompletely effective due to steam channeling rather than even permeation through the bed. This phenomenon can usually be avoided by using hot water sanitization. It is also important to note that microbial biofilm development on the surface of the granular carbon particles (as well as on other particles such as found in deionizer beds and even multimedia beds) can cause adjacent bed granules to "stick" together. When large masses of granules are agglomerated in this fashion, normal backwashing and bed fluidization flow parameters may not be sufficient to disperse them, leading to ineffective removal of trapped debris, loose biofilm, and penetration of microbial controlling conditions (as well as regenerant chemicals as in the case of agglomerated deionizer resins). Alternative technologies to activated carbon beds can be used in order to avoid their microbial problems, such as disinfectant neutralizing chemical additives and regenerable organic scavenging devices. However, these alternatives do not function by the same mechanisms as activated carbon, may not be as effective at removing disinfectants and some organics, and have a different set of operating concerns and control measures that may be nearly as troublesome as activated carbon beds.

Additives

Chemical additives are used in water systems (a) to control microorganisms by use of sanitants such as chlorine compounds and ozone, (b) to enhance the removal of suspended solids by use of flocculating agents, (c) to remove chlorine compounds, (d) to avoid scaling on reverse osmosis membranes, and (e) to adjust pH for more effective removal of carbonate and ammonia compounds by reverse osmosis. These additives do not constitute "added substances" as long as they are either removed by subsequent processing steps or are otherwise absent from the

finished water. Control of additives to ensure a continuously effective concentration and subsequent monitoring to ensure their removal should be designed into the system and included in the monitoring program.

Organic Scavengers

Organic scavenging devices use macroreticular weakly basic anion exchange resins capable of removing organic material and endotoxins from the water. They can be regenerated with appropriate biocidal caustic brine solutions. Operating concerns are associated with organic scavenging capacity; particulate, chemical and microbiological fouling of the reactive resin surface; flow rate; regeneration frequency; and shedding of resin fragments. Control measures include TOC testing of influent and effluent, backwashing, monitoring hydraulic performance, and using downstream filters to remove resin fines.

Softeners

Water softeners may be located either upstream or downstream of disinfectant removal units. They utilize sodium-based cation exchange resins to remove water hardness ions, such as calcium and magnesium, that could foul or interfere with the performance of downstream processing equipment such as reverse osmosis membranes, deionization devices, and distillation units. Water softeners can also be used to remove other lower affinity cations, such as the ammonium ion, that may be released from chloramine disinfectants commonly used in drinking water and which might otherwise carryover through other downstream unit operations. If ammonium removal is one of its purposes, the softener must be located downstream of the disinfectant removal operation, which itself may liberate ammonium from neutralized chloramine disinfectants. Water softener resin beds are regenerated with concentrated sodium chloride solution (brine). Concerns include microorganism proliferation, channeling caused by biofilm agglomeration of resin particles, appropriate water flow rates and contact time, ion exchange capacity, organic and particulate resin fouling, organic leaching from new resins, fracture of the resin beads, resin degradation by excessively chlorinated water, and contamination from the brine solution used for regeneration. Control measures involve recirculation of water during periods of low water use, periodic sanitization of the resin and brine system, use of microbial control devices (e.g., UV light and chlorine), locating the unit upstream of the disinfectant removal step (if used only for softening), appropriate regeneration frequency, effluent chemical monitoring (e.g., hardness ions and possibly ammonium), and downstream filtration to remove resin fines. If a softener is used for ammonium removal from chloramine-containing source water, then capacity, contact time, resin surface fouling, pH, and regeneration frequency are very important.

Deionization

Deionization (DI), and continuous electrodeionization (CEDI) are effective methods of improving the chemical quality attributes of water by removing cations and anions. DI systems have charged resins that require periodic regeneration with an acid and base. Typically, cationic resins are regenerated with either hydrochloric or sulfuric acid, which replace the captured positive ions with hydrogen ions. Anionic resins are regenerated with sodium or potassium hydroxide, which replace captured negative ions with hydroxide ions. Because free endotoxin is negatively charged, there is some removal of endotoxin achieved by the anionic resin. Both regenerant chemicals are biocidal and offer a measure of microbial control. The system can be designed so that the cation and anion resins are in separate or "twin" beds or they can be

mixed together to form a mixed bed. Twin beds are easily regenerated but deionize water less efficiently than mixed beds, which have a considerably more complex regeneration process. Rechargeable resin canisters can also be used for this purpose.

The CEDI system uses a combination of mixed resin, selectively permeable membranes, and an electric charge, providing continuous flow (product and waste concentrate) and continuous regeneration. Water enters both the resin section and the waste (concentrate) section. As it passes through the resin, it is deionized to become product water. The resin acts as a conductor enabling the electrical potential to drive the captured cations and anions through the resin and appropriate membranes for concentration and removal in the waste water stream. The electrical potential also separates the water in the resin (product) section into hydrogen and hydroxide ions. This permits continuous regeneration of the resin without the need for regenerant additives. However, unlike conventional deionization, CEDI units must start with water that is already partially purified because they generally cannot produce *Purified Water* quality when starting with the heavier ion load of unpurified source water.

Concerns for all forms of deionization units include microbial and endotoxin control, chemical additive impact on resins and membranes, and loss, degradation, and fouling of resin. Issues of concern specific to DI units include regeneration frequency and completeness, channeling caused by biofilm agglomeration of resin particles, organic leaching from new resins, complete resin separation for mixed bed regeneration, and mixing air contamination (mixed beds). Control measures vary but typically include recirculation loops, effluent microbial control by UV light, conductivity monitoring, resin testing, microporous filtration of mixing air, microbial monitoring, frequent regeneration to minimize and control microorganism growth, sizing the equipment for suitable water flow and contact time, and use of elevated temperatures. Internal distributor and regeneration piping for mixed bed units should be configured to ensure that regeneration chemicals contact all internal bed and piping surfaces and resins. Rechargeable canisters can be the source of contamination and should be carefully monitored. Full knowledge of previous resin use, minimum storage time between regeneration and use, and appropriate sanitizing procedures are critical factors ensuring proper performance.

Reverse Osmosis

Reverse osmosis (RO) units employ semipermeable membranes. The "pores" of RO membranes are actually intersegmental spaces among the polymer molecules. They are big enough for permeation of water molecules, but too small to permit passage of hydrated chemical ions. However, many factors including pH, temperature, and differential pressure across the membrane affect the selectivity of this permeation. With the proper controls, RO membranes can achieve chemical, microbial, and endotoxin quality improvement. The process streams consist of supply water, product water (permeate), and wastewater (reject). Depending on source water, pretreatment and system configuration variations and chemical additives may be necessary to achieve desired performance and reliability.

A major factor affecting RO performance is the permeate recovery rate, that is, the amount of the water passing through the membrane compared to the amount rejected. This is influenced by the several factors, but most significantly by the pump pressure. Recoveries of 75% are typical, and can accomplish a 1–2 log purification of most impurities. For most feed waters, this is usually not enough to meet *Purified Water* conductivity specifications. A second pass of this permeate water through another RO stage usually achieves the necessary permeate purity if other factors such as pH and temperature have been appropriately adjusted and the ammonia

from chloraminated source water has been previously removed. Increasing recoveries with higher pressures in order to reduce the volume of reject water will lead to reduced permeate purity. If increased pressures are needed over time to achieve the same permeate flow, this is an indication of partial membrane blockage that needs to be corrected before it becomes irreversibly fouled, and expensive membrane replacement is the only option.

Other concerns associated with the design and operation of RO units include membrane materials that are extremely sensitive to sanitizing agents and to particulate, chemical, and microbial membrane fouling; membrane and seal integrity; the passage of dissolved gases, such as carbon dioxide and ammonia; and the volume of wastewater, particularly where water discharge is tightly regulated by local authorities. Failure of membrane or seal integrity will result in product water contamination. Methods of control involve suitable pretreatment of the influent water stream, appropriate membrane material selection, integrity challenges, membrane design and heat tolerance, periodic sanitization, and monitoring of differential pressures, conductivity, microbial levels, and TOC.

The development of RO units that can tolerate sanitizing water temperatures as well as operate efficiently and continuously at elevated temperatures has added greatly to their microbial control and to the avoidance of biofouling. RO units can be used alone or in combination with DI and CEDI units as well as ultrafiltration for operational and quality enhancements.

Ultrafiltration

Ultrafiltration is a technology most often employed in pharmaceutical water systems for removing endotoxins from a water stream. It can also use semipermeable membranes, but unlike RO, these typically use polysulfone membranes whose intersegmental "pores" have been purposefully exaggerated during their manufacture by preventing the polymer molecules from reaching their smaller equilibrium proximities to each other. Depending on the level of equilibrium control during their fabrication, membranes with differing molecular weight "cutoffs" can be created such that molecules with molecular weights above these cutoff ratings are rejected and cannot penetrate the filtration matrix.

Ceramic ultrafilters are another molecular sieving technology. Ceramic ultrafilters are self supporting and extremely durable, backwashable, chemically cleanable, and steam sterilizable. However, they may require higher operating pressures than membrane type ultrafilters.

All ultrafiltration devices work primarily by a molecular sieving principle. Ultrafilters with molecular weight cutoff ratings in the range of 10,000–20,000 Da are typically used in water systems for removing endotoxins. This technology may be appropriate as an intermediate or final purification step. Similar to RO, successful performance is dependent upon pretreatment of the water by upstream unit operations.

Issues of concern for ultrafilters include compatibility of membrane material with heat and sanitizing agents, membrane integrity, fouling by particles and microorganisms, and seal integrity. Control measures involve filtration medium selection, sanitization, flow design (dead end vs. tangential), integrity challenges, regular cartridge changes, elevated feed water temperature, and monitoring TOC and differential pressure. Additional flexibility in operation is possible based on the way ultrafiltration units are arranged such as in a parallel or series configurations. Care should be taken to avoid stagnant water conditions that could promote microorganism growth in back-up or standby units.

Charge-Modified Filtration

Charge-modified filters are usually microbially retentive filters that are treated during their manufacture to have a positive charge on their surfaces. Microbial retentive filtration will be described in a subsequent section, but the significant feature of these membranes is their electrostatic surface charge. Such charged filters can reduce endotoxin levels in the fluids passing through them by their adsorption (owing to the endotoxin's negative charge) onto the membrane surfaces. Although ultrafilters are more often employed as a unit operation for endotoxin removal in water systems, charge-modified filters may also have a place in endotoxin removal particularly where available upstream pressures are not sufficient for ultrafiltration and for a single, relatively short-term use. Charge-modified filters may be difficult to validate for long-term or large-volume endotoxin retention. Even though their purified standard endotoxin retention can be well characterized, their retention capacity for "natural" endotoxins is difficult to gauge. Nevertheless, utility could be demonstrated and validated as short-term, single-use filters at points of use in water systems that are not designed for endotoxin control or where only an endotoxin "polishing" (removal of only slight or occasional endotoxin levels) is needed. Control and validation concerns include volume and duration of use, flow rate, water conductivity and purity, and constancy and concentration of endotoxin levels being removed. All of these factors may have to be evaluated and challenged prior to using this approach, making this a difficult-to-validate application. Even so, there may still be a possible need for additional backup endotoxin testing both upstream and downstream of the filter.

Microbial-Retentive Filtration

Microbial-retentive membrane filters have experienced an evolution of understanding in the past decade that has caused previously held theoretical retention mechanisms to be reconsidered. These filters have a larger effective "pore size" than ultrafilters and are intended to prevent the passage of microorganisms and similarly sized particles without unduly restricting flow. This type of filtration is widely employed within water systems for filtering the bacteria out of both water and compressed gases as well as for vent filters on tanks and stills and other unit operations. However, the properties of the water system microorganisms seem to challenge a filter's microbial retention from water with phenomena absent from other aseptic filtration applications, such as filter sterilizing of pharmaceutical formulations prior to packaging. In the latter application, sterilizing grade filters are generally considered to have an assigned rating of 0.2 or 0.22 μm . This rather arbitrary rating is associated with filters that have the ability to retain a high level challenge of a specially prepared inoculum of *Brevundimonas* (formerly *Pseudomonas*) *diminuta*. This is a small microorganism originally isolated decades ago from a product that had been "filter sterilized" using a 0.45 μm rated filter. Further study revealed that a percentage of cells of this microorganism could reproducibly penetrate the 0.45 μm sterilizing filters. Through historic correlation of *B. diminuta* retaining tighter filters, thought to be twice as good as a 0.45 μm filter, assigned ratings of 0.2 or 0.22 μm with their successful use in product solution filter sterilization, both this filter rating and the associated high level *B. diminuta* challenge have become the current benchmarks for sterilizing filtration. New evidence now suggests that for microbial-retentive filters used for pharmaceutical water, *B. diminuta* may not be the best model microorganism.

An archaic understanding of microbial-retentive filtration would lead one to equate a filter's rating with the false impression of a simple sieve or screen that absolutely retains particles sized at or above the filter's rating. A current understanding of the mechanisms involved in microbial retention and the variables that can affect those mechanisms has yielded a far more

complex interaction of phenomena than previously understood. A combination of simple sieve retention and surface adsorption are now known to contribute to microbial retention.

The following all interact to create some unusual and surprising retention phenomena for water system microorganisms: the variability in the range and average pore sizes created by the various membrane fabrication processes, the variability of the surface chemistry and three-dimensional structures related to the different polymers used in these filter matrices, and the size and surface properties of the microorganism intended to be retained by the filters. *B. diminuta* may not be the best challenge microorganisms for demonstrating bacterial retention for 0.2- to 0.22- μm rated filters for use in water systems because it appears to be more easily retained by these filters than some water system flora. The well-documented appearance of water system microorganisms on the downstream sides of some 0.2- to 0.22- μm rated filters after a relatively short period of use seems to support that some penetration phenomena are at work. Unknown for certain is if this downstream appearance is caused by a "blow-through" or some other pass-through phenomenon as a result of tiny cells or less cell "stickiness", or by a "growth through" phenomenon as a result of cells hypothetically replicating their way through the pores to the downstream side. Whatever is the penetration mechanism, 0.2- to 0.22- μm rated membranes may not be the best choice for some water system uses.

Microbial retention success in water systems has been reported with the use of some manufacturers' filters arbitrarily rated as 0.1 μm . There is general agreement that for a given manufacturer, their 0.1- μm rated filters are tighter than their 0.2- to 0.22- μm rated filters. However, comparably rated filters from different manufacturers in water filtration applications may not perform equivalently owing to the different filter fabrication processes and the nonstandardized microbial retention challenge processes currently used for defining the 0.1- μm filter rating. It should be noted that use of 0.1- μm rated membranes generally results in a sacrifice in flow rate compared to 0.2- to 0.22- μm membranes, so whatever membranes are chosen for a water system application, the user must verify that the membranes are suitable for their intended application, use period, and use process, including flow rate.

For microbial retentive gas filtrations, the same sieving and adsorptive retention phenomena are at work as in liquid filtration, but the adsorptive phenomenon is enhanced by additional electrostatic interactions between particles and filter matrix. These electrostatic interactions are so strong that particle retention for a given filter rating is significantly more efficient in gas filtration than in water or product solution filtrations. These additional adsorptive interactions render filters rated at 0.2-0.22- μm unquestionably suitable for microbial retentive gas filtrations. When microbially retentive filters are used in these applications, the membrane surface is typically hydrophobic (non-wettable by water). A significant area of concern for gas filtration is blockage of tank vents by condensed water vapor, which can cause mechanical damage to the tank. Control measures include electrical or steam tracing and a self-draining orientation of vent filter housings to prevent accumulation of vapor condensate. However, a continuously high filter temperature will take an oxidative toll on polypropylene components of the filter, so sterilization of the unit prior to initial use, and periodically thereafter, as well as regular visual inspections, integrity tests, and changes are recommended control methods.

In water applications, microbial retentive filters may be used downstream of unit operations that tend to release microorganisms or upstream of unit operations that are sensitive to microorganisms. Microbial retentive filters may also be used to filter water feeding the distribution system. It should be noted that regulatory authorities allow the use of microbial-

retentive filters within distribution systems or even at use points if they have been properly validated and are appropriately maintained. A point-of-use filter should only be intended to "polish" the microbial quality of an otherwise well-maintained system and not to serve as the primary microbial control device. The efficacy of system microbial control measures can only be assessed by sampling the water upstream of the filters. As an added measure of protection, in-line UV lamps, appropriately sized for the flow rate (see *Sanitization*), may be used just upstream of microbial retentive filters to inactivate microorganisms prior to their capture by the filter. This tandem approach tends to greatly delay potential microbial penetration phenomena and can substantially extend filter service life.

Ultraviolet Light

The use of low-pressure UV lights that emit a 254-nm wavelength for microbial control is discussed under *Sanitization*, but the application of UV light in chemical purification is also emerging. This 254-nm wavelength is also useful in the destruction of ozone. With intense emissions at wavelengths around 185 nm (as well as at 254 nm), medium-pressure UV lights have demonstrated utility in the destruction of the chlorine-containing disinfectants used in source water as well as for interim stages of water pretreatment. High intensities of this wavelength alone or in combination with other oxidizing sanitants, such as hydrogen peroxide, have been used to lower TOC levels in recirculating distribution systems. The organics are typically converted to carbon dioxide, which equilibrates to bicarbonate, and incompletely oxidized carboxylic acids, both of which can easily be removed by polishing ion-exchange resins. Areas of concern include adequate UV intensity and residence time, gradual loss of UV emissivity with bulb age, gradual formation of UV-absorbing film at the water contact surface, incomplete photodegradation during unforeseen source water hyperchlorination, release of ammonia from chloramine photodegradation, unapparent UV bulb failure, and conductivity degradation in distribution systems using 185-nm UV lights. Control measures include regular inspection or emissivity alarms to detect bulb failures or film occlusions, regular UV bulb sleeve cleaning and wiping, downstream chlorine detectors, downstream polishing deionizers, and regular (approximately yearly) bulb replacement.

Distillation

Distillation units provide chemical and microbial purification via thermal vaporization, mist elimination, and water vapor condensation. A variety of designs is available including single effect, multiple effect, and vapor compression. The latter two configurations are normally used in larger systems because of their generating capacity and efficiency. Distilled water systems require different feed water controls than required by membrane systems. For distillation, due consideration must be given to prior removal of hardness and silica impurities that may foul or corrode the heat transfer surfaces as well as prior removal of those impurities that could volatilize and condense along with the water vapor. In spite of general perceptions, even the best distillation process cannot afford absolute removal of contaminating ions and endotoxin. Most stills are recognized as being able to accomplish at least a 3-4 log reduction in these impurity concentrations. Areas of concern include carry-over of volatile organic impurities such as trihalomethanes (see *Source or Feed Water Considerations*) and gaseous impurities such as ammonia and carbon dioxide, faulty mist elimination, evaporator flooding, inadequate blowdown, stagnant water in condensers and evaporators, pump and compressor seal design, pinhole evaporator and condenser leaks, and conductivity (quality) variations during start-up and operation.

Methods of control may involve preliminary decarbonation steps to remove both dissolved carbon dioxide and other volatile or noncondensable impurities; reliable mist elimination to minimize feedwater droplet entrainment; visual or automated high water level indication to detect boiler flooding and boil over; use of sanitary pumps and compressors to minimize microbial and lubricant contamination of feedwater and condensate; proper drainage during inactive periods to minimize microbial growth and accumulation of associated endotoxin in boiler water; blowdown control to limit the impurity concentration effect in the boiler to manageable levels; on-line conductivity sensing with automated diversion to waste to prevent unacceptable water upon still startup or still malfunction from getting into the finished water distribute system; and periodic integrity testing for pinhole leaks to routinely assure condensate is not compromised by nonvolatized source water contaminants.

Storage Tanks

Storage tanks are included in water distribution systems to optimize processing equipment capacity. Storage also allows for routine maintenance within the pretreatment train while maintaining continuous supply to meet manufacturing needs. Design and operation considerations are needed to prevent or minimize the development of biofilm, to minimize corrosion, to aid in the use of chemical sanitization of the tanks, and to safeguard mechanical integrity. These considerations may include using closed tanks with smooth interiors, the ability to spray the tank headspace using sprayballs on recirculating loop returns, and the use of heated, jacketed/insulated tanks. This minimizes corrosion and biofilm development and aids in thermal and chemical sanitization. Storage tanks require venting to compensate for the dynamics of changing water levels. This can be accomplished with a properly oriented and heat-traced filter housing fitted with a hydrophobic microbial retentive membrane filter affixed to an atmospheric vent. Alternatively, an automatic membrane-filtered compressed gas blanketing system may be used. In both cases, rupture disks equipped with a rupture alarm device should be used as a further safeguard for the mechanical integrity of the tank. Areas of concern include microbial growth or corrosion due to irregular or incomplete sanitization and microbial contamination from unalarmed rupture disk failures caused by condensate-occluded vent filters.

Distribution Systems

Distribution system configuration should allow for the continuous flow of water in the piping by means of recirculation. Use of nonrecirculating, dead-end, or one-way systems or system segments should be avoided whenever possible. If not possible, these systems should be periodically flushed and more closely monitored. Experience has shown that continuously recirculated systems are easier to maintain. Pumps should be designed to deliver fully turbulent flow conditions to facilitate thorough heat distribution (for hot water sanitized systems) as well as thorough chemical sanitant distribution. Turbulent flow also appear to either retard the development of biofilms or reduce the tendency of those biofilms to shed bacteria into the water. If redundant pumps are used, they should be configured and used to avoid microbial contamination of the system.

Components and distribution lines should be sloped and fitted with drain points so that the system can be completely drained. In stainless steel distribution systems where the water is circulated at a high temperature, dead legs and low flow conditions should be avoided, and valved tie-in points should have length-to-diameter ratios of six or less. If constructed of heat-tolerant plastic, this ratio should be even less to avoid cool points where biofilm development

could occur. In ambient temperature distribution systems, particular care should be exercised to avoid or minimize dead leg ratios of any size and provide for complete drainage. If the system is intended to be steam sanitized, careful sloping and low point drainage is crucial to condensate removal and sanitization success. If drainage of components or distribution lines is intended as a microbial control strategy, they should also be configured to be completely dried using dry compressed air (or nitrogen if appropriate employee safety measures are used). Drained but still moist surfaces will still support microbial proliferation. Water exiting from the distribution system should not be returned to the system without first passing through all or a portion of the purification train.

The distribution design should include the placement of sampling valves in the storage tank and at other locations, such as in the return line of the recirculating water system. Where feasible, the primary sampling sites for water should be the valves that deliver water to the points of use. Direct connections to processes or auxiliary equipment should be designed to prevent reverse flow into the controlled water system. Hoses and heat exchangers that are attached to points of use in order to deliver water for a particular use must not chemically or microbiologically degrade the water quality. The distribution system should permit sanitization for microorganism control. The system may be continuously operated at sanitizing conditions or sanitized periodically.

INSTALLATION, MATERIALS OF CONSTRUCTION, AND COMPONENT SELECTION

Installation techniques are important because they can affect the mechanical, corrosive, and sanitary integrity of the system. Valve installation attitude should promote gravity drainage. Pipe supports should provide appropriate slopes for drainage and should be designed to support the piping adequately under worst case thermal and flow conditions. The methods of connecting system components including units of operation, tanks, and distribution piping require careful attention to preclude potential problems. Stainless steel welds should provide reliable joints that are internally smooth and corrosion free. Low carbon stainless steel, compatible wire filler, where necessary, inert gas, automatic welding machines, and regular inspection and documentation help to ensure acceptable weld quality. Follow up cleaning and passivation are important for removing contamination and corrosion products and to re-establish the passive corrosion resistant surface. Plastic materials can be fused (welded) in some cases and also require smooth, uniform internal surfaces. Adhesive glues and solvents should be avoided due to the potential for voids and extractables. Mechanical methods of joining, such as flange fittings, require care to avoid the creation of offsets, gaps, penetrations, and voids. Control measures include good alignment, properly sized gaskets, appropriate spacing, uniform sealing force, and the avoidance of threaded fittings.

Materials of construction should be selected to be compatible with control measures such as sanitizing, cleaning, and passivating. Temperature rating is a critical factor in choosing appropriate materials because surfaces may be required to handle elevated operating and sanitization temperatures. Should chemicals or additives be used to clean, control, or sanitize the system, materials resistant to these chemicals or additives must be utilized. Materials should be capable of handling turbulent flow and elevated velocities without wear of the corrosion resistant film such as the passive chromium oxide surface of stainless steel. The finish on metallic materials such as stainless steel, whether it is a refined mill finish, polished to a specific grit, or an electropolished treatment, should complement system design and provide satisfactory corrosion and microbial activity resistance as well as chemical sanitizability.

Auxiliary equipment and fittings that require seals, gaskets, diaphragms, filter media, and membranes should exclude materials that permit the possibility of extractables, shedding, and microbial activity. Insulating materials exposed to stainless steel surfaces should be free of chlorides to avoid the phenomenon of stress corrosion cracking that can lead to system contamination and the destruction of tanks and critical system components.

Specifications are important to ensure proper selection of materials and to serve as a reference for system qualification and maintenance. Information such as mill reports for stainless steel and reports of composition, ratings, and material handling capabilities for nonmetallic substances should be reviewed for suitability and retained for reference. Component (auxiliary equipment) selection should be made with assurance that it does not create a source of contamination intrusion. Heat exchangers should be constructed to prevent leakage of heat transfer medium to the pharmaceutical water and, for heat exchanger designs where prevention may fail, there should be a means to detect leakage. Pumps should be of sanitary design with seals that prevent contamination of the water. Valves should have smooth internal surfaces with the seat and closing device exposed to the flushing action of water, such as occurs in diaphragm valves. Valves with pocket areas or closing devices (e.g., ball, plug, gate, globe) that move into and out of the flow area should be avoided.

SANITIZATION

Microbial control in water systems is achieved primarily through sanitization practices. Systems can be sanitized using either thermal or chemical means. Thermal approaches to system sanitization include periodic or continuously circulating hot water and the use of steam. Temperatures of at least 80° are most commonly used for this purpose, but continuously recirculating water of at least 65° has also been used effectively in insulated stainless steel distribution systems when attention is paid to uniformity and distribution of such self-sanitizing temperatures. These techniques are limited to systems that are compatible with the higher temperatures needed to achieve sanitization. Although thermal methods control biofilm development by either continuously inhibiting their growth or, in intermittent applications, by killing the microorganisms within biofilms, they are not effective in removing established biofilms. Killed but intact biofilms can become a nutrient source for rapid biofilm regrowth after the sanitizing conditions are removed or halted. In such cases, a combination of routine thermal and periodic supplementation with chemical sanitization might be more effective. The more frequent the thermal sanitization, the more likely biofilm development and regrowth can be eliminated. Chemical methods, where compatible, can be used on a wider variety of construction materials. These methods typically employ oxidizing agents such as halogenated compounds, hydrogen peroxide, ozone, peracetic acid, or combinations thereof. Halogenated compounds are effective sanitizers but are difficult to flush from the system and may leave biofilms intact. Compounds such as hydrogen peroxide, ozone, and peracetic acid oxidize bacteria and biofilms by forming reactive peroxides and free radicals (notably hydroxyl radicals). The short half-life of ozone in particular, and its limitation on achievable concentrations require that it be added continuously during the sanitization process. Hydrogen peroxide and ozone rapidly degrade to water and oxygen; peracetic acid degrades to acetic acid in the presence of UV light. In fact, ozone's ease of degradation to oxygen using 254 nm UV light at use points allow it to be most effectively used on a continuous basis to provide continuously sanitizing conditions.

In-line UV light at a wavelength of 254 nm can also be used to continuously "sanitize" water

circulating in the system, but these devices must be properly sized for the water flow. Such devices inactivate a high percentage (but not 100%) of microorganisms that flow through the device but cannot be used to directly control existing biofilm upstream or downstream of the device. However, when coupled with conventional thermal or chemical sanitization technologies or located immediately upstream of a microbially retentive filter, it is most effective and can prolong the interval between system sanitizations.

It is important to note that microorganisms in a well-developed biofilm can be extremely difficult to kill, even by aggressive oxidizing biocides. The less developed and therefore thinner the biofilm, the more effective the biocidal action. Therefore, optimal biocide control is achieved by frequent biocide use that does not allow significant biofilm development between treatments.

Sanitization steps require validation to demonstrate the capability of reducing and holding microbial contamination at acceptable levels. Validation of thermal methods should include a heat distribution study to demonstrate that sanitization temperatures are achieved throughout the system, including the body of use point valves. Validation of chemical methods requires demonstrating adequate chemical concentrations throughout the system, exposure to all wetted surfaces, including the body of use point valves, and complete removal of the sanitant from the system at the completion of treatment. Methods validation for the detection and quantification of residues of the sanitant or its objectionable degradants is an essential part of the validation program. The frequency of sanitization should be supported by, if not triggered by, the results of system microbial monitoring. Conclusions derived from trend analysis of the microbiological data should be used as the alert mechanism for maintenance. The frequency of sanitization should be established in such a way that the system operates in a state of microbiological control and does not routinely exceed alert levels (see *Alert and Action Levels and Specifications*).

OPERATION, MAINTENANCE, AND CONTROL

A preventive maintenance program should be established to ensure that the water system remains in a state of control. The program should include (1) procedures for operating the system, (2) monitoring programs for critical quality attributes and operating conditions including calibration of critical instruments, (3) schedule for periodic sanitization, (4) preventive maintenance of components, and (5) control of changes to the mechanical system and to operating conditions.

Operating Procedures—Procedures for operating the water system and performing routine maintenance and corrective action should be written, and they should also define the point when action is required. The procedures should be well documented, detail the function of each job, assign who is responsible for performing the work, and describe how the job is to be conducted. The effectiveness of these procedures should be assessed during water system validation.

Monitoring Program—Critical quality attributes and operating parameters should be documented and monitored. The program may include a combination of in-line sensors or automated instruments (e.g., for TOC, conductivity, hardness, and chlorine), automated or manual documentation of operational parameters (such as flow rates or pressure drop across a carbon bed, filter, or RO unit), and laboratory tests (e.g., total microbial counts). The frequency of sampling, the requirement for evaluating test results, and the necessity for initiating corrective action should be included.

Sanitization—Depending on system design and the selected units of operation, routine periodic sanitization may be necessary to maintain the system in a state of microbial control. Technologies for sanitization are described above.

Preventive Maintenance—A preventive maintenance program should be in effect. The program should establish what preventive maintenance is to be performed, the frequency of maintenance work, and how the work should be documented.

Change Control—The mechanical configuration and operating conditions must be controlled. Proposed changes should be evaluated for their impact on the whole system. The need to requalify the system after changes are made should be determined. Following a decision to modify a water system, the affected drawings, manuals, and procedures should be revised.

SAMPLING CONSIDERATIONS

Water systems should be monitored at a frequency that is sufficient to ensure that the system is in control and continues to produce water of acceptable quality. Samples should be taken from representative locations within the processing and distribution system. Established sampling frequencies should be based on system validation data and should cover critical areas including unit operation sites. The sampling plan should take into consideration the desired attributes of the water being sampled. For example, systems for *Water for Injection*, because of their more critical microbiological requirements, may require a more rigorous sampling frequency.

Analyses of water samples often serve two purposes: in-process control assessments and final quality control assessments. In-process control analyses are usually focused on the attributes of the water within the system. Quality control is primarily concerned with the attributes of the water delivered by the system to its various uses. The latter usually employs some sort of transfer device, often a flexible hose, to bridge the gap between the distribution system use-point valve and the actual location of water use. The issue of sample collection location and sampling procedure is often hotly debated because of the typically mixed use of the data generated from the samples, for both in-process control and quality control. In these single-sample and mixed-data use situations, the worst-case scenario should be utilized. In other words, samples should be collected from use points using the same delivery devices, such as hoses, and procedures, such as preliminary hose or outlet flushing, as are employed by production from those use points. Where use points per se cannot be sampled, such as hard-piped connections to equipment, special sampling ports may be used. In all cases, the sample must represent as closely as possible the quality of the water used in production. If a point-of-use filter is employed, sampling of the water prior to and after the filter is needed, because the filter will mask the microbial control achieved by the normal operating procedures of the system.

Samples containing chemical sanitizing agents require neutralization prior to microbiological analysis. Samples for microbiological analysis should be tested immediately, or suitably refrigerated to preserve the original microbial attributes until analysis can begin. Samples of flowing water are only indicative of the concentration of planktonic (free floating) microorganisms present in the system. Biofilm microorganisms (those attached to water system surfaces) are usually present in greater numbers and are the source of the planktonic population recovered from grab samples. Microorganisms in biofilms represent a continuous

source of contamination and are difficult to directly sample and quantify. Consequently, the planktonic population is usually used as an indicator of system contamination levels and is the basis for system *Alert and Action Levels and Specifications*. The consistent appearance of elevated planktonic levels is usually an indication of advanced biofilm development in need of remedial control. System control and sanitization are key in controlling biofilm formation and the consequent planktonic population.

Sampling for chemical analyses is also done for in-process control and for quality control purposes. However, unlike microbial analyses, chemical analyses can be and often are performed using on-line instrumentation. Such on-line testing has unequivocal in-process control purposes because it is not performed on the water delivered from the system. However, unlike microbial attributes, chemical attributes are usually not significantly degraded by hoses. Therefore, through verification testing, it may be possible to show that the chemical attributes detected by the on-line instrumentation (in-process testing) are equivalent to those detected at the ends of the use-point hoses (quality control testing). This again creates a single-sample and mixed-data use scenario. It is far better to operate the instrumentation in a continuous mode, generating large volumes of in-process data, but only using a defined small sampling of that data for QC purposes. Examples of acceptable approaches include using highest values for a given period, highest time-weighted average for a given period (from fixed or rolling sub-periods), or values at a fixed daily time. Each approach has advantages and disadvantages relative to calculation complexity and reflection of continuous quality, so the user must decide which approach is most suitable or justifiable.

CHEMICAL CONSIDERATIONS

The chemical attributes of *Purified Water* and *Water for Injection* in effect prior to *USP 23* were specified by a series of chemistry tests for various specific and nonspecific attributes with the intent of detecting chemical species indicative of incomplete or inadequate purification. While these methods could have been considered barely adequate to control the quality of these waters, they nevertheless stood the test of time. This was partly because the operation of water systems was, and still is, based on on-line conductivity measurements and specifications generally thought to preclude the failure of these archaic chemistry attribute tests.

USP moved away from these chemical attribute tests to contemporary analytical technologies for the bulk waters *Purified Water* and *Water for Injection*. The intent was to upgrade the analytical technologies without tightening the quality requirements. The two contemporary analytical technologies employed were TOC and conductivity. The TOC test replaced the test for *Oxidizable Substances* that primarily targeted organic contaminants. A multistaged *Conductivity* test which detects ionic (mostly inorganic) contaminants replaced, with the exception of the test for *Heavy metals*, all of the inorganic chemical tests (i.e., *Ammonia*, *Calcium*, *Carbon dioxide*, *Chloride*, *Sulfate*).

Replacing the heavy metals attribute was considered unnecessary because (a) the source water specifications (found in the NPDWR) for individual *Heavy metals* were tighter than the approximate limit of detection of the *Heavy metals* test for *USP XXII Water for Injection* and *Purified Water* (approximately 0.1 ppm), (b) contemporary water system construction materials do not leach heavy metal contaminants, and (c) test results for this attribute have uniformly been negative—there has not been a confirmed occurrence of a singular test failure (failure of only the *Heavy metals* test with all other attributes passing) since the current heavy metal

drinking water standards have been in place. Nevertheless, because the presence of heavy metals in *Purified Water* or *Water for Injection* could have dire consequences, its absence should at least be documented during new water system commissioning and validation or through prior test results records.

Total solids and *pH* were the only tests not covered by conductivity testing. The test for *Total solids* was considered redundant because the nonselective tests of conductivity and TOC could detect most chemical species other than silica, which could remain undetected in its colloidal form. Colloidal silica in *Purified Water* and *Water for Injection* is easily removed by most water pretreatment steps and even if present in the water, constitutes no medical or functional hazard except under extreme and rare situations. In such extreme situations, other attribute extremes are also likely to be detected. It is, however, the user's responsibility to ensure fitness for use. If silica is a significant component in the source water, and the purification unit operations could be operated or fail and selectively allow silica to be released into the finished water (in the absence of co-contaminants detectable by conductivity), then either silica-specific or a total solids type testing should be utilized to monitor and control this rare problem.

The *pH* attribute was eventually recognized to be redundant to the conductivity test (which included *pH* as an aspect of the test and specification); therefore, *pH* was dropped as a separate attribute test.

The rationale used by USP to establish its *Purified Water* and *Water for Injection* conductivity specifications took into consideration the conductivity contributed by the two least conductive former attributes of *Chloride* and *Ammonia*, thereby precluding their failure had those wet chemistry tests been performed. In essence, the *Stage 3* conductivity specifications (see *Water Conductivity* (645), *Bulk Water*) were established from the sum of the conductivities of the limit concentrations of chloride ions (from pH 5.0–6.2) and ammonia ions (from pH 6.3–7.0), plus the unavoidable contribution of other conductivity-contributing ions from water (H^+ and OH^-), dissolved atmospheric CO_2 (as HCO_3^-), and an electro-balancing quantity of either Na^+ or Cl^- , depending on the pH-induced ionic imbalance (see *Table 1*). The *Stage 2* conductivity specification is the lowest value on this table, 2.1 $\mu S/cm$. The *Stage 1* specifications, designed primarily for on-line measurements, were derived essentially by summing the lowest values in the contributing ion columns for each of a series of tables similar to *Table 1*, created for each 5° increment between 0° and 100°. For example purposes, the italicized values in *Table 1*, the conductivity data table for 25°, were summed to yield a conservative value of 1.3 $\mu S/cm$, the *Stage 1* specification for a nontemperature compensated, nonatmosphere-equilibrated water sample that actually had a measured temperature of 25°–29°. Each 5° increment in the table was similarly treated to yield the individual values listed in the table of *Stage 1* specifications (see *Water Conductivity* (645), *Bulk Water*).

Table 1. Contributing Ion Conductivities of the Chloride–Ammonia Model as a Function of pH

(in atmosphere-equilibrated water at 25°)

pH	H ⁺	OH ⁻	HCO ₃ ⁻	Conductivity (μ S/cm)		NH ₄ ⁺	Combined Conductivities	Stage 3 Limit
				Cl ⁻	Na ⁺			
5.0	3.49	0	0.02	1.01	0.19	0	4.71	4.7
5.1	2.77	0	0.02	1.01	0.29	0	4.09	4.1
5.2	2.20	0	0.03	1.01	0.38	0	3.62	3.6
5.3	1.75	0	0.04	1.01	0.46	0	3.26	3.3
5.4	1.39	0	0.05	1.01	0.52	0	2.97	3.0
5.5	1.10	0	0.06	1.01	0.58	0	2.75	2.8
5.6	0.88	0	0.08	1.01	0.63	0	2.60	2.6
5.7	0.70	0	0.10	1.01	0.68	0	2.49	2.5
5.8	0.55	0	0.12	1.01	0.73	0	2.41	2.4
5.9	0.44	0	0.16	1.01	0.78	0	2.39	2.4
6.0	0.35	0	0.20	1.01	0.84	0	2.40	2.4
6.1	0.28	0	0.25	1.01	0.90	0	2.44	2.4
6.2	0.22	0	0.31	1.01	0.99	0	2.53	2.5
6.3	0.18	0	0.39	0.63	0	1.22	2.42	2.4
6.4	0.14	0.01	0.49	0.45	0	1.22	2.31	2.3
6.5	0.11	0.01	0.62	0.22	0	1.22	2.18	2.2
6.6	0.09	0.01	0.78	0	0.04	1.22	2.14	2.1
6.7	0.07	0.01	0.99	0	0.27	1.22	2.56	2.6
6.8	0.06	0.01	1.24	0	0.56	1.22	3.09	3.1
6.9	0.04	0.02	1.56	0	0.93	1.22	3.77	3.8
7.0	0.03	0.02	1.97	0	1.39	1.22	4.63	4.6

As stated above, this rather radical change to utilizing a conductivity attribute as well as the inclusion of a TOC attribute allowed for on-line measurements. This was a major philosophical change and allowed major savings to be realized by industry. The TOC and conductivity tests can also be performed "off-line" in the laboratories using collected samples, although sample collection tends to introduce opportunities for adventitious contamination that can cause false high readings. The collection of on-line data is not, however, without challenges. The continuous readings tend to create voluminous amounts of data where before only a single data point was available. As stated under *Sampling Considerations*, continuous in-process data sampling is excellent for understanding how a water system performs during all of its various usage and maintenance events in real time, but is too much data for QC purposes. Therefore, a justifiable fraction or averaging of the data can be used that is still representative of the overall water quality being used.

Packaged/sterile waters present a particular dilemma relative to the attributes of conductivity and TOC. The package itself is the source of chemicals (inorganics and organics) that leach over time into the packaged water and can easily be detected by the conductivity and TOC tests. The irony of organic leaching from plastic packaging is that before the advent of bulk water TOC testing when the *Oxidizable Substances* test was the only "organic purity" test for both bulk and packaged/sterile water monographs in *USP*, that test's insensitivity to many of the organic leachables from plastic and elastomeric packaging materials was largely unrealized, allowing organic levels in packaged/sterile water to be quite high (possibly many times the TOC specification for bulk water). Similarly, glass containers can also leach inorganics, such as sodium, which are easily detected by conductivity, but poorly detected by the former wet chemistry attribute tests. Most of these leachables are considered harmless by current

perceptions and standards at the rather significant concentrations present. Nevertheless, they effectively degrade the quality of the high-purity waters placed into these packaging systems. Some packaging materials contain more leachables than others and may not be as suitable for holding water and maintaining its purity.

The attributes of conductivity and TOC tend to reveal more about the packaging leachables than they do about the water's original purity. These currently "allowed" leachables could render the sterile packaged versions of originally equivalent bulk water essentially unsuitable for many uses where the bulk waters are perfectly adequate.

Therefore, to better control the ionic packaging leachables, *Water Conductivity* (645) is divided into two sections. The first is titled *Bulk Water*, which applies to *Purified Water*, *Water for Injection*, *Water for Hemodialysis*, and *Pure Steam*, and includes the three-stage conductivity testing instructions and specifications. The second is titled *Sterile Water*, which applies to *Sterile Purified Water*, *Sterile Water for Injection*, *Sterile Water for Inhalation*, and *Sterile Water for Irrigation*. The *Sterile Water* section includes conductivity specifications similar to the *Stage 2* testing approach because it is intended as a laboratory test, and these sterile waters were made from bulk water that already complied with the three-stage conductivity test. In essence, packaging leachables are the primary target "analytes" of the conductivity specifications in the *Sterile Water* section of *Water Conductivity* (645). The effect on potential leachables from different container sizes is the rationale for having two different specifications, one for small packages containing nominal volumes of 10 mL or less and another for larger packages. These conductivity specifications are harmonized with the *European Pharmacopoeia* conductivity specifications for *Sterile Water for Injection*. All monographed waters, except *Bacteriostatic Water for Injection*, have a conductivity specification that directs the user to either the *Bulk Water* or the *Sterile Water* section of *Water Conductivity* (645). For the sterile packaged water monographs, this water conductivity specification replaces the redundant wet chemistry limit tests intended for inorganic contaminants that had previously been specified in these monographs.

Controlling the organic purity of these sterile packaged waters, particularly those in plastic packaging, is more challenging. Although the TOC test can better detect and therefore be better used to monitor and control these impurities than the current *Oxidizable Substances* test, the latter has many decades-old precedents and flexibility with the variety of packaging types and volumes applicable to these sterile packaged waters. Nevertheless, TOC testing of these currently allowed sterile, plastic-packaged waters reveals substantial levels of plastic-derived organic leachables that render the water perhaps orders of magnitude less organically pure than typically achieved with bulk waters. Therefore, usage of these packaged waters for analytical, manufacturing, and cleaning applications should only be exercised after suitability of the waters' purity for the application has been assured.

There is an analogous partitioning of *Total Organic Carbon* (643) to better control the organic packaging leachables. The first part is titled *Bulk Water*, which applies to the TOC method for *Purified Water*, *Water for Injection*, *Water for Hemodialysis*, and *Pure Steam*. The second part is titled *Sterile Water*, which applies to the TOC method for *Sterile Purified Water*, *Sterile Water for Injection*, *Sterile Water for Inhalation*, and *Sterile Water for Irrigation*. For these sterile waters, the TOC method is provided as an alternative test to the *Oxidizable Substances* test. The TOC limits for the sterile waters are set to higher values than the TOC limits for bulk waters.

MICROBIAL CONSIDERATIONS

The major exogenous source of microbial contamination of bulk pharmaceutical water is source or feed water. Feed water quality must, at a minimum, meet the quality attributes of Drinking Water for which the level of coliforms is regulated. A wide variety of other microorganisms, chiefly Gram-negative bacteria, may be present in the incoming water. These microorganisms may compromise subsequent purification steps. Examples of other potential exogenous sources of microbial contamination include unprotected vents, faulty air filters, ruptured rupture disks, backflow from contaminated outlets, unsanitized distribution system "openings" including routine component replacements, inspections, repairs, and expansions, inadequate drain and air breaks, and replacement activated carbon, deionizer resins, and regenerant chemicals. In these situations, the exogenous contaminants may not be normal aquatic bacteria but rather microorganisms of soil or even of human origin. The detection of nonaquatic microorganisms may be an indication of a system component failure, which should trigger investigations that will remediate their source. Sufficient care should be given to system design and maintenance in order to minimize microbial contamination from these exogenous sources.

Unit operations can be a major source of endogenous microbial contamination. Microorganisms present in feed water may adsorb to carbon bed, deionizer resins, filter membranes, and other unit operation surfaces and initiate the formation of a biofilm. In a high-purity water system, biofilm is an adaptive response by certain microorganisms to survive in this low-nutrient environment. Downstream colonization can occur when microorganisms are shed from existing biofilm colonized surfaces and carried to other areas of the water system. Microorganisms may also attach to suspended particles such as carbon bed fines or fractured resin particles. When the microorganisms become planktonic, they serve as a source of contamination to subsequent purification equipment (compromising its functionality) and to distribution systems.

Another source of endogenous microbial contamination is the distribution system itself. Microorganisms can colonize pipe surfaces, rough welds, badly aligned flanges, valves, and unidentified dead legs, where they proliferate, forming a biofilm. The smoothness and composition of the surface may affect the rate of initial microbial adsorption, but once adsorbed, biofilm development, unless otherwise inhibited by sanitizing conditions, will occur regardless of the surface. Once formed, the biofilm becomes a continuous source of microbial contamination.

ENDOTOXIN CONSIDERATIONS

Endotoxins are lipopolysaccharides found in and shed from the cell envelope that is external to the cell wall of Gram-negative bacteria. Gram-negative bacteria that form biofilms can become a source of endotoxins in pharmaceutical waters. Endotoxins may occur as clusters of lipopolysaccharide molecules associated with living microorganisms, fragments of dead microorganisms or the polysaccharide slime surrounding biofilm bacteria, or as free molecules. The free form of endotoxins may be released from cell surfaces of the bacteria that colonize the water system, or from the feed water that may enter the water system. Because of the multiplicity of endotoxin sources in a water system, endotoxin quantitation in a water system is not a good indicator of the level of biofilm abundance within a water system.

Endotoxin levels may be minimized by controlling the introduction of free endotoxins and microorganisms in the feed water and minimizing microbial proliferation in the system. This may

be accomplished through the normal exclusion or removal action afforded by various unit operations within the treatment system as well as through system sanitization. Other control methods include the use of ultrafilters or charge modified filters, either in-line or at the point of use. The presence of endotoxins may be monitored as described in the chapter *Bacterial Endotoxins Test* (85).

MICROBIAL ENUMERATION CONSIDERATIONS

The objective of a water system microbiological monitoring program is to provide sufficient information to control and assess the microbiological quality of the water produced. Product quality requirements should dictate water quality specifications. An appropriate level of control may be maintained by using data trending techniques and, if necessary, limiting specific contraindicated microorganisms. Consequently, it may not be necessary to detect all of the microorganisms species present in a given sample. The monitoring program and methodology should indicate adverse trends and detect microorganisms that are potentially harmful to the finished product, process, or consumer. Final selection of method variables should be based on the individual requirements of the system being monitored.

It should be recognized that there is no single method that is capable of detecting all of the potential microbial contaminants of a water system. The methods used for microbial monitoring should be capable of isolating the numbers and types of organisms that have been deemed significant relative to in-process system control and product impact for each individual system. Several criteria should be considered when selecting a method to monitor the microbial content of a pharmaceutical water system. These include method sensitivity, range of organisms types or species recovered, sample processing throughput, incubation period, cost, and methodological complexity. An alternative consideration to the use of the classical "culture" approaches is a sophisticated instrumental or rapid test method that may yield more timely results. However, care must be exercised in selecting such an alternative approach to ensure that it has both sensitivity and correlation to classical culture approaches, which are generally considered the accepted standards for microbial enumeration.

Consideration should also be given to the timeliness of microbial enumeration testing after sample collection. The number of detectable planktonic bacteria in a sample collected in a scrupulously clean sample container will usually drop as time passes. The planktonic bacteria within the sample will tend to either die or to irretrievably adsorb to the container walls, reducing the number of viable planktonic bacteria that can be withdrawn from the sample for testing. The opposite effect can also occur if the sample container is not scrupulously clean and contains a low concentration of some microbial nutrient that could promote microbial growth within the sample container. Because the number of recoverable bacteria in a sample can change positively or negatively over time after sample collection, it is best to test the samples as soon as possible after being collected. If it is not possible to test the sample within about 2 h of collection, the sample should be held at refrigerated temperatures (2°–8°) for a maximum of about 12 h to maintain the microbial attributes until analysis. In situations where even this is not possible (such as when using off-site contract laboratories), testing of these refrigerated samples should be performed within 48 h after sample collection. In the delayed testing scenario, the recovered microbial levels may not be the same as would have been recovered had the testing been performed shortly after sample collection. Therefore, studies should be performed to determine the existence and acceptability of potential microbial enumeration aberrations caused by protracted testing delays.

The Classical Culture Approach

Classical culture approaches for microbial testing of water include but are not limited to pour plates, spread plates, membrane filtration, and most probable number (MPN) tests. These methods are generally easy to perform, are less expensive, and provide excellent sample processing throughput. Method sensitivity can be increased via the use of larger sample sizes. This strategy is used in the membrane filtration method. Culture approaches are further defined by the type of medium used in combination with the incubation temperature and duration. This combination should be selected according to the monitoring needs presented by a specific water system as well as its ability to recover the microorganisms of interest: those that could have a detrimental effect on the product or process uses as well as those that reflect the microbial control status of the system.

There are two basic forms of media available for traditional microbiological analysis: "high nutrient" and "low nutrient". High nutrient media, such as plate count agar (TGYA) and m-HPG agar (formerly m-SPC agar), are intended as general media for the isolation and enumeration of heterotrophic or "copiotrophic" bacteria. Low nutrient media, such as R2A agar and NWRI agar (HPCA), may be beneficial for isolating slow growing "oligotrophic" bacteria and bacteria that require lower levels of nutrients to grow optimally. Often some facultative oligotrophic bacteria are able to grow on high nutrient media and some facultative copiotrophic bacteria are able to grow on low nutrient media, but this overlap is not complete. Low nutrient and high nutrient cultural approaches may be concurrently used, especially during the validation of a water system, as well as periodically thereafter. This concurrent testing could determine if any additional numbers or types of bacteria can be preferentially recovered by one of the approaches. If so, the impact of these additional isolates on system control and the end uses of the water could be assessed. Also, the efficacy of system controls and sanitization on these additional isolates could be assessed.

Duration and temperature of incubation are also critical aspects of a microbiological test method. Classical methodologies using high nutrient media are typically incubated at 30°–35° for 48–72 h. Because of the flora in certain water systems, incubation at lower temperatures (e.g., 20°–25°) for longer periods (e.g., 5–7 days) can recover higher microbial counts when compared to classical methods. Low nutrient media are designed for these lower temperature and longer incubation conditions (sometimes as long as 14 days to maximize recovery of very slow growing oligotrophs or sanitant-injured microorganisms), but even high nutrient media can sometimes increase their recovery with these longer and cooler incubation conditions. Whether or not a particular system needs to be monitored using high or low nutrient media with higher or lower incubation temperatures or shorter or longer incubation times should be determined during or prior to system validation and periodically reassessed as the microbial flora of a new water system gradually establish a steady state relative to its routine maintenance and sanitization procedures. The establishment of a "steady state" can take months or even years and can be perturbed by a change in use patterns, a change in routine and preventative maintenance or sanitization procedures, and frequencies, or any type of system intrusion, such as for component replacement, removal, or addition. The decision to use longer incubation periods should be made after balancing the need for timely information and the type of corrective actions required when an alert or action level is exceeded with the ability to recover the microorganisms of interest.

The advantages gained by incubating for longer times, namely recovery of injured microorganisms, slow growers, or more fastidious microorganisms, should be balanced against

the need to have a timely investigation and to take corrective action, as well as the ability of these microorganisms to detrimentally affect products or processes. In no case, however, should incubation at 30°–35° be less than 48 h or less than 96 h at 20°–25°.

Normally, the microorganisms that can thrive in extreme environments are best cultivated in the laboratory using conditions simulating the extreme environments from which they were taken. Therefore, thermophilic bacteria might be able to exist in the extreme environment of hot pharmaceutical water systems, and if so, could only be recovered and cultivated in the laboratory if similar thermal conditions were provided. Thermophilic aquatic microorganisms do exist in nature, but they typically derive their energy for growth from harnessing the energy from sunlight, from oxidation/reduction reactions of elements such as sulfur or iron, or indirectly from other microorganisms that do derive their energy from these processes. Such chemical/nutritional conditions do not exist in high-purity water systems, whether ambient or hot. Therefore, it is generally considered pointless to search for thermophiles from hot pharmaceutical water systems owing to their inability to grow there.

The microorganisms that inhabit hot systems tend to be found in much cooler locations within these systems, for example, within use-point heat exchangers or transfer hoses. If this occurs, the kinds of microorganisms recovered are usually of the same types that might be expected from ambient water systems. Therefore, the mesophilic microbial cultivation conditions described later in this chapter are usually adequate for their recovery.

“Instrumental” Approaches

Examples of instrumental approaches include microscopic visual counting techniques (e.g., epifluorescence and immunofluorescence) and similar automated laser scanning approaches and radiometric, impedometric, and biochemically based methodologies. These methods all possess a variety of advantages and disadvantages. Advantages could be their precision and accuracy or their speed of test result availability as compared to the classical cultural approach. In general, instrument approaches often have a shorter lead time for obtaining results, which could facilitate timely system control. This advantage, however, is often counterbalanced by limited sample processing throughput due to extended sample collection time, costly and/or labor-intensive sample processing, or other instrument and sensitivity limitations.

Furthermore, instrumental approaches are typically destructive, precluding subsequent isolate manipulation for characterization purposes. Generally, some form of microbial isolate characterization, if not full identification, may be a required element of water system monitoring. Consequently, culturing approaches have traditionally been preferred over instrumental approaches because they offer a balance of desirable test attributes and post-test capabilities.

Suggested Methodologies

The following general methods were originally derived from *Standard Methods for the Examination of Water and Wastewater*, 17th Edition, American Public Health Association, Washington, DC 20005. Although this publication has undergone several revisions since its first citation in this chapter, the methods are still considered appropriate for establishing trends in the number of colony-forming units observed in the routine microbiological monitoring of pharmaceutical waters. It is recognized, however, that other combinations of media and incubation time and temperature may occasionally or even consistently result in higher numbers of colony-forming units being observed and/or different species being recovered.

The extended incubation periods that are usually required by some of the alternative methods available offer disadvantages that may outweigh the advantages of the higher counts that may be obtained. The somewhat higher baseline counts that might be observed using alternate cultural conditions would not necessarily have greater utility in detecting an excursion or a trend. In addition, some alternate cultural conditions using low nutrient media tend to lead to the development of microbial colonies that are much less differentiated in colonial appearance, an attribute that microbiologists rely on when selecting representative microbial types for further characterization. It is also ironic that the nature of some of the slow growers and the extended incubation times needed for their development into visible colonies may also lead to those colonies being largely nonviable, which limits their further characterization and precludes their subculture and identification.

Methodologies that can be suggested as generally satisfactory for monitoring pharmaceutical water systems are as follows. However, it must be noted that these are not referee methods nor are they necessarily optimal for recovering microorganisms from all water systems. The users should determine through experimentation with various approaches which methodologies are best for monitoring their water systems for in-process control and quality control purposes as well as for recovering any contraindicated species they may have specified.

<i>Drinking Water</i>	Pour Plate Method or Membrane Filtration Method ^a
-	Sample volume—1.0 mL minimum ^b Growth medium—Plate Count Agar ^c Incubation time—48–72 h minimum Incubation temperature—30°–35°
<i>Purified Water</i>	Pour Plate Method or Membrane Filtration Method ^a
-	Sample volume—1.0 mL minimum ^b Growth medium—Plate Count Agar ^c Incubation time—48–72 h minimum Incubation temperature—30°–35°
<i>Water for Injection</i>	Membrane Filtration Method ^a
-	Sample volume—100 mL minimum ^b Growth medium—Plate Count Agar ^c Incubation time—48–72 h minimum Incubation temperature—30°–35°
<p>^a A membrane filter with a rating of 0.45 µm is generally considered preferable, although the cellular width of some of the bacteria in the sample may be narrower than this. The efficiency of the filtration process still allows the retention of a very high percentage of these smaller cells and is adequate for this application. Filters with smaller ratings may be used if desired, but for a variety of reasons the ability of the retained cells to develop into visible colonies may be compromised, so count accuracy must be verified by a reference approach.</p> <p>^b When colony counts are low to undetectable using the indicated minimum sample volume, it is generally recognized that a larger sample volume should be tested in order to gain better assurance that the resulting colony count is more statistically representative. The sample volume to consider testing is dependent on the user's need to know (which is related to the established alert and action levels and the water system's microbial control capabilities) and the statistical reliability of the resulting colony count. In order to test a larger sample volume, it may be necessary to change testing techniques, e.g., changing from a pour plate to a membrane filtration approach. Nevertheless, in a very low to nil count scenario, a maximum sample volume of around 250–300 mL is usually considered a reasonable balance of sample collecting and processing ease and increased statistical reliability. However, when sample volumes larger than about 2 mL are needed, they can only be processed using the membrane filtration method.</p> <p>^c Also known as Standard Methods Agar, Standard Methods Plate Count Agar, or TGYA, this medium contains tryptone (pancreatic digest of casein), glucose, and yeast extract.</p>	

IDENTIFICATION OF MICROORGANISMS

Identifying the isolates recovered from water monitoring methods may be important in instances where specific waterborne microorganisms may be detrimental to the products or processes in which the water is used. Microorganism information such as this may also be useful when identifying the source of microbial contamination in a product or process. Often a limited group of microorganisms is routinely recovered from a water system. After repeated recovery and

characterization, an experienced microbiologist may become proficient at their identification based on only a few recognizable traits such as colonial morphology and staining characteristics. This may allow for a reduction in the number of identifications to representative colony types, or, with proper analyst qualification, may even allow testing shortcuts to be taken for these microbial identifications.

ALERT AND ACTION LEVELS AND SPECIFICATIONS

Although the use of alert and action levels is most often associated with microbial data, they can be associated with any attribute. In pharmaceutical water systems, almost every quality attribute, other than microbial quality, can be very rapidly determined with near real time results. These short delay data can give immediate system performance feedback, serving as ongoing process control indicators. However, because some attributes may not continuously be monitored or have a long delay in data availability (like microbial monitoring data), properly established *Alert and Action Levels and Specifications* can serve as an early warning or indication of a potentially approaching quality shift occurring between or at the next periodic monitoring. In a validated water system, process controls should yield relatively constant and more than adequate values for these monitored attributes such that their *Alert and Action Levels and Specifications* are infrequently breached.

As process control indicators, alert and action levels are designed to allow remedial action to occur that will prevent a system from deviating completely out of control and producing water unfit for its intended use. This "intended use" minimum quality is sometimes referred to as a "specification" or "limit". In the opening paragraphs of this chapter, rationale was presented for no microbial specifications being included within the body of the bulk water (*Purified Water* and *Water for Injection*) monographs. This does not mean that the user should not have microbial specifications for these waters. To the contrary, in most situations such specifications should be established by the user. The microbial specification should reflect the maximum microbial level at which the water is still fit for use without compromising the quality needs of the process or product where the water is used. Because water from a given system may have many uses, the most stringent of these uses should be used to establish this specification.

Where appropriate, a microbial specification could be qualitative as well as quantitative. In other words, the number of total microorganisms may be as important as the number of a specific microorganism or even the absence of a specific microorganism. Microorganisms that are known to be problematic could include opportunistic or overt pathogens, nonpathogenic indicators of potentially undetected pathogens, or microorganisms known to compromise a process or product, such as by being resistant to a preservative or able to proliferate in or degrade a product. These microorganisms comprise an often ill defined group referred to as "objectionable microorganisms". Because objectionable is a term relative to the water's use, the list of microorganisms in such a group should be tailored to those species with the potential to be present and problematic. Their negative impact is most often demonstrated when they are present in high numbers, but depending on the species, an allowable level may exist, below which they may not be considered objectionable.

As stated above, alert and action levels for a given process control attribute are used to help maintain system control and avoid exceeding the pass/fail specification for that attribute. Alert and action levels may be both quantitative and qualitative. They may involve levels of total microbial counts or recoveries of specific microorganisms. Alert levels are events or levels that,

when they occur or are exceeded, indicate that a process may have drifted from its normal operating condition. Alert level excursions constitute a warning and do not necessarily require a corrective action. However, alert level excursions usually lead to the alerting of personnel involved in water system operation as well as QA. Alert level excursions may also lead to additional monitoring with more intense scrutiny of resulting and neighboring data as well as other process indicators. Action levels are events or higher levels that, when they occur or are exceeded, indicate that a process is probably drifting from its normal operating range. Examples of kinds of action level "events" include exceeding alert levels repeatedly; or in multiple simultaneous locations, a single occurrence of exceeding a higher microbial level; or the individual or repeated recovery of specific objectionable microorganisms. Exceeding an action level should lead to immediate notification of both QA and personnel involved in water system operations so that corrective actions can immediately be taken to bring the process back into its normal operating range. Such remedial actions should also include efforts to understand and eliminate or at least reduce the incidence of a future occurrence. A root cause investigation may be necessary to devise an effective preventative action strategy. Depending on the nature of the action level excursion, it may also be necessary to evaluate its impact on the water uses during that time. Impact evaluations may include delineation of affected batches and additional or more extensive product testing. It may also involve experimental product challenges.

Alert and action levels should be derived from an evaluation of historic monitoring data called a trend analysis. Other guidelines on approaches that may be used, ranging from "inspectional" to statistical evaluation of the historical data have been published. The ultimate goal is to understand the normal variability of the data during what is considered a typical operational period. Then, trigger points or levels can be established that will signal when future data may be approaching (alert level) or exceeding (action level) the boundaries of that "normal variability". Such alert and action levels are based on the control capability of the system as it was being maintained and controlled during that historic period of typical control.

In new water systems where there is very limited or no historic data from which to derive data trends, it is common to simply establish initial alert and action levels based on a combination of equipment design capabilities but below the process and product specifications where water is used. It is also common, especially for ambient water systems, to microbiologically "mature" over the first year of use. By the end of this period, a relatively steady state microbial population (microorganism types and levels) will have been allowed or promoted to develop as a result of the collective effects of routine system maintenance and operation, including the frequency of unit operation rebeddings, backwashings, regenerations, and sanitizations. This microbial population will typically be higher than was seen when the water system was new, so it should be expected that the data trends (and the resulting alert and action levels) will increase over this "maturation" period and eventually level off.

A water system should be designed so that performance based alert and action levels are well below water specifications. With poorly designed or maintained water systems, the system owner may find that initial new system microbial levels were acceptable for the water uses and specifications, but the mature levels are not. This is a serious situation, which if not correctable with more frequent system maintenance and sanitization, may require expensive water system renovation or even replacement. Therefore, it cannot be overemphasized that water systems should be designed for ease of microbial control, so that when monitored against alert and action levels, and maintained accordingly, the water continuously meets all applicable

specifications.

An action level should not be established at a level equivalent to the specification. This leaves no room for remedial system maintenance that could avoid a specification excursion. Exceeding a specification is a far more serious event than an action level excursion. A specification excursion may trigger an extensive finished product impact investigation, substantial remedial actions within the water system that may include a complete shutdown, and possibly even product rejection.

Another scenario to be avoided is the establishment of an arbitrarily high and usually nonperformance-based action level. Such unrealistic action levels deprive users of meaningful indicator values that could trigger remedial system maintenance. Unrealistically high action levels allow systems to grow well out of control before action is taken, when their intent should be to catch a system imbalance before it goes wildly out of control.

Because alert and action levels should be based on actual system performance, and the system performance data are generated by a given test method, it follows that those alert and action levels should be valid only for test results generated by the same test method. It is invalid to apply alert and action level criteria to test results generated by a different test method. The two test methods may not equivalently recover microorganisms from the same water samples. Similarly invalid is the use of trend data to derive alert and action levels for one water system, but applying those alert and action levels to a different water system. Alert and action levels are water system and test method specific.

Nevertheless, there are certain maximum microbial levels above which action levels should never be established. Water systems with these levels should unarguably be considered out of control. Using the microbial enumeration methodologies suggested above, generally considered maximum action levels are 100 cfu/mL for *Purified Water* and 10 cfu/100 mL for *Water for Injection*. However, if a given water system controls microorganisms much more tightly than these levels, appropriate alert and action levels should be established from these tighter control levels so that they can truly indicate when water systems may be starting to trend out of control. These in-process microbial control parameters should be established well below the user-defined microbial specifications that delineate the water's fitness for use.

Special consideration is needed for establishing maximum microbial action levels for *Drinking Water* because the water is often delivered to the facility in a condition over which the user has little control. High microbial levels in *Drinking Water* may be indicative of a municipal water system upset, broken water main, or inadequate disinfection, and therefore, potential contamination with objectionable microorganisms. Using the suggested microbial enumeration methodology, a reasonable maximum action level for *Drinking Water* is 500 cfu/mL. Considering the potential concern for objectionable microorganisms raised by such high microbial levels in the feed water, informing the municipality of the problem so they may begin corrective actions should be an immediate first step. In-house remedial actions may or may not also be needed, but could include performing additional coliform testing on the incoming water and pretreating the water with either additional chlorination or UV light irradiation or filtration, or a combination of approaches.



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1. INTRODUCTION

Water is widely used as a raw material, inactive ingredient, medicinal vehicle, and solvent in the processing, formulation, and manufacture of pharmaceutical products (dosage forms), active pharmaceutical ingredients (APIs), API intermediates, compendial articles, and analytical reagents as well as in cleaning applications.

This is an informational chapter on pharmaceutical water topics and includes some of the chemical and microbiological concerns unique to water and its preparation and uses. The chapter provides information about water quality attributes (that may or may not be included within a water monograph) and processing techniques that can be used to improve water quality. It also discusses water system validation and gives a description of minimum water quality standards that should be considered when selecting a water source including sampling and system controls. It is equally important for water systems to be operated and maintained in a state of control to provide assurance of operational stability and therefore the capability to provide water that meets established water quality standards.

This informational chapter is intended to be educational, and the user should also refer to existing regulations or guidelines that cover U.S. and international [International Conference on Harmonisation (ICH) or World Health Organization (WHO)] good manufacturing practice (GMP) issues, as well as operational and engineering guides and/or other regulatory guidance for water [e.g., from the Food and Drug Administration (FDA), Environmental Protection Agency (EPA), or WHO]. This chapter is not, and should not be considered, an all-inclusive document on pharmaceutical waters. It contains basic information and points to be considered for the processing, holding, monitoring, and use of water. It is the user's responsibility to ensure that:

1. The selection of the type and specifications of water is appropriate for its intended use.
2. Water production and quality meet applicable governmental regulations and guidance.
3. The pharmacopeial specifications for the types of water used in monographed articles are met.
4. Water used in the preparation of reagents for analysis or the performance of required tests meets USP requirements.

Control and monitoring of the chemical and endotoxin purity of waters are important for complying with the requirements of the monographs in this compendium. Attributes listed in *USP* monographs should be considered the *minimum* requirements. More stringent requirements may be needed for some applications to ensure suitability for particular uses. Basic guidance on the appropriate applications of waters can be found in the monographs and is also discussed further in this chapter.

Control of the microbiological quality of water is also important for many of its uses. This attribute is intentionally not specified in most water monographs. Microbiological control is discussed throughout this chapter, but especially in sections 4, 5, 6, 8, and 9.

This chapter contains various chemical, microbiological, processing, and engineering concepts of importance to users of water. Water system validation, process control levels, and specifications are also presented later in this chapter.

2. SOURCE WATER CONSIDERATIONS

Source water is the water that enters the facility. The origin of this source water can be from natural surface waters like rivers and reservoirs, deep-bed well waters, sea waters, or some combination of these, potentially including multiple locations of each type of source water. Thus, source water can be supplied from these various origins (public or private), from municipalities' on-site water sourcing, or by external delivery such as a truck. It is possible that source water may not be potable and safe to drink. Such water may require pretreatment to ensure that it meets drinking water standards. It is the responsibility of the users of any source water to ensure that the water used in the production of drug substances (API), as well as water for indirect drug product contact or for purification system feed water purposes meets, at a minimum, drinking (potable) water standards as defined by the requirements of the National Primary Drinking Water Regulations (NPDWR) (40 CFR 141) issued by the U.S. EPA or the drinking water regulations of the European Union (EU) or Japan, or the WHO drinking water guidelines (see 3.3.1 *Drinking Water*). These regulations establish limits on the types and quantities of certain chemical and microbiological contaminants and ensure that the water will contain safe quantities of chemical and microbial species.

Where water supplies are from regulated water utility companies, less stringent monitoring may be possible because the attributes may be tested regularly and ensured by the supplier (see 9.4.5 *Source Water Control*). Water being withdrawn from a nonregulated supply should be sampled and tested appropriately at a suitable frequency that takes into account local environmental and seasonal changes and other quality fluctuations. Testing should ensure conformance with one of the drinking water standards discussed above.

The use of water complying with one of these designated drinking waters as a source water allows water pretreatment systems to only be challenged to remove small quantities of potentially difficult-to-remove chemicals. Control of objectionable chemical contaminants at the source water stage eliminates the need to specifically test for some of them [e.g., trihalomethanes and elemental impurities (see *Elemental Impurities—Limits* (232))] after the water has been further purified, assuming there is no opportunity for recontamination.

Source waters can be used for nonproduct contact purposes such as for non-contact cooling systems. Such water may not normally be required to meet drinking water standards. Under such circumstances, the quality standards for this water when used in a pharmaceutical facility should be subject to quality standards established by the user and defensible to regulatory agencies.

3. WATERS USED FOR PHARMACEUTICAL MANUFACTURING AND TESTING PURPOSES

There are many different grades of water used for pharmaceutical purposes. Several are described in *USP* monographs that specify uses, acceptable methods of preparation, and quality attributes. These waters can be divided into two general types: bulk waters, which are typically produced on site where they are used; and sterile waters, which are produced, packaged, and sterilized to preserve microbial quality throughout their packaged shelf life. There are several specialized types of sterile waters that differ in their designated applications, packaging limitations, and other quality attributes. Monographed waters must meet the quality attributes as specified in the related monographs, and any *Notes* appearing in those monographs should be considered and addressed.

With the exception of *Bacteriostatic Water for Injection*, the monographed bulk and sterile waters have a statement indicating that there are no added substances, or no added

antimicrobial agents. In the case of antimicrobial agents, the purpose is to ensure that the sterile water product is rendered sterile based solely on its preparation, packaging, and storage. In the case of the more general statement, "no added substances", this requirement is intended to mean "no added substances that aren't sufficiently removed". Two specific examples support this intention, but there are many examples. First, the use of softeners is commonplace. A softener replaces calcium and magnesium ions with sodium, so technically you are adding two sodium ions for each hard ion. The purpose of sodium displacement is to protect downstream equipment from the hard water. The sodium ions are eventually removed sufficiently, and this is proven when the water sample passes the test for *Water Conductivity* (645). Another specific example is the use of ozone as a sanitant that is added to the storage tank for microbial control. This could be considered an added substance, unless the ozone is destroyed before use, as is normally the case. Other notable examples include the addition of chlorine to kill bacteria in the pretreatment system, use of bisulfite to chemically reduce chlorine to chloride and protect downstream equipment, and use of a nitrogen blanket for protection from atmospheric contamination.

There are also other types of water for which there are no monographs. These are waters with names given for descriptive purposes only. Many of these waters are used in specific analytical methods. The descriptive titles may imply certain quality attributes or modes of preparation, but these nonmonographed waters may not necessarily adhere strictly to the stated or implied modes of preparation or specified attributes. Waters produced by other means or controlled by other test attributes, or even a monographed water, may equally satisfy the intended uses for these waters. It is the user's responsibility to ensure that such waters, even if produced and controlled exactly as stated, are suitable for their intended use. Wherever the term "water" is used within this compendium without other descriptive adjectives or clauses, the intent is that water of no less purity than USP Purified Water be used (see 3.1.1 *Purified Water*). A brief description of the various types of waters commonly associated with pharmaceutical applications and their significant uses or attributes follows.

Figure 1 may be helpful in understanding some of the various types of waters, their preparation, and uses.

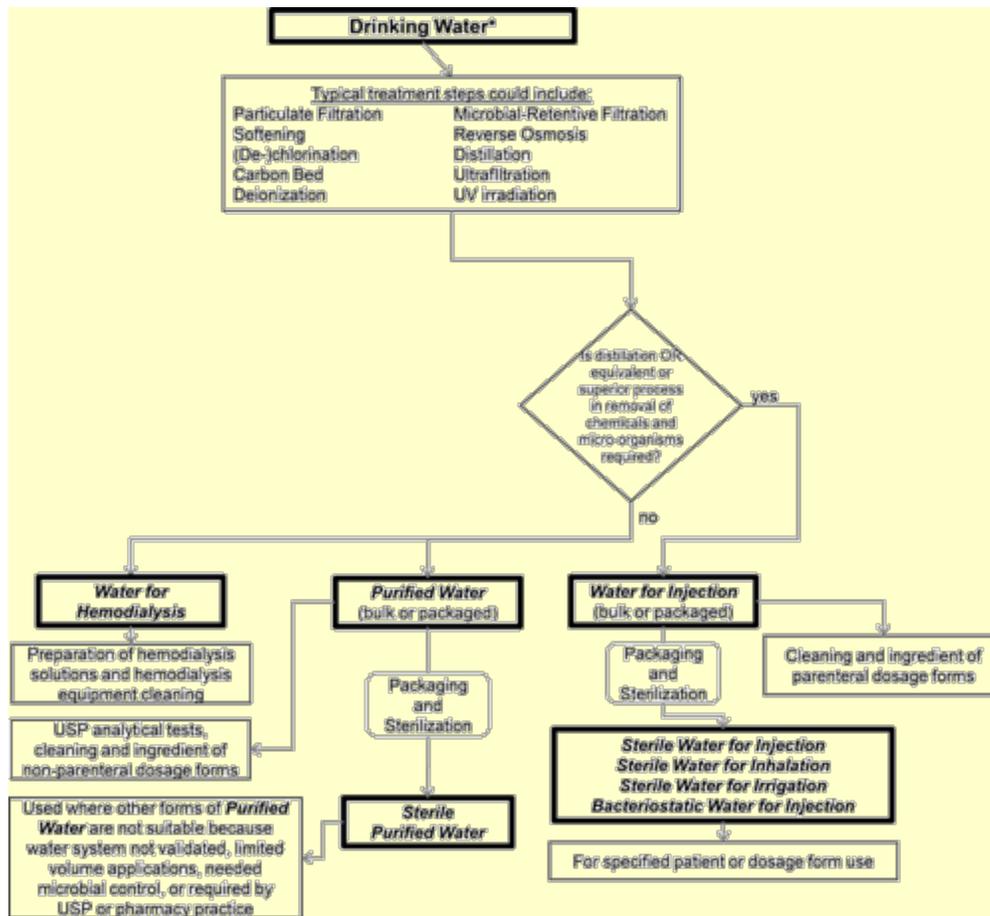


Figure 1. Water for pharmaceutical purposes. *Complying with U.S. EPA NPDWR or the drinking water regulations of EU or Japan or WHO.

3.1 Bulk Monographed Waters and Steam

The following waters are generally produced in large volumes using a multiple-unit operation water system. These waters are typically distributed in a piping system for use at the same site.

PURIFIED WATER

Purified Water (see the *USP* monograph) is used as an excipient in the production of nonparenteral preparations and in other pharmaceutical applications, such as the cleaning of nonparenteral product-contact components and equipment. Unless otherwise specified, Purified Water is also to be used as the minimum water quality for all tests and assays in which “water” is indicated (see *General Notices and Requirements 8.230.30*). This applies regardless of the font and letter case used in its spelling.

The minimal quality of source water for the production of *Purified Water* is Drinking Water whose attributes are prescribed by the U.S. EPA, EU, Japan, or WHO. This source water may be purified using unit operations that include deionization, distillation, ion exchange, reverse osmosis, filtration, or other suitable purification procedures. Purified Water must meet the requirements for ionic and organic chemical purity and must be protected from microbial contamination. Purified Water systems must be validated to reliably and consistently produce and distribute water of acceptable chemical and microbiological quality. Purified Water systems that function under ambient conditions are particularly susceptible to the establishment of

biofilms of microorganisms, which can be the source of undesirable levels of viable microorganisms or endotoxins in the water. These ambient Purified Water systems require frequent sanitization and microbiological monitoring to ensure that the water reaching the points of use has appropriate microbiological quality.

The *Purified Water* monograph also allows bulk packaging for commercial use elsewhere. In contrast to *Sterile Purified Water*, packaged *Purified Water* is not required to be sterile. Because there is potential for microbial contamination and other quality changes in this packaged nonsterile water, this form of Purified Water should be prepared and stored in a manner that limits microbial growth, and/or should be used in a timely fashion before microbial proliferation renders it unsuitable for its intended use. Also, depending on the material used for packaging, extractable compounds could be leaching into the water from the packaging. Although this article is required to meet the same chemical purity standards as the bulk water, extractables from the packaging will likely render the packaged water less chemically pure than the bulk water. The nature of these impurities may even render the water an inappropriate choice for some applications. It is the user's responsibility to ensure fitness for use of this packaged article when it is used in manufacturing, clinical, or analytical applications where the purer bulk form of the water is indicated.

WATER FOR INJECTION

Water for Injection (see the *USP* monograph) is used as an excipient in the production of parenteral and other preparations where product endotoxin content must be controlled, and in other pharmaceutical applications, such as the cleaning of certain equipment and parenteral product-contact components.

The minimal quality of source water for the production of *Water for Injection* is Drinking Water whose attributes are prescribed by the U.S. EPA, EU, Japan, or WHO. This source water may be treated to render it suitable for subsequent final purification steps, such as distillation (or whatever other validated process is used, according to the monograph). The finished water must meet all of the chemical requirements specified in the monograph, as well as an additional bacterial endotoxin specification. Because endotoxins are produced by the kinds of microorganisms that are prone to inhabit water systems, the equipment and procedures used by the system to purify, store, and distribute *Water for Injection* should be designed to control microbial contamination and must be designed to remove incoming endotoxins from the source water. *Water for Injection* systems must be validated to reliably and consistently produce and distribute this quality of water.

The *Water for Injection* monograph also allows bulk packaging for commercial use. In contrast to *Sterile Water for Injection*, packaged *Water for Injection* is not required to be sterile. However, to preclude significant changes in its microbial and endotoxins content during storage, this form of Water for Injection should be prepared and stored in a manner that limits microbial introduction and growth and/or should be used in a timely fashion before microbial proliferation renders it unsuitable for its intended use. Also, depending on the material used for packaging, extractable compounds could be leaching into the water from the packaging. Although this article is required to meet the same chemical purity standards as the bulk water, extractables from the packaging will likely render the packaged water less chemically pure than the bulk water. The nature of these impurities may even render the water an inappropriate choice for some applications. It is the user's responsibility to ensure fitness for use of this packaged article when it is used in manufacturing, clinical, or analytical applications where the

purier bulk form of the water is indicated.

WATER FOR HEMODIALYSIS

Water for Hemodialysis (see the *USP* monograph) is used for hemodialysis applications, primarily the dilution of hemodialysis concentrate solutions. The minimal quality of source water for the production of *Water for Hemodialysis* is Drinking Water whose attributes are prescribed by the U.S. EPA, EU, Japan, or WHO. *Water for Hemodialysis* has been further purified to reduce chemical and microbiological components, and it is produced and used on site. This water contains no added antimicrobial agents, and it is not intended for injection. *Water for Hemodialysis* must meet all of the chemical requirements specified in the monograph as well as an additional bacterial endotoxin specification. The microbial limits attribute for this water is unique among the "bulk" water monographs, but is justified on the basis of this water's specific application, which has microbial content requirements related to its safe use. The bacterial endotoxins attribute is likewise established at a level related to its safe use.

PURE STEAM

Pure Steam (see the *USP* monograph) is also sometimes referred to as "clean steam". It is used where the steam or its condensate would directly contact official articles or article-contact surfaces, such as during their preparation, sterilization, or cleaning where no subsequent processing step is used to remove any impurity residues. These Pure Steam applications include, but are not limited to, porous load sterilization processes, product or cleaning solutions heated by direct steam injection, or humidification of processes where steam injection is used to control the humidity inside processing vessels where the official articles or their in-process forms are exposed. The primary intent of using this quality of steam is to ensure that official articles or article-contact surfaces exposed to it are not contaminated by residues within the steam.

The minimal quality of source water for the production of *Pure Steam* is Drinking Water whose attributes are prescribed by the U.S. EPA, EU, Japan, or WHO, and which has been suitably treated. The water is then vaporized with suitable mist elimination, and distributed under pressure. The sources of undesirable contaminants within *Pure Steam* could arise from entrained source water droplets, anticorrosion steam additives, or residues from the steam production and distribution system itself. The chemical tests in the *Pure Steam* monograph should detect most of the contaminants that could arise from these sources. If an official article is exposed to *Pure Steam* and it is intended for parenteral use or other applications where the pyrogenic content must be controlled, the *Pure Steam* must additionally meet the specification for *Bacterial Endotoxins Test* (85).

These purity attributes are measured in the condensate of the article, rather than the article itself. This, of course, imparts great importance to the cleanliness of the process for *Pure Steam* condensate generation and collection, because it must not adversely impact the quality of the resulting condensed fluid.

Other steam attributes not detailed in the monograph, particularly the presence of even small quantities of noncondensable gases or the existence of a superheated or dry state, may also be important for applications such as sterilization. The large release of energy (latent heat of condensation) as water changes from the gaseous to the liquid state is the key to steam's sterilization efficacy and its efficiency, in general, as a heat transfer agent. If this phase change (condensation) is not allowed to happen because the steam is extremely hot and is in a

persistent superheated, dry state, then its usefulness could be seriously compromised. Noncondensable gases in steam tend to stratify or collect in certain areas of a steam sterilization chamber or its load. These surfaces would thereby be at least partially insulated from the steam condensation phenomenon, preventing them from experiencing the full energy of the sterilizing conditions. Therefore, control of these kinds of steam attributes, in addition to its chemical purity, may also be important for certain Pure Steam applications. However, because these additional attributes are use-specific, they are not mentioned in the *Pure Steam* monograph.

Note that lower-purity "plant steam" may be used in the following applications: 1) for steam sterilization of nonproduct-contact nonporous loads, 2) for general cleaning of nonproduct-contact equipment, 3) as a nonproduct-contact heat-exchange medium, and 4) in all compatible applications involved in bulk pharmaceutical chemical and API manufacture.

Finally, because *Pure Steam* is lethal to microbes, monitoring of microbial control within a steam system is unnecessary, as is microbial analysis of the steam condensate.

3.2 Sterile Monographed Waters

The following monographed waters are packaged forms of either *Purified Water* or *Water for Injection* that have been sterilized to preserve their microbiological properties. These waters may have specific intended uses as indicated by their names, and may also have restrictions on the packaging configurations related to those uses. In general, these sterile waters may be used in a variety of applications in lieu of the bulk forms of water from which they were derived. However, there is a substantial difference between the acceptance criteria for the chemical purities of these bulk waters versus sterile waters. The specifications for sterile waters differ from those of bulk waters to accommodate a wide variety of packaging types, properties, volumes, and uses. As a result, the inorganic and organic impurity specifications are not equivalent for bulk and packaged waters. The packaging materials and elastomeric closures are the primary sources of these impurities, which tend to increase over the shelf life of these packaged articles. Therefore, due consideration must be given to the chemical purity suitability at the time of use of the sterile forms of water when used in manufacturing, analytical, and cleaning applications in lieu of the bulk waters from which these waters were derived. It is the user's responsibility to ensure fitness for use of these sterile packaged waters in these applications. Nevertheless, for the applications discussed below for each sterile water, their respective purities and packaging restrictions generally render them suitable by definition.

STERILE PURIFIED WATER

Sterile Purified Water (see the *USP* monograph) is *Purified Water*, packaged and rendered sterile. It can be used in the preparation of nonparenteral compendial dosage forms or in analytical applications requiring *Purified Water* where 1) access to a validated *Purified Water* system is not practical, 2) only a relatively small quantity is needed, 3) *Sterile Purified Water* is required by specific monograph or pharmacy practice, or 4) bulk packaged *Purified Water* is not suitably controlled for the microbiological quality for its intended use.

STERILE WATER FOR INJECTION

Sterile Water for Injection (see the *USP* monograph) is *Water for Injection* packaged and rendered sterile. It is used for extemporaneous prescription compounding and as a sterile diluent for parenteral products. It may also be used for other applications where bulk *Water for*

Injection or *Purified Water* is indicated but access to a validated water system is not practical, or where only a relatively small quantity is needed. *Sterile Water for Injection* is packaged in single-dose containers not larger than 1 L.

BACTERIOSTATIC WATER FOR INJECTION

Bacteriostatic Water for Injection (see the *USP* monograph) is *Water for Injection*, packaged and rendered sterile, to which has been added one or more suitable antimicrobial preservatives. It is intended to be used as a diluent in the preparation of parenteral products, most typically for multi-dose products that require repeated content withdrawals. It may be packaged in single-dose or multiple-dose containers not larger than 30 mL.

STERILE WATER FOR IRRIGATION

Sterile Water for Irrigation (see the *USP* monograph) is *Water for Injection* packaged and sterilized in single-dose containers which may be larger than 1 L and allow rapid delivery of their contents. Due to its usage, *Sterile Water for Irrigation* is not required to meet *Particulate Matter in Injections* (788). It may also be used in other applications that do not have particulate matter specifications, where bulk *Water for Injection* or *Purified Water* is indicated but where access to a validated water system is not practical, or where somewhat larger quantities are needed than are provided as *Sterile Water for Injection*.

STERILE WATER FOR INHALATION

Sterile Water for Inhalation (see the *USP* monograph) is *Water for Injection* that is packaged and rendered sterile and is intended for use in inhalators and in the preparation of inhalation solutions. This monograph has no requirement to meet (788); it carries a less stringent specification for bacterial endotoxins than *Sterile Water for Injection*, and therefore is not suitable for parenteral applications.

3.3 Nonmonographed Waters

In addition to the bulk monographed waters described above, nonmonographed waters can also be used in pharmaceutical processing steps such as cleaning and synthetic steps, and also as a starting material for further purification or testing purposes. Unless otherwise specified in the compendium, the minimum quality of water is *Purified Water*. [Note—The information in this chapter is not an all-inclusive discussion of all nonmonographed waters identified in the *USP-NF*.]

DRINKING WATER

Drinking Water can be referred to as Potable Water (meaning drinkable or fit to drink), National Primary Drinking Water, Primary Drinking Water, or EPA Drinking Water. Except where a singular drinking water specification is stated (such as the U.S. EPA's NPDWR, as cited in 40 CFR Part 141), this water must comply with the quality attributes of either the NPDWR or the drinking water regulations of the EU or Japan, or the *WHO Guidelines for Drinking-Water Quality*. Drinking Water may originate from a variety of sources including a public water supply, a private water supply (e.g., a well), or a combination of these sources (see 2. *Source Water Considerations*).

Drinking Water may be used in the early stages of cleaning pharmaceutical manufacturing equipment and product-contact components. Drinking Water is also the minimum quality of water that should be used for the preparation of official substances and other bulk pharmaceutical ingredients. Where compatible with the processes, the contaminant levels

allowed in Drinking Water are generally considered safe for use in preparing official substances and other drug substances. However, where required by the processing of the materials to achieve their required final purity, higher qualities of water may be needed for these manufacturing steps, perhaps even water as pure as *Water for Injection* or *Purified Water*. Such higher-purity waters, however, might require only selected attributes to be of higher purity than Drinking Water (see *Figure 2a* and *Figure 2b*). Drinking Water is the prescribed source or feed water for the production of bulk monographed pharmaceutical waters. The use of Drinking Water specifications establishes a reasonable set of maximum allowable levels of chemical and microbiological contaminants with which a water purification system will be challenged. Because seasonal variations in the quality attributes of the Drinking Water supply can occur, it is important to give due consideration to its uses. The processing steps in the production of pharmaceutical waters must be designed to accommodate this variability.

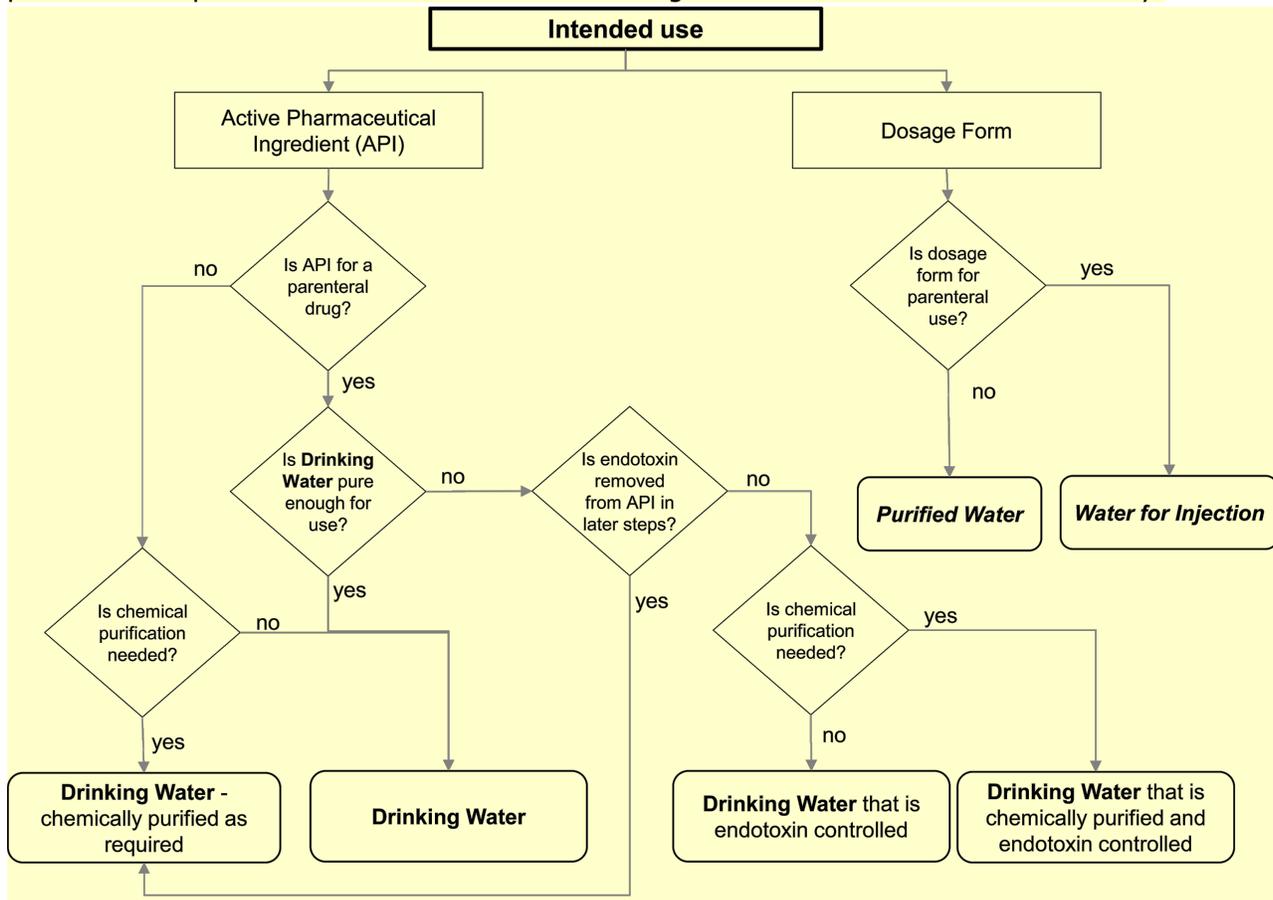


Figure 2a. Selection of water for pharmaceutical purposes: APIs and dosage forms.

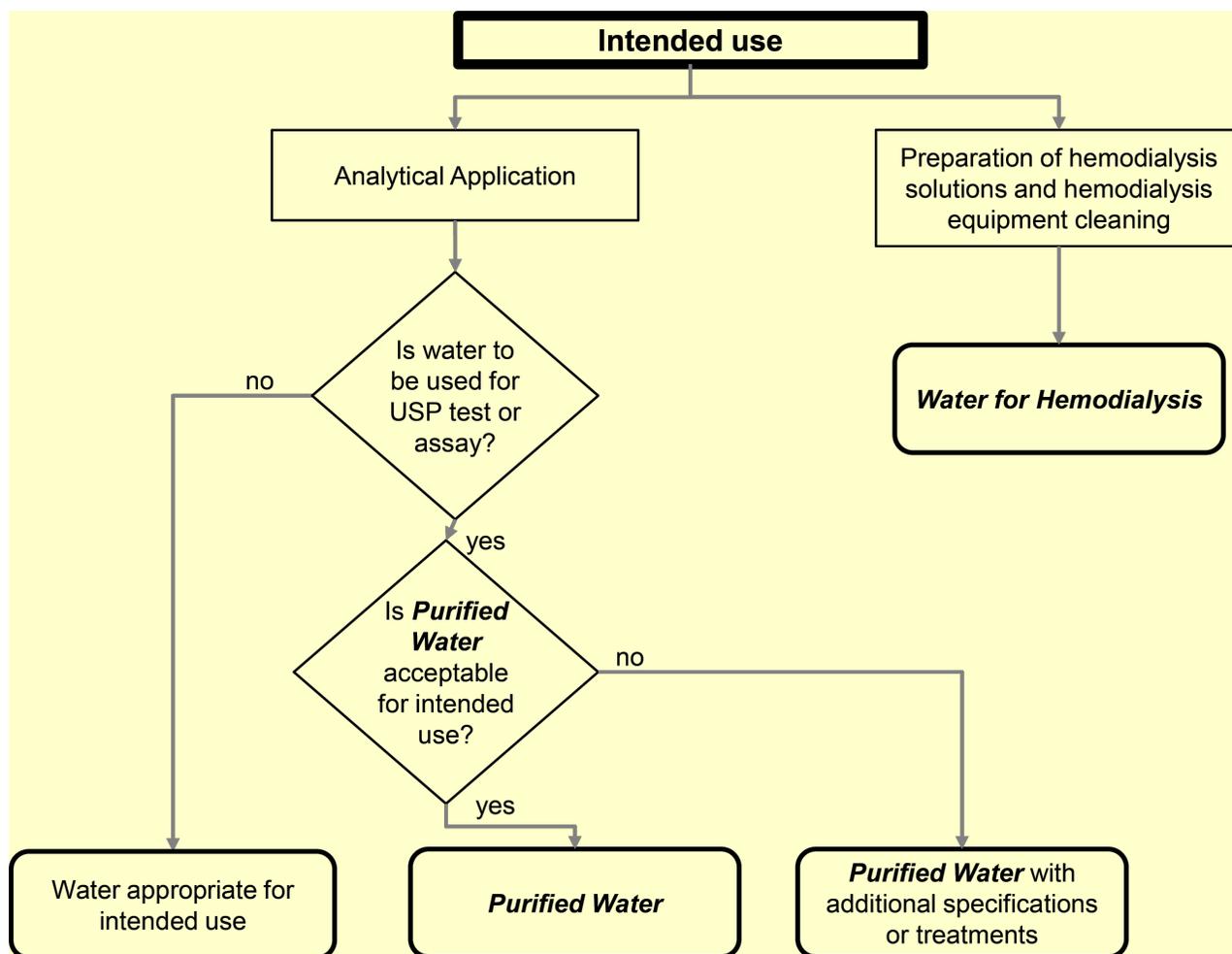


Figure 2b. Selection of water for pharmaceutical purposes: Analytical reagents.

OTHER NONMONOGRAPHED WATERS

In addition to Drinking Water, this compendium discusses waters with various other designations. These include waters of various quality levels for special uses such as, but not limited to, cleaning and testing purposes.

Both *General Notices and Requirements* (see *General Notices and Requirements 8.230.30*) and *Reagents, Indicators, and Solutions* (see introductory *RIS* section 4.7) clearly state that where the term “water” is indicated for use in analyses without grammatical qualification or other specification, the quality of the water must be *Purified Water*. However, numerous such qualifications do exist. Some of these qualifications involve adjectives describing methods of preparation, ranging from specifying the primary purification step to specifying additional purification. Other qualifications call for specific attribute “absences” to be met that might otherwise interfere with analytical processes. In most of these cases, the required attribute absences are not specifically tested. Sometimes, a further “purification process” is specified that ostensibly allows the water to adequately meet this required “absence attribute”.

However, preparation instructions for many reagents were carried forward from the innovator's laboratories to the originally introduced monograph for a particular *USP–NF* article or general test chapter. The quality of the reagent water described in these tests may reflect the water quality designation of the innovator's laboratory. These specific water designations may have originated without the innovator's awareness of the requirement for *Purified Water* in *USP–NF*

tests. Regardless of the original reason for the creation of these numerous special analytical waters, it is possible that the attributes of these special waters could now be met by the basic preparation steps and current specifications of *Purified Water*. In some cases, however, some of the cited post-processing steps are still necessary to reliably achieve the required attributes.

Users are not obligated to utilize specific and perhaps archaically generated forms of analytical water where alternatives with equal or better quality, availability, or analytical performance may exist. The consistency and reliability of operations for producing these alternative analytical waters should be verified so that the desired attributes are produced. In addition, any alternative analytical water must be evaluated on an application-by-application basis by the user to ensure its suitability. The following is a summary of the various types of nonmonographed analytical waters that are cited in the *USP-NF*. This is not an exhaustive listing. Those listed below are used in multiple locations. Several nonmonographed analytical waters and not included below because they are only found in one or perhaps two locations within this compendium.

Note that the names of many of the waters below imply a very low chemical impurity level. For example, "deionized water" implies that all the ions have been removed. However, in most cases discussed below, exposure of the water to air will result in the ingress of carbon dioxide (CO_2), leading to the formation of bicarbonate and hydrogen ions. Therefore, the removal of ions cannot be completely maintained for most analytical applications.

AMMONIA-FREE WATER

From a functional standpoint, *Ammonia-Free Water* must have a negligible ammonia concentration to avoid interference in tests sensitive for or to ammonia. Due to the nature of the uses of this water, *Purified Water* could be a reasonable alternative for these applications.

CARBON DIOXIDE-FREE WATER

Carbon Dioxide-Free Water is defined in the introductory portion of the *Reagents, Indicators, and Solutions* section of *USP-NF* as *Purified Water* that has been vigorously boiled for NLT 5 min, then cooled and protected from absorption of atmospheric carbon dioxide. Alternatively, this could be *Purified Water* that has a resistivity of NLT 18 Mohm-cm at 25°.

Because the absorption of atmospheric carbon dioxide lowers the pH of high-purity waters, most of the uses of Carbon Dioxide-Free Water are either associated as a solvent in pH-related or pH-sensitive determinations or as a solvent in bicarbonate-sensitive reagents or determinations.

The term *Carbon Dioxide-Free Water* is sometimes used improperly. Besides its use for pH or acidity/alkalinity tests, the purpose for using this water is not always clear. The intention could be to use water that was deaerated (free of dissolved air) or deionized (free of extraneous ions), or even *Purified Water* with an additional boiling step. Although boiling is highly effective for removing carbon dioxide (CO_2) as well as all other dissolved gasses, these gases are readily re-absorbed unless the water is protected. Even with protection, such as use of a stoppered container, re-absorption will occur over time as air will readily transmit through seals and diffuse through most materials. Deionization is also an efficient process for removing dissolved carbon dioxide. Carbon dioxide forms ionic bicarbonate in water, and will be subsequently removed by ion-exchange resins. However, the same problem of carbon dioxide re-absorption

will occur after the deionized water is exposed to air. Also, the deionization approach for creating Carbon Dioxide-Free Water does not deaerate the water or remove other dissolved gases such as oxygen (O₂); it only removes carbon dioxide and other ions.

Depending on the application, *Purified Water* may meet the requirements where Carbon Dioxide-Free Water is called for. This could also include pH or acidity or alkalinity tests. The pH of a sample of pure Deionized Water is, by definition, 7.0. When that same sample is exposed to typical environmental atmospheric conditions, the water sample will absorb carbon dioxide and result in a pH range of approximately 5.4–6.2 ([H⁺] is in the range of 4.0 × 10⁻⁶ M to 6.3 × 10⁻⁷ M). The added acidity caused by carbon dioxide absorption may be insignificant compared to the material being analyzed.

DISTILLED WATER

Distilled Water is produced by vaporizing Drinking Water or a higher quality of water and condensing it into a purer state. It is used primarily as a solvent for reagent preparation, and it is also specified in the execution of other aspects of tests, such as for rinsing an analyte, transferring a test material as a slurry, as a calibration standard or analytical blank, and for test apparatus cleaning. Distilled Water is also cited as the starting water to be used for making *High-Purity Water*. Because none of the cited uses of this water imply a need for a particular purity attribute that can only be derived by distillation, water meeting the requirements for *Purified Water* derived by other means of purification or *Water for Injection* could be equally suitable where Distilled Water is specified. It is the user's responsibility to verify the suitability of *Purified Water* or *Water for Injection*.

FRESHLY DISTILLED WATER

Freshly Distilled Water or "recently distilled water" is produced in the same manner as Distilled Water and should be used soon after its generation. This implies the need to avoid endotoxin contamination, as well as any other forms of contamination from the air or containers, that could arise with prolonged storage. Freshly Distilled Water is used for preparing solutions for subcutaneous test-animal injections and for a reagent solvent in tests for which there appears to be no particularly high water purity needed that could be ascribable to being "freshly distilled". In the test-animal application, the term "freshly distilled" and its testing use imply a chemical, endotoxin, and microbiological purity that could be equally satisfied by Water for Injection (although no reference is made to these chemical, endotoxin, or microbial attributes or specific protection from recontamination). For non-animal uses, water meeting the requirements for *Purified Water* derived by other means of purification and/or storage periods could be equally suitable where "recently distilled water" or Freshly Distilled Water is specified. It is the user's responsibility to verify the suitability of *Purified Water* or *Water for Injection*.

DEIONIZED WATER

Deionized Water can be produced by starting with either Drinking Water or Purified Water, depending upon monograph or testing procedures defined in the compendia. Deionized Water is produced by an ion-exchange process in which the cations and anions are replaced with H⁺ and OH⁻ ions by use of ion-exchange resins. Similar to *Distilled Water*, Deionized Water is used primarily as a solvent for reagent preparation, but it is also specified in the execution of other aspects of tests, such as for transferring an analyte within a test procedure, as a calibration standard or analytical blank, and for test apparatus cleaning. Also, none of the cited uses of this water imply any needed purity attribute that can only be achieved by deionization.

Therefore, water meeting the requirements for *Purified Water* that is derived by other means of purification could be equally suitable where Deionized Water is specified. It is the user's responsibility to verify the suitability of *Purified Water*.

DEIONIZED DISTILLED WATER

Deionized Distilled Water is produced by deionizing (see *Deionized Water*) Distilled Water. This water is used as a reagent in a liquid chromatography test that requires a low ionic or organic impurity level. Because of the importance of this high purity, water that meets the requirements for *Purified Water* may not be acceptable. High-Purity Water (see 3.3.10 *High-Purity Water*) could be a reasonable alternative to this water. It is the user's responsibility to verify the suitability of the alternative water used.

FILTERED WATER

Filtered Water is *Purified Water* that has been filtered to remove particles that could interfere with the analysis where this water is specified. It is sometimes used synonymously with Particle-Free Water and Ultra-Filtered Water and is cited in some monographs and general chapters as well as in *Reagents, Indicators, and Solutions*. Depending on its referenced location in *USP-NF*, it is variously defined as water that has been passed through filters rated as 1.2, 0.22, or 0.2 μm , or unspecified porosity rating. Even though the water names and the filter ratings used to produce these waters are defined inconsistently, the use of 0.2- μm filtered *Purified Water* should be universally acceptable for all applications where Particle-Free Water, Filtered Water, or Ultra-Filtered Water are specified.

HIGH-PURITY WATER

High-Purity Water may be prepared by deionizing previously distilled water and then filtering it through a 0.45- μm rated membrane. This water must have an in-line conductivity of NMT 0.15 $\mu\text{S}/\text{cm}$ (NLT 6.67 Megohm-cm) at 25°. If the water of this purity contacts the atmosphere even briefly as it is being used or drawn from its purification system, its conductivity will immediately increase by as much as about 1.0 $\mu\text{S}/\text{cm}$ as atmospheric carbon dioxide dissolves in the water and equilibrates to hydrogen and bicarbonate ions. Therefore, if the analytical use requires that water conductivity remains as high as possible or the bicarbonate/carbon dioxide levels be as low as possible, the water should be protected from atmospheric exposure. High-Purity Water is used as a reagent, as a solvent for reagent preparation, and for test apparatus cleaning where less stringent water specifications would not be considered acceptable. However, if a user's routinely available *Purified Water* is filtered and meets or exceeds the conductivity specifications of High-Purity Water, it could be used in lieu of High-Purity Water.

DEAERATED WATER

Deaerated Water or "degassed water" is *Purified Water* that has been treated to reduce the content of dissolved air by "suitable means" such as boiling, sonication, and/or stirring during the application of a partial vacuum, followed by immediate use or protection from air reabsorption.

OXYGEN-FREE WATER

Oxygen-Free Water is *Purified Water* that has been treated to remove or reduce dissolved oxygen. Such treatment could involve deaerating by boiling or sparging with an inert gas such as nitrogen or helium, followed by inert gas blanketing to prevent oxygen reabsorption. Any

procedure used for removing oxygen should be verified as reliably producing water that is fit for use.

WATER FOR BACTERIAL ENDOTOXINS TEST

Water for BET is also referred to as Limulus Amebocyte Lysate (LAL) Reagent Water. This type of water is often *Water for Injection*, which may have been sterilized. It is free from a level of endotoxin that would yield any detectable reaction or interference with the LAL reagent used in the BET (see (85)).

4. VALIDATION AND QUALIFICATION OF WATER PURIFICATION, STORAGE, AND DISTRIBUTION SYSTEMS

4.1 Validation Requirement

Establishing the reliability of pharmaceutical water purification, storage, and distribution systems requires demonstrating control of the process through an appropriate period of monitoring and observation. Finished water is typically continuously produced and used, while product and process attributes may only be periodically assessed. The quality of bulk finished water cannot be established by only testing monograph attributes. The unit operations in the pharmaceutical water system need to demonstrate that they are in control through monitoring of the process parameters and water quality. The advent of using conductivity and total organic carbon (TOC) to define chemical purity allows the user to more quantitatively assess the water's chemical purity and its variability as a function of routine treatment system maintenance and regeneration. Treatment processes must also demonstrate control of microbial attributes within the overall system. Some unit operations that are needed for chemical treatment may significantly increase microbial and bacterial endotoxin levels. These are later controlled by downstream unit operations. Knowledge of the treatment system processes and the effectiveness of control measures is needed to ensure that the pharmaceutical waters are acceptable for use.

Efficacy of the design, operation, sanitization, and control of the pharmaceutical water system is demonstrated through the monitoring of chemical and microbial attributes. A typical water system validation program involves an initial increased frequency of monitoring of the treatment system process parameters and sampling and testing of major process points to demonstrate the ability to produce the acceptable water and to characterize the operation of the system. This is followed by a life cycle approach of validation maintenance and monitoring.

4.2 Validation Approach

Validation is the program of documenting, to a high level of assurance, that a specific process is capable of consistently delivering product conforming to an established set of quality attributes. A validation program qualifies and documents the design, installation, operation, and performance of the system. A graphical representation of a typical water system validation life cycle is shown in *Figure 3*.

The validation protocol should be based on the boundaries of the water system and the critical water quality and process attributes needed to maintain consistent performance. The system boundary may stop at the point of use or may include the water transfer process. If the transfer process from the distribution system outlets to the water use locations (typically either with hoses or hard-piped equipment connections) is defined as outside the water system

boundary, then this transfer process still needs to be validated to not adversely affect the quality of the water as it is delivered for use. Because routine quality control (QC) microbial monitoring is performed for the same transfer process and components (e.g., hoses and heat exchangers) as that of routine water use [see 6.1.2 *Quality Control (QC) Sampling*], there is some logic to include this water transfer process within the distribution system validation.

VALIDATION ELEMENTS

Validation is accomplished through the use of a structured, documented process. The phases of this process include Design Qualification (DQ), Installation Qualification (IQ), Operational Qualification (OQ), Performance Qualification (PQ), and Validation Maintenance. The process is documented in a validation protocol. The elements may be in individual protocols for each phase, or integrated into variations of a DQ/IQ/OQ/PQ combined document format. The protocols are formally approved quality documents. Factory Acceptance Testing (FAT), Site Acceptance Testing (SAT), and commissioning testing of the system may supplement qualification tests for IQ or OQ provided that they are properly documented and reviewed; and if it can be shown that the system functionality is not affected by the transport and installation.

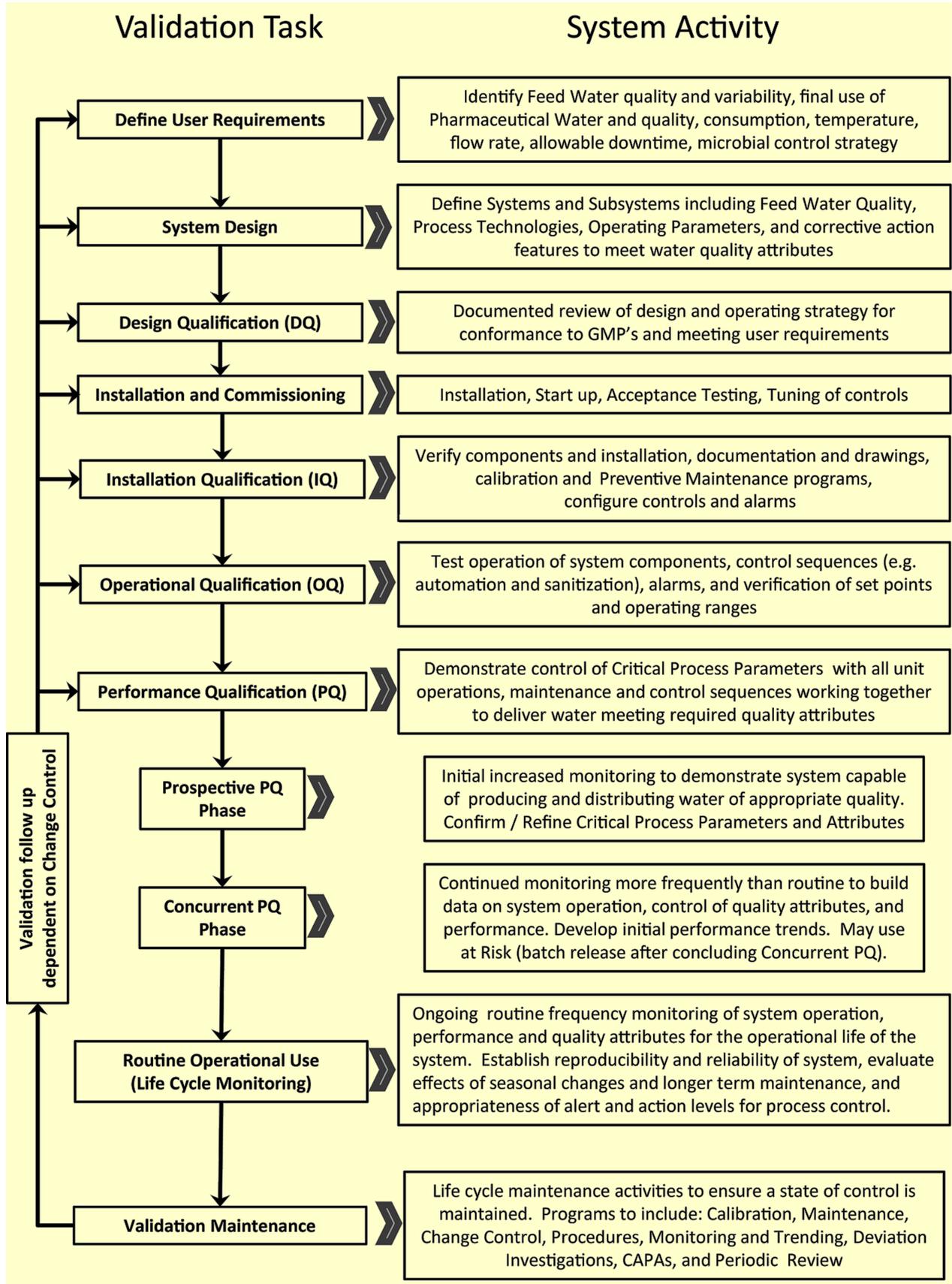


Figure 3. Water system validation life cycle.

USER REQUIREMENTS AND DESIGN QUALIFICATION, URS, FAT, SAT

The user requirements for the water system should identify the design, operation, maintenance, and quality elements needed to produce the desired water type from the available source water, including its anticipated attribute variability. The essential elements of quality need to be built in at this stage and any GMP risks mitigated to an acceptable level.

The review of the specifications, system design, components, functions, and operation should be performed to demonstrate that the system complies with GMPs and verify that the design meets the user requirements. This documented review may be performed as part of the overall design process or as a separate DQ.

INSTALLATION QUALIFICATION

An IQ protocol for a water system confirms that the system has been properly installed and documented. This may include verification of components, piping, installation, and weld quality; documentation of the specifications for all system components present; inspections to verify that the drawings accurately depict the final configuration of the water system and, where necessary, special tests to verify that the installation meets the design requirements. Additionally, the water system is readied for operational testing, including calibration of instruments, configuration of alarm levels and adjustment of operating parameters (e.g., flow rate, pressure).

OPERATIONAL QUALIFICATION

The OQ phase consisting of tests and inspections to verify that the equipment, system alerts, and controls are operating reliably and that appropriate Alert and Action Levels are established (this phase of qualification may overlap with aspects of IQ and PQ). During this phase of validation specific testing is performed for alarms, verifying control sequences, equipment functional checks, and verification of operating ranges. SOPs for all aspects of water system operation, maintenance, water use, water sampling, and testing, etc. should be in place and operator training completed. At the completion of the OQ, the water system has demonstrated that the components are operational and the system is producing suitable water.

PERFORMANCE QUALIFICATION

The prospective PQ stage considers two aspects of the water system: critical process parameters and critical water attribute parameters. These are evaluated in parallel by monitoring the water quality and demonstrating acceptable quality attributes while demonstrating control of the process parameters (see 6.3 *Validation Sampling Plans*). The initial PQ stage may result in refinement of process parameters to yield appropriate water quality. This PQ stage includes an increased frequency of monitoring for approximately 2–4 weeks, or sufficient time to generate adequate data to demonstrate that water meeting the appropriate quality attributes is produced and distributed. One of the reasons for this duration is that biofilm, the source of planktonic organisms in water samples, takes time to develop and to determine if the sanitization unit operations and processes are adequate to control microbial proliferation. The chemical control program adequacy is typically apparent in less time than it takes to see microbial control adequacy. However, chemical purification can be compromised by poor microbial control and, to a lesser degree, vice versa.

Once a level of control of microbial and chemical attributes has been demonstrated, the next phase of PQ is to continue the frequency of monitoring for approximately 2–4 weeks at a somewhat reduced level that will still give adequate data on system performance while using

the pharmaceutical water. The water may be used for manufacturing at risk, and the associated products may be released only after water quality attributes have been determined to be acceptable and this validation phase has been completed. At the completion of the second phase, the data should be formally reviewed and the system approved for operational use.

4.3 Operational Use

When the water system has been placed into operational use, monitoring of the water quality attributes and the system process parameters is performed at a routine frequency (see 6.4 *Routine Sampling Plans*) to ensure that they remain with a state of control during long-term variability from seasonal variations in source water quality, unit operation maintenance, system sanitization processes, and earlier-established Alert and Action Levels.

The water system should continue to be monitored and evaluated on an on-going basis following a life cycle approach using online instruments or samples for laboratory-based testing. The use of online instruments and process automation technology, such as conductivity, TOC, temperature, flow rate, and pressure can facilitate improved operational control of the attributes and parameters and for process release. Manual observation of operating parameters and laboratory-based testing is also appropriate and acceptable for monitoring and trend evaluation.

MONITORING

The frequency of routine monitoring should be based on the criticality of the finished water, capabilities of the process, and ability to maintain product water quality trends. Monitoring may be adjusted from the initial validation monitoring program when there is sufficient data to support a change (see 6.4 *Routine Sampling Plans*).

VALIDATION MAINTENANCE

Maintaining the validated state of control requires a life cycle approach. After the completion of the PQ and release of the water system for use, ongoing activities and programs have to be in place to maintain the validated state of control after the system has been validated and placed into service (see 5.4 *Operation, Maintenance, and Control*). This included unit operation, calibration, corrective maintenance, preventive maintenance, procedures, manuals and drawings, standardization of instruments, process parameter and quality attribute trending, change control, deviations, corrective and preventive actions (CAPA), training, records retention, logbooks, etc.

CHANGE CONTROL

Identification and control of changes made to unit operations and other system components, operation parameters, system sanitization, and laboratory processes or procedures need to be established. Not all changes will require validation follow up, but even minor ones, such as gasket elastomer changes could have an impact on quality attributes. The impact of the change on process parameters and quality attributes must be identified, evaluated and remediated. This may result in a selective validation activity to demonstrate the ongoing state of control for the system and ability to maintain water quality attributes.

Certain calibration and preventive maintenance activities may be considered routine tasks if they do not impact on system operation or water quality. Replacement of components needs to

be carefully evaluated. Replacement of components using exact parts generally does not affect system operation or control. Replacement of components with ones that have the similar functional specifications can be performed at risk with the critical specifications (e.g., material of construction, dimensions, flow rate, response factors) having been evaluated and the differences determined to be acceptable and documented within the change control system.

PERIODIC REVIEW

The water system qualification, maintenance history, calibration records, quality and process data, issues with the unit operations and any process variability, change control, and other validation maintenance data should be assessed periodically to determine impact on the state of control.

The review may result in adjustments to operating or sanitization processes, calibration or maintenance plans, or monitoring plans. This may also result in additional testing or repeating certain qualification tasks (re-qualification).

5. DESIGN AND OPERATION OF PURIFIED WATER AND WATER FOR INJECTION SYSTEMS

The design, installation, and operation of systems to produce *Purified Water* and *Water for Injection* include similar components, control techniques, and procedures. The quality attributes of the two waters differ only in the presence of a bacterial endotoxin requirement for *Water for Injection* and in their methods of preparation. The similarities in the quality attributes provide considerable common ground in the design of water systems to meet either requirement. The critical difference is the degree of control of the system and the final purification steps needed to ensure removal of bacteria and bacterial endotoxins and reductions in opportunities for biofilm re-development within those purification steps which could become *in situ* sources of bacteria and endotoxin in the finished water.

Many aspects of system design and operation relate to control and elimination of biofilm. Unit operations can cause the deterioration of water microbial attributes and the formation of biofilm on unit operation surfaces, even when properly maintained (see 8.2. *Biofilm Formation in Water Systems*).

Production of pharmaceutical water involves sequential unit operations (processing steps) that address specific water quality attributes and protect the operation of subsequent treatment steps. A typical evaluation process for selecting an appropriate water quality for a particular pharmaceutical purpose is shown in the decision trees in *Figure 2a* and *Figure 2b*. This diagram may be used to assist in defining requirements for specific water uses and in the selection of unit operations. The final unit operation used to produce *Water for Injection* is limited to distillation or other processes equivalent or superior to distillation in the removal of chemical impurities as well as microorganisms and their components, such as bacterial endotoxins. Distillation coupled with suitable pretreatment technologies has a long history of generally reliable performance (though not completely infallible) and can be validated as a unit operation for the production of *Water for Injection*. Other combinations of purification technologies may also be suitable in the production of *Water for Injection* if they can be shown through validation to be as effective and reliable as distillation in the removal of chemicals and microorganisms. The development of new designs and materials of construction for other technologies (such as reverse osmosis, electrodeionization, and ultrafiltration) that allow intermittent or continuous operation at hot bactericidal conditions show promise for a valid use

in producing *Water for Injection*.

5.1 Unit Operations Considerations

To achieve the quality attributes for pharmaceutical waters, multiple-unit operations are required. The design of the water purification system needs to take into consideration different aspects, including the source water quality, sanitization, pharmaceutical water quality attributes, uses of the water, and maintenance programs. Each unit operation contributes specific purification attributes associated with chemical and microbiological parameters.

The following is a brief description of selected unit operations and the design, installation, operation, maintenance, and monitoring parameter considerations associated with them. Not all unit operations are discussed, nor are all potential shortcomings addressed.

PREFILTRATION

The purpose of prefiltration—also referred to as initial, coarse, particulate, or depth filtration—is to remove solid contaminants from the incoming source water supply and protect downstream system components from particulates that can inhibit equipment performance and shorten their effective life. This coarse filtration technology primarily uses sieving effects for particle capture and a depth of filtration medium that has a high “dirt load” capacity. Such filtration units are available in a wide range of designs and for various applications. Removal efficiencies and capacities differ significantly, from granular bed filters such as multimedia or sand for larger water systems, to depth cartridges for smaller water systems. Unit and system configurations vary widely in the type of filtering media and the location in the process. Granular or cartridge prefilters are often situated at the beginning of the water purification system prior to unit operations designed to remove the source water disinfectants. Cartridge-type coarse filters may also be used to capture fines released from granular beds such as activated carbon and deionization beds. These locations, however, do not preclude the need for periodic microbial evaluation.

Design and operational issues that may impact the performance of depth filters include channeling of the filtering media, blockage from silt, microbial growth, and filtering-media loss during improper backwashing. Control methods involve pressure and flow monitoring during use and backwashing, sanitizing, and replacing filtering media. An important design concern is sizing of the filter to prevent channeling or media loss resulting from inappropriate water flow rates as well as proper sizing to minimize excessively frequent or infrequent backwashing or cartridge filter replacement.

ACTIVATED CARBON

Activated carbon beds, depending on the type and placement, are used to adsorb low-molecular-weight organic material, bacterial endotoxins, and oxidizing additives such as chlorine and chloramine compounds, removing them from the water. They are used to achieve certain quality attributes and to protect against reactions with downstream unit operations, stainless steel surfaces, resins, and membranes.

The chief operating concerns regarding activated carbon beds include the propensity to support bacterial growth, the potential for hydraulic channeling, the organic adsorption capacity, and insufficient contact time. Operation deficiencies may result in the release of bacteria, endotoxins, organic chemicals, and fine carbon particles.

Control measures may involve monitoring water flow rates and differential pressures, sanitizing with hot water or steam, backwashing, testing for adsorption capacity, and frequent replacement of the carbon bed. Monitoring of carbon bed unit operation may also include microbial loading, disinfectant chemical reduction, and TOC if used for TOC reduction. The use of hot water or steam for carbon bed sanitization is ineffective if there is channeling rather than even permeation through the bed. Channeling can be mitigated through design and proper flow rates during sanitization.

Microbial biofilm development on the surface of the granular carbon particles can cause adjacent bed granules to agglomerate. This may result in ineffective removal of trapped debris and fragile biofilm during backwashing, and ineffective sanitization.

Alternative technologies to activated carbon beds can be used to avoid their microbial challenges. These include disinfectant-neutralizing chemical additives and intense ultraviolet (UV) light for removal of chlorine, and regenerable organic scavenging deionizing resins for removal of organics.

ADDITIVES

Chemical additives are used in water systems 1) to control microorganisms by use of sanitizing agents, such as chlorine compounds and ozone; 2) to enhance the removal of suspended solids by use of flocculating agents; 3) to remove chlorine compounds; 4) to avoid scaling on reverse osmosis membranes; and 5) to adjust pH for more effective removal of carbonate and ammonia compounds by reverse osmosis. These additives do not constitute "added substances" as long as they are either removed by subsequent processing steps or are otherwise absent from the finished water. Control of additives to ensure a continuously effective concentration and subsequent monitoring to ensure their removal should be designed into the system and included in the monitoring program.

ORGANIC SCAVENGERS

Organic scavenging devices use macroreticular, weakly basic anion-exchange resins capable of removing negatively charged organic material and endotoxins from the water. Organic scavenger resins can be regenerated with appropriate biocidal caustic brine solutions. Operating concerns are associated with organic scavenging capacity; particulate, chemical, and microbiological fouling of the reactive resin surface; flow rate; regeneration frequency; and shedding of fines from the fragile resins. Control measures include TOC testing of influent and effluent, backwashing, monitoring hydraulic performance, and using downstream filters to remove resin fines.

SOFTENERS

Water softeners may be located either upstream or downstream of disinfectant removal units. They utilize sodium-based cation-exchange resins to remove water-hardness ions, such as calcium and magnesium, that could foul or interfere with the performance of downstream processing equipment such as reverse osmosis membranes, deionization devices, and distillation units. Water softeners can also be used to remove other lower affinity cations, such as the ammonium ion, that may be released from chloramine disinfectants commonly used in drinking water. If ammonium removal is one of its purposes, the softener must be located downstream of the disinfectant removal operation. Water softener resin beds are regenerated with concentrated sodium chloride solution (brine).

Concerns include microorganism proliferation, channeling, appropriate water flow rates and contact time, ion-exchange capacity, organic and particulate resin fouling, organic leaching from new resins, fracture of the resin beads, resin degradation by excessively chlorinated water, and contamination from the brine solution used for regeneration.

Control measures involve recirculation of water during periods of low water use; periodic sanitization of the resin and brine system; use of microbial control devices (e.g., UV light and chlorine); locating the unit upstream of the disinfectant removal step (if used only for softening); appropriate regeneration frequency; effluent chemical monitoring (e.g., hardness ions and possibly ammonium); and downstream filtration to remove resin fines. If a softener is used for ammonium removal from chloramine-containing source water, then the capacity, contact time, resin surface fouling, pH, and regeneration frequency are very important.

DEIONIZATION

Deionization (DI) and continuous electrodeionization (CEDI) are effective methods of improving the chemical quality attributes of water by removing cations and anions. DI systems have charged resins that require periodic regeneration with an acid and base. Typically, cation resins are regenerated with either hydrochloric or sulfuric acid, which replace the captured positive ions with hydrogen ions. Anion resins are regenerated with sodium hydroxide or potassium hydroxide, which replace captured negative ions with hydroxide ions. Because free endotoxin is negatively charged, some removal of endotoxin is achieved by the anion resin. The system can be designed so that the cation and anion resins are in separate or "twin" beds, or they can be blended together to form a "mixed" bed.

The CEDI system uses a combination of ion-exchange resin, selectively permeable membranes, and an electric charge, providing continuous flow (of product and waste concentrate) and continuous regeneration. Water enters both the resin section and the waste (concentrate) section. The resin acts as a conductor, enabling the electrical potential to drive the captured cations and anions through the resin and appropriate membranes for concentration and removal in the waste water stream. As the water passes through the resin, it is deionized to become product water. The electrical potential also separates the water in the resin (product) section into hydrogen and hydroxide ions. This permits continuous regeneration of the resin without the need for regenerant additives. However, unlike conventional deionization, CEDI units must start with water that is already partially purified because they generally cannot achieve the conductivity attribute of Purified Water when starting with the heavier ion load of source water.

Concerns for all forms of DI units include microbial and endotoxin control; chemical additive impact on resins and membranes; and loss, degradation, and fouling of resin. Issues of concern specific to DI units include regeneration frequency and completeness; channeling caused by biofilm agglomeration of resin particles; organic leaching from new resins; complete resin separation for mixed bed regeneration; and bed fluidization air contamination (mixed beds).

Control measures may include continuous recirculation loops, effluent microbial control by UV light, conductivity monitoring, resin testing, microporous filtration of bed fluidization air, microbial monitoring, frequent regeneration to minimize and control microorganism growth, sizing the equipment for suitable water flow and contact time, and use of elevated temperatures. Internal distributor and regeneration piping for DI bed units should be configured to ensure that regeneration chemicals contact all internal bed and piping surfaces and resins.

Rechargeable canisters can be the source of contamination and should be carefully monitored. Full knowledge of previous resin use, minimum storage time between regeneration and use, and appropriate sanitizing procedures are critical factors for ensuring proper performance.

REVERSE OSMOSIS

Reverse osmosis (RO) units use semipermeable membranes. The "pores" of RO membranes are intersegmental spaces among the polymer molecules. They are big enough for permeation of water molecules, but they limit the passage of hydrated chemical ions, organic compounds, and microorganisms. RO membranes can achieve chemical, microbial, and endotoxin quality improvement. Many factors, including pH, temperature, source water hardness, permeate and reject flow rate, and differential pressure across the membrane, affect the selectivity and effectiveness of this permeation. The process streams consist of supply water, product water (permeate), and waste water (reject). Depending on the source water, pretreatment and system configuration variations and chemical additives may be necessary to achieve the desired performance and reliability. For most source waters, a single stage of RO filtration is usually not enough to meet *Purified Water* conductivity specifications. A second pass of this permeate water through another RO stage usually achieves the necessary permeate purity if other factors such as pH and temperature have been appropriately adjusted and the ammonia from chloraminated source water has been previously removed.

Concerns associated with the design and operation of RO units include membrane materials that are sensitive to sanitizing agents and to particulate, chemical, and microbial membrane fouling; membrane and seal integrity; and the passage of dissolved gases, such as carbon dioxide and ammonia. Failure of membrane or seal integrity will result in product water contamination. Methods of control involve suitable pretreatment of the influent water stream; appropriate membrane material selection; membrane design and heat tolerance; periodic sanitization; and monitoring of differential pressures, conductivity, microbial levels, and TOC.

The development of RO units that can tolerate sanitizing water temperatures and also operate efficiently and continuously at elevated temperatures has added greatly to their microbial control ability and to the avoidance of biofouling. RO units can be used alone or in combination with DI and CEDI units, as well as ultrafiltration, for operational and quality enhancements.

ULTRAFILTRATION

Ultrafiltration is a technology that is often used near the end of a pharmaceutical water purification system for removing endotoxins from a water stream. Ultrafiltration can use semipermeable membranes, but unlike RO, these typically use polysulfone membranes with intersegmental "pores" that have been purposefully enlarged. Membranes with differing molecular weight "cutoffs" can be created to preferentially reject molecules with molecular weights above these ratings.

Ceramic ultrafilters are another molecular sieving technology. Ceramic ultrafilters are self-supporting and extremely durable; they can be backwashed, chemically cleaned, and steam sterilized. However, they may require higher operating pressures than do membrane-type ultrafilters.

All ultrafiltration devices work primarily by a molecular sieving principle. Ultrafilters with molecular weight cutoff ratings in the range of 10,000–20,000 Da are typically used in water systems for removing endotoxins. This technology may be appropriate as an intermediate or

final purification step. As with RO, successful performance is dependent upon pretreatment of the water by upstream unit operations.

Issues of concern for ultrafilters include compatibility of membrane material with heat and sanitizing agents, membrane integrity, fouling by particles and microorganisms, and seal integrity. Control measures involve filter membrane composition, sanitization, flow design (dead end vs. tangential), cartridge replacement, elevated feed water temperature, and monitoring TOC and differential pressure.

MICROBIAL-RETENTIVE FILTRATION

Microbial-retentive membrane filters have a larger effective "pore size" than ultrafilters and are intended to prevent the passage of microorganisms and similarly sized particles without unduly restricting flow. This type of filtration is widely employed within water systems for filtering the bacteria out of both water and compressed gases as well as for vent filters on tanks and stills and other unit operations.

In water systems, a filter's microbial retention characteristics exhibit different phenomena than in other aseptic filtration applications.

The following factors interact to create the retention phenomena for water system microorganisms: the variability in the range and average pore sizes created by the various membrane fabrication processes; the variability of the surface chemistry and three-dimensional structure related to the different polymers used in these filter matrices; and the size and surface properties of the microorganism intended to be retained by the filters. The typical challenge organism for these filters, *Brevundimonas diminuta*, may not be the best challenge microorganism for demonstrating bacterial retention by 0.2- to 0.22- μm rated filters for use in water systems because this challenge microorganism appears to be more easily retained by these filters than some other water system bacteria. In some situations, the appearance of water system microorganisms on the downstream sides of some 0.2- to 0.22- μm rated filters after a relatively short period of use (days to weeks) seems to support the idea that some penetration phenomenon is at work. It is not known whether this downstream appearance is caused by a "pass-through" phenomenon resulting from tiny cells or less cell "stickiness," or perhaps by a "grow through" phenomenon where cells hypothetically replicate their way through the pores to the downstream side. Whatever the penetration mechanism, 0.2- to 0.22- μm rated membranes may not be the best choice for some water system uses (see *Sterilization and Sterility Assurance of Compendial Articles* (1211)).

Nevertheless, microbial retention success in water systems has been reported with the use of filters rated as 0.2 or 0.1 μm . There is general agreement that, for a given manufacturer, their 0.1- μm rated filters are tighter than their 0.2- to 0.22- μm rated filters. However, comparably rated filters from different manufacturers may not have equivalent performance in water filtration applications because of the different filter materials, different fabrication processes, and nonstandardized microbial retention challenge processes currently used for defining the 0.1- μm filter rating. It should be noted that filters with a 0.1- μm rating may result in a lower flow rate compared to 0.2- to 0.22- μm filters, so whatever filters are chosen for a water system application, the user must verify that they are suitable for their intended application, use period, and use process, including flow rate.

For microbial retentive gas filtrations, the same sieving and adsorptive retention phenomena are at work as in liquid filtration, but the adsorptive phenomenon is enhanced by additional

electrostatic interactions between the particles and filter matrix. These electrostatic interactions are so strong, particle retention for a given filter rating is significantly more efficient in gas filtration than in water or product-solution filtrations. These additional adsorptive interactions render filters rated at 0.2–0.22 μm unquestionably suitable for microbial retentive gas filtrations. When microbial retentive filters are used in these applications, the membrane surface is typically hydrophobic (non-wettable by water). A significant area of concern for gas filtration is blockage of tank vents by condensed water vapor, which can cause mechanical damage to the tank. Control measures include electrical or steam tracing and a self-draining orientation of vent filter housings to prevent accumulation of vapor condensate. However, a continuously high filter temperature will take an oxidative toll on polypropylene components of the filter, so sterilization of the unit prior to initial use, and periodically thereafter, as well as regular visual inspections, integrity tests, and filter cartridge changes are recommended control methods.

In water applications, microbial retentive filters may be used downstream of unit operations that tend to release microorganisms or upstream of unit operations that are sensitive to microorganisms. Microbial retentive filters may also be used to filter water feeding the distribution system. It should be noted that regulatory authorities allow the use of microbial retentive filters within distribution systems or even at use points if they have been properly validated and are appropriately maintained. A point-of-use filter should only be intended to “polish” the microbial quality of an otherwise well-maintained system and not to serve as the primary microbial control device. The efficacy of system microbial control measures can only be assessed by sampling the water upstream of the filters. As an added measure of protection, in-line UV lamps, appropriately sized for the flow rate (see 5.3 *Sanitization*), may be used just upstream of microbial retentive filters to inactivate microorganisms prior to their capture by the filter. This tandem approach tends to greatly delay potential microbial penetration phenomena and can substantially extend filter service life.

ULTRAVIOLET LIGHT

The use of low-pressure UV lights that emit a 254-nm wavelength for microbial control is discussed under 5.3 *Sanitization*, but the application of UV light in chemical purification is also emerging. This 254-nm wavelength is also useful in the destruction of ozone. At wavelengths around 185 nm (as well as at 254 nm), medium-pressure UV lights have demonstrated utility in the destruction of the chlorine-containing disinfectants used in source water as well as for interim stages of water pretreatment. High intensities of 254 nm or in combination with other oxidizing sanitants, such as hydrogen peroxide, have been used to lower TOC levels in recirculating distribution systems. The organics are typically converted to carbon dioxide, which equilibrates to bicarbonate, and incompletely oxidized carboxylic acids, both of which can easily be removed by polishing ion-exchange resins.

Areas of concern include inadequate UV intensity and residence time, gradual loss of UV emissivity with bulb age, gradual formation of a UV-absorbing film at the water contact surface, incomplete photodegradation during unforeseen source water hyperchlorination, release of ammonia from chloramine photodegradation, unapparent UV bulb failure, and conductivity degradation in distribution systems using 185-nm UV lights.

Control measures include regular inspection or emissivity alarms to detect bulb failures or film occlusions, regular UV bulb sleeve cleaning and wiping, downstream chlorine detectors (when used for dechlorination), downstream polishing deionizers (when used for TOC reduction), and

regular (approximately yearly) bulb replacement. UV lamps generate heat during operation, which can cause failure of the lamps or increase the temperature of the water. Precautions should be in place to ensure that water flow is present to control excessive temperature increase.

DISTILLATION

Distillation units provide chemical and microbial purification via thermal vaporization, mist elimination, and water vapor condensation. A variety of designs is available, including single effect, multiple effect, and vapor compression. The latter two configurations are normally used in larger systems because of their generating capacity and efficiency. Source water controls must provide for the removal of hardness and silica impurities that may foul or corrode the heat transfer surfaces, as well as the removal of those impurities that could volatilize and condense along with the water vapor. In spite of general perceptions, even the best distillation process does not ensure absolute removal of contaminating ions, organics, and endotoxins. Most stills are recognized as being able to accomplish at least a 3–4 log reduction in these impurity concentrations. They are highly effective in sterilizing the feed water.

Areas of concern include carry-over of volatile organic impurities such as trihalomethanes (see 2. *Source Water Considerations*) and gaseous impurities such as ammonia and carbon dioxide, faulty mist elimination, evaporator flooding, inadequate blow down, stagnant water in condensers and evaporators, pump and compressor seal design, pinhole evaporator and condenser leaks, and conductivity (quality) variations during start-up and operation.

Methods of control may involve the following: preliminary steps to remove both dissolved carbon dioxide and other volatile or noncondensable impurities; reliable mist elimination to minimize feed water droplet entrainment; visual or automated high-water-level indication to detect boiler flooding and boil over; use of sanitary pumps and compressors to minimize microbial and lubricant contamination of feed water and condensate; proper drainage during inactive periods to minimize microbial growth and accumulation of associated endotoxin in boiler water; blow down control to limit the impurity concentration effect in the boiler to manageable levels; on-line conductivity sensing with automated diversion to waste to prevent unacceptable water upon still startup or still malfunction from getting into the finished water distribution system; and periodic testing for pinhole leaks to routinely ensure that condensate is not compromised by nonvolatilized source water contaminants.

STORAGE TANKS

Storage tanks are included in water distribution systems to optimize processing equipment capacity. Storage also allows for routine maintenance within the purification system while maintaining continuous supply to meet manufacturing needs. Design and operation considerations are needed to prevent or minimize the development of biofilm, to minimize corrosion, to aid in the use of chemical sanitization of the tanks, and to safeguard mechanical integrity.

Areas of concern include microbial growth or corrosion due to irregular or incomplete sanitization and microbial contamination from unalarmed rupture disk failures caused by condensate-occluded vent filters.

Control considerations may include using closed tanks with smooth interiors, the ability to spray the tank headspace using sprayballs on recirculating loop returns, and the use of heated,

jacketed/insulated tanks. This minimizes corrosion and biofilm development and aids in thermal or chemical sanitization. Storage tanks require venting to compensate for the dynamics of changing water levels. This can be accomplished with a properly oriented and heat-traced filter housing fitted with a hydrophobic microbial retentive membrane filter affixed to an atmospheric vent. Alternatively, an automatic membrane-filtered compressed gas blanketing system may be used. In both cases, rupture disks equipped with a rupture alarm device should be used as a further safeguard for the mechanical integrity of the tank.

DISTRIBUTION SYSTEMS

Distribution system configuration should allow for the continuous flow of water in the piping by means of recirculation. Use of no recirculating, dead-end, or one-way systems or system segments should be avoided whenever possible. If not possible, these systems should be flushed periodically and monitored more closely. Experience has shown that continuously recirculated systems are easier to maintain. Pumps should be designed to deliver fully turbulent flow conditions to facilitate thorough heat distribution (for hot-water sanitized systems) as well as thorough chemical sanitant distribution. Turbulent flow also appears to either retard the development of biofilms or reduce the tendency of those biofilms to shed bacteria into the water. If redundant components, such as pumps or filters, are used, they should be configured and used to avoid microbial contamination of the system.

Components and distribution lines should be sloped and fitted with drain points so that the system can be completely drained. In distribution systems, dead legs and low-flow conditions should be avoided, and valved tie-in points should have length-to-diameter ratios of six or less. In systems that operate at self-sanitizing temperatures, precautions should be taken to avoid cool points where biofilm development could occur. If drainage of components or distribution lines is intended as a microbial control strategy, they should also be configured to be dried completely using dry compressed gas because drained but still moist surfaces will still support microbial proliferation. Water exiting from the distribution system should not be returned to the system without first passing through all or a portion of the purification system.

The distribution design should include the placement of sampling valves in the storage tank and at other locations, such as in the return line of the recirculating water system. Direct connections to processes or auxiliary equipment should be designed to prevent reverse flow into the controlled water system. Hoses and heat exchangers that are attached to points of use to deliver water must not chemically or microbiologically degrade the water quality. The distribution system should permit sanitization for microorganism control. The system may be continuously operated at sanitizing conditions or sanitized periodically.

NOVEL/EMERGING TECHNOLOGIES

New water treatment technologies are being developed continuously. Before these technologies are utilized in pharmaceutical water systems, they should be evaluated for acceptable use in a GMP environment. Other considerations should include the treatment process, reliability and robustness, use of added substances, materials of construction, and ability to validate. Consideration should be given to recognize the areas of concern during the evaluation and to identify control measures for the technology. This should include impact on chemical and microbial attributes.

5.2 Installation, Materials of Construction, and Component Selection

Installation techniques are important because they can affect the mechanical, corrosive, and sanitary integrity of the system. Valve installation should promote gravity drainage. Pipe supports should provide appropriate slopes for drainage and should be designed to support the piping adequately under worst-case thermal and flow conditions. The methods of connecting system components—including units of operation, tanks, and distribution piping—require careful attention to preclude potential operational and microbial problems.

Stainless steel welds should provide reliable joints that are internally smooth and corrosion-free. Low-carbon stainless steel, compatible wire filler where necessary, inert gas, automatic welding machines, and regular inspection and documentation help to ensure acceptable weld quality. Follow-up cleaning and passivation of metal surfaces after installation are important for removing contamination and corrosion products and to re-establish the passive corrosion-resistant surface.

Plastic materials can be fused (welded) in some cases, and also require smooth, uniform internal surfaces. Adhesive glues and solvents should be avoided due to the potential for voids and organic extractables. Mechanical methods of joining, such as flange fittings, require care to avoid the creation of offsets, gaps, penetrations, and voids. Use of plastic materials may contribute to TOC levels.

Control measures include good alignment, properly sized gaskets, appropriate spacing, uniform sealing force, and the avoidance of threaded fittings.

Materials of construction should be selected to be compatible with control measures such as sanitizing, cleaning, or passivation. Temperature rating is a critical factor in choosing appropriate materials because surfaces may be required to handle elevated operating and sanitization temperatures. If chemicals or additives will be used to clean, passivate, or sanitize the system, materials resistant to these chemicals or additives must be utilized. Materials should be capable of handling turbulent flow and elevated velocities without erosion of the corrosion-resistant film (such as the passive chromium oxide surface of stainless steel) or reduction in wall thickness for plastics. The finish on metallic materials such as stainless steel, whether it is a refined mill finish, polished to a specific grit, or an electropolished treatment, should complement the system design and provide satisfactory corrosion and microbial activity resistance. The finish should also be a material that can be chemically sanitized. Auxiliary equipment and fittings that require seals, gaskets, diaphragms, filter media, and membranes should exclude materials that permit the possibility of extractables, shedding, and microbial activity. Insulating materials exposed to stainless steel surfaces should be free of chlorides to avoid the phenomenon of stress corrosion cracking that can lead to system contamination and the destruction of tanks and critical system components.

Specifications are important to ensure proper selection of materials and to serve as a reference for system qualification and maintenance. Information such as the manufacturer's metallurgical reports for stainless steel and reports of composition, ratings, and material-handling capabilities for nonmetallic substances should be reviewed for suitability and retained for reference. Component (auxiliary equipment) selection should be made with assurance that it does not create a source of contamination intrusion. Heat exchangers should be constructed to prevent leakage of heat transfer medium into the pharmaceutical water and, for heat exchanger designs where prevention may fail, there should be a means to detect leakage. Pumps should be of sanitary design with seals that prevent contamination of the water. Valves should have smooth internal surfaces with the seat and closing device exposed to the flushing action of water, such

as occurs in diaphragm valves. Valves with pocket areas or closing devices (e.g., ball, plug, gate, globe) that move into and out of the flow area should be avoided.

5.3 Sanitization

Microbial control in water systems is achieved primarily through sanitization practices. Systems can be sanitized using either thermal or chemical means.

THERMAL SANITIZATION

Thermal approaches to system sanitization include periodic or continuously circulating hot water and the use of steam. Temperatures of 65°–80° are most commonly used for thermal sanitization. Continuously recirculating water of at least 65° at the coldest location in the distribution system has also been used effectively in stainless steel distribution systems when attention is paid to uniformity and distribution of such self-sanitizing temperatures. These techniques are limited to systems that are compatible with the higher temperatures needed to achieve sanitization. Frequent use of thermal sanitization at appropriate temperatures should eliminate the need for other sanitization methods.

The use of thermal methods at temperatures significantly above 80° is contraindicated because it does not add to microbial control of the system or reduction of biofilm. Some methods (e.g., steam sanitizing, hot water circulation at temperatures $\geq 100^\circ$) can be less effective or even destructive because of the need to eliminate condensate or manipulate system components, stress materials of construction, deform filters, and its adverse impact on instrumentation.

Although thermal methods control biofilm development by either continuously inhibiting its growth or, in intermittent applications, by killing the microorganisms within developing biofilms, they are not effective in removing established biofilms. Killed but intact biofilms can become a nutrient source for rapid biofilm regrowth after the sanitizing conditions are removed or halted. In cases of infrequent thermal sanitizations that allow biofilm development between treatments, a combination of routine thermal treatment and periodic supplementation with chemical sanitization may be more effective. The more frequent the thermal sanitization, the more likely it is that biofilm re-development can be eliminated.

CHEMICAL SANITIZATION

Chemical methods, where compatible, can be used on a wider variety of construction materials. These methods typically use oxidizing agents such as ozone, hydrogen peroxide, peracetic acid, or combinations thereof. Halogenated compounds can be effective sanitizers but are less aggressive oxidizing agents and may be difficult to flush from the system. Chemical agents may not penetrate the full biofilm matrix or extend into all biofilm locations (such as crevices at gasketed fittings) and may leave biofilms incompletely inactivated. Compounds such as ozone, hydrogen peroxide, and peracetic acid oxidize bacteria and biofilms with reactive peroxides and by forming very reactive free radicals (notably hydroxyl radicals). The short half-life of ozone in particular, and its limitation on achievable concentrations, require that it be added continuously during the sanitization process. Hydrogen peroxide and ozone rapidly degrade to water and/or oxygen, and peracetic acid degrades to oxygen and acetic acid. The ease of degradation of ozone to oxygen using 254-nm UV lights in circulating loops allow it to be used effectively on a continuously sanitizing basis in holding tanks and on an intermittent basis (e.g., daily or weekly) in the distribution loops. The highly reactive nature of ozone requires the use of system materials and components that are even more oxidation resistant than those typically used with

the other oxidizing agents.

It is important to note that microorganisms in a well-developed biofilm can be extremely difficult to kill, even by using aggressive oxidizing chemicals. The less developed and therefore thinner the biofilm, the more effective the biofilm inactivation. Therefore, optimal microbial control is achieved by using oxidizing chemicals at a frequency that does not permit significant biofilm development between treatments.

Validation of chemical sanitization requires demonstration of adequate chemical concentrations throughout the system, exposure to all wetted surfaces including the body of use point valves, and complete removal of the sanitant from the system at the completion of treatment. Methods validation for the detection and quantification of residues of the sanitant or its objectionable degradants is an essential part of the validation program.

UV SANITIZATION

In-line UV light at a wavelength of 254 nm can also be used to continuously "sanitize" only the water circulating in the system, but these devices must be properly sized for the water flow. Such devices inactivate a high percentage (but not 100%) of microorganisms that flow through the device but cannot be used to directly control existing biofilm upstream or downstream of the device. However, when coupled with conventional thermal or chemical sanitization technologies or located immediately upstream of a microbially retentive filter, UV light is most effective and can prolong the interval between needed system re-sanitizations.

SANITIZATION PROCEDURES

Sanitization steps require validation to demonstrate the ability to reduce and hold microbial contamination at acceptable levels. Validation of thermal methods should include a heat distribution study to demonstrate that sanitization temperatures are achieved throughout the system, including the body of use point valves; sampling ports; instrument side branches; and fittings, couplings, and adapters, relying on water convection and thermal conduction through system materials for heat transfer to wetted surfaces.

The routine frequency of sanitization should be supported by the results of system microbial monitoring. Conclusions derived from trend analysis of the microbiological data should be used as the alert mechanism for the need for extraordinary maintenance. The routine frequency of sanitization should be established in such a way that the system operates in a state of microbiological control and does not regularly exceed Alert and Action Levels (see 9.4. *Defining Alert and Action Levels and Specifications*).

5.4 Operation, Maintenance, and Control

A preventive maintenance program should be established to ensure that the water system remains in a state of control. The program should include 1) procedures for operating the system, 2) monitoring programs for critical quality attributes and operating conditions including calibration of critical instruments, 3) schedule for periodic sanitization, 4) preventive maintenance of components, and 5) control of changes to the mechanical system and to operating conditions.

OPERATING PROCEDURES

Operating procedures for the water system and for performing routine maintenance and corrective action should be written, and they should also define the point when action is

required. The procedures should be well documented, and should detail the function of each job, assign who is responsible for performing the work, describe how the job is to be done, and identify acceptable operating parameters. The effectiveness of these procedures should be assessed during water system validation.

PROCESS MONITORING PROGRAM

A process-monitoring program should establish the critical quality attributes and operating parameters that are documented and monitored. The program may include a combination of in-line sensors and/or automated instruments (e.g., for temperature, TOC conductivity, hardness, and chlorine), automated or manual documentation of operational parameters (such as flow rates or pressure drop across a carbon bed, filter, or RO unit), and laboratory tests (e.g., total microbial counts). The frequency of sampling, the requirement for evaluating test results, and the necessity of initiating corrective action should be included.

ROUTINE MICROBIAL CONTROL

Sanitization may be integral to operation and maintenance, and necessary on a routine basis, depending on system design and the selected units of operation, to maintain the system in a state of microbial control. Technologies for sanitization are described above in more detail in 5.3 *Sanitization*.

PREVENTIVE MAINTENANCE

A preventive maintenance program should be in effect. The program should establish what preventive maintenance is to be performed, the frequency of maintenance work, and how the work should be documented.

CHANGE CONTROL

The mechanical configuration, operating conditions, and maintenance activities of the water system must be controlled. Proposed changes should be evaluated for their impact on the whole system. The need to requalify the system after changes are made should be determined. After a decision is made to modify a water system, the affected drawings, manuals, and procedures should be revised. Portions or operations of the water system that are affected by the modification should be tested to demonstrate a continued state of control. The extent and duration of testing should be related to the risk impact of the change to the system.

6. SAMPLING

The testing of water samples from a water system is critical to the ongoing control of the system and assessment of the quality of the water being used. If improperly collected, a sample could yield a test result that is unrepresentative of the sample's purpose. This could lead to inaction when remediation is needed or to unnecessary remediation when none is necessary. It could also lead to misinterpretations of product impact. Therefore, properly collecting water samples, understanding their purpose, and establishing appropriate water system sampling plans are essential to water quality control and system control.

6.1 Purposes and Procedures

To assess a particular water quality attribute, a sample of the water usually must be removed from a water system for specific quality attribute testing. This sample may be analyzed by in-

line/on-line instruments or it may be completely removed from the system as a "grab sample" in a container for off-line testing. In-line/on-line testing avoids the exogenous contamination potential of grab samples that could lead to artifactually variable data trends and incorrect decisions on system performance, maintenance, and utilized water quality, as well as initiating fruitless causative investigations. However, in-line/on-line testing may not be suitable for the purpose of the resulting data, making the off-line testing of grab samples the only suitable approach.

The data from water testing are generally used for one of two purposes: for process control (PC) of the water purification and distribution system or for quality control (QC) of the water being drawn from the system for some application or use. In many cases, depending on the sampling location and sampling process, the resulting data can be used for both PC and QC purposes.

PROCESS CONTROL (PC) SAMPLING

Because PC sampling is intended to reflect the quality of the water behind the valve and within the distribution system, coming from the purification system, or between its purification steps, efforts should be made to avoid contaminating the water as it drawn from the system so that its test results accurately reflect the water quality within the system at that location. This may require the use of strategically located sampling ports, in addition to points of use.

If microbial testing is needed for PC purposes, the sampling valve should have a properly installed, sanitary design that uses vigorous pre-sampling flushing. This flushing shears off fragile biofilm structures growing on surfaces within the valve and water path before the sample is collected. This avoids biasing the microbial count of perhaps pristine water in the system behind that valve. A fully open valve flush (at >8 ft/s velocity within the valve and connector) for at least 30 s typically provides sufficient shear forces to adequately remove any fragile biofilm structures. Additional control measures for preventing sample contamination could also include stringent pre- and post-sampling outlet sanitation, the use of sterile hoses and gaskets or other connectors to direct the water flow, and other measures.

The data from PC sampling indicate how well the system is maintaining the water quality at that sampling location. These data are subsequently used to signal when some extraordinary intervention might be needed, in addition to normal maintenance and system sanitization operations, to restore the system to the expected level of purity.

PC sampling can only be used to indicate the quality of the water being delivered to the points of use (for QC purposes) if it has been shown to be representative of that point-of-use quality. This may be possible with chemical attributes which are typically not affected by the fluid path of the water delivery process, but is generally not possible with microbial attributes, which can be greatly affected by localized biofilms along that fluid path. If this fluid path is not utilized for PC sampling, then the resulting data typically cannot be used for QC purposes.

QUALITY CONTROL (QC) SAMPLING

QC sampling is intended to reflect the quality of water that is being used. These samples should be collected at the true point of use, that is, where the water is delivered for use, not where it leaves the water system. QC sampling must utilize that same delivery path and components utilized for a water transfer during actual water use. This includes the same valves, hoses, heat exchangers, flow totalizers, hard-piped connections, and other components utilized during

water use.

In addition to the water transfer components, QC sampling must also use the same water transfer process employed during water use, including the same pre-use outlet and delivery path flushing procedure and the same outlet, fitting, and hose sanitization practices employed during actual water use. The water delivery process and components used for QC sampling must be identical to manufacturing practices at every system outlet for the QC sample to mimic the quality of water being used by accumulating the same chemical and microbial contaminant levels it would during actual use from that outlet location.

Where permanent connections from the water system to equipment are present, accommodation should be made in the design to collect samples from locations as close to the equipment as possible. For example, samples can be collected from special sample ports or other valves near the equipment connection that allow the collected water sample to accurately reflect the water quality that is used. Where the water transfer conduit is designed and/or definitively treated to eliminate all contaminating influences prior to water transfer through that conduit, QC sampling locations within the distribution system can reflect the quality of the water that is actually used for QC purposes at those permanent connections. However, the success of the design and treatments intended to eliminate these contaminating influences must be verified. This is typically done during water system validation.

Where routine water use practices involve contamination-prone activities, such as no pre-use flushing or poor hose storage/sanitization/replacement practices, these water use practices should be improved to reduce the potential for delivering contaminated water from the water system and for unacceptable QC sample testing results that reflect that same contamination.

6.2 Attributes and Sampling Locations

The tests being performed on the samples are relevant to the sampling location and purpose of the sample. In-process monitoring of nonmonograph attributes may be indicated for specific unit operations. For instance, before and after a softener, it may be important to determine water hardness to verify softener efficacy. Before and after an activated carbon bed/filter, it may be important to verify chlorine or TOC removal and/or reduction or test for an increase in microbial count. Before a distillation unit, it may be important to quantitate the incoming bacterial endotoxin level to ensure that the still is not being over-challenged beyond its typical 3–4 log purification capability. However, once the water is in the distribution system, the compendial attributes of importance typically include at least conductivity, TOC, and microbial count. In Water for Injection systems and other systems or system locations where bacterial endotoxin control is important, endotoxin is also assayed. Other tests may be necessary depending on the intended uses of the water.

CHEMICAL ATTRIBUTES

Dissolved chemical contaminants detected by conductivity or TOC testing tend to be uniformly distributed in the water throughout the water system. However, there are exceptions where localized chemical contamination sources can occur, such as from a coolant-leaking heat exchanger in a sub-loop, or at a point of use, or within a dead leg. These chemical contaminants may only be seen at the associated outlets and not systemically. However, in the absence of localized contamination influences, chemical attributes are candidates for on-line testing at fixed strategic locations within the distribution system, such as near a circulating loop return, and are generally reflective of the same chemical quality at all locations and points

of use within the distribution system. Nevertheless, the suitability of the on-line locations of these instruments for QC release purposes must be verified as being representative of the use-point water quality. This is usually done during water system validation.

MICROBIAL ATTRIBUTES

The same uniformity scenario cannot be assumed for microbial attributes. Planktonic organisms in a water sample could have originated from biofilms in the purification or distribution systems releasing more or less uniform levels of planktonic organisms into the circulating water, as detectable in samples from all outlets. However, a local biofilm developing within a water delivery conduit (such as a use-point outlet valve and transfer hose) in an otherwise pristine biofilm-free water system could release planktonic organisms detectable only in water delivered through that conduit. Therefore, QC release samples for assessing the quality of water that is delivered by the system during water use must be collected after the water has traversed the same fluid conduit (including the same preparatory activities such as outlet sanitization and pre-flushing) from the water distribution system to the specific locations where the water is used.

On-line microbial water sampling/testing has value in pharmaceutical water systems only for PC purposes unless the water is taken from the point of use in the same manner as routine water usage, in which case the data can also have a QC release purpose. Microbial counts detected from strategic sampling ports continue to have PC and investigational value, but generally cannot be substituted for QC release testing except in certain scenarios, as described in 6.1.2 *Quality Control (QC) Sampling*.

6.3 Validation Sampling Plans

The initial sampling plan for a pharmaceutical water system is usually developed for a validation program (see 4. *Validation and Qualification of Water Purification, Storage, and Distribution Systems*). This strategy is for characterization of the system's ability to purify, distribute, and deliver pharmaceutical water. Typically, the initial validation sampling is for a short duration (e.g., at least 2–4 weeks) at a high sampling frequency to generate a significant body of data that will allow detection of short-term or localized chemical or microbial quality deviations from all outlets. These data provide an initial assessment of system performance to guide decisions about using the water for operational purposes.

The initial validation sampling plan is re-evaluated when the pharmaceutical water is placed into operation, typically to reduce the amount of data being generated while not compromising the ability to identify anomalous operations/events, especially during the early life cycle of the water system. In the absence of such quality deviations during the initial sampling period, the sampling frequency can be lessened for a period of time (e.g., at least 2–4 additional weeks) to ensure that somewhat longer-term adverse quality trends are not apparent. During this second period of time, the water may be considered for at-risk routine use, pending the acceptable completion of the second validation sampling period. After successful completion, monitoring can eventually be lessened again to what will become the routine sampling plan.

Periodic review of the water system operation and monitoring needs to be performed to assess seasonal source water variability, effectiveness of sanitization, and routine maintenance events. Periodic review should be performed during the complete life cycle of the water system, typically annually, for evidence of longer-term data trends and quality deviations.

The routine sampling plan should be re-evaluated periodically based on the available data to determine the appropriate frequency and sample locations. This review offers an opportunity to improve data evaluation and reduce workloads based on what that data indicate relative to process and quality control. The routine sampling plan should have a rationale for the frequency and locations that are selected to justify how the resulting data will be used to characterize the overall operation of the system and the release of the water for use.

6.4 Routine Sampling Plans

SOURCE WATER SAMPLING

As mentioned in earlier sections, the source water for pharmaceutical water systems must comply with the standards for one of the Drinking Waters listed in the associated compendial water monograph or in *General Notices and Requirements*. When a municipality or other water authority is providing this Drinking Water, they are required to comply with the local Drinking Water Regulations for the water supplied to a drinking or potable water distribution piping grid for that region. The quality of that water by the time it reaches the pharmaceutical user is dependent on a number of factors including distance from the input source, duration of travel within the piping, and condition of the piping in that potable water distribution grid, any of which could have adversely affected some of its initial chemical and/or microbial attributes. Based on a risk assessment, it may be prudent to verify full compliance with regulations using water collected from sample ports prior to the pretreatment system, or other equivalent Drinking Water outlets within the facility. If the water complies, then continued assurance of compliance could be verified using Drinking Water Regulation test results provided by the water authority or by periodic retesting of selected or all the Drinking Water attributes by the user or by both the user and the water authority. If private sourced water is utilized, it is the user's responsibility to demonstrate full Drinking Water regulation compliance, using water samples from such sampling ports on a periodic basis as determined by a risk analysis.

These pre-pretreatment sampling ports could, at the user's discretion, be used to periodically monitor other source water attributes that could affect specific pretreatment or purification unit operations. Depending on the user's source water quality consistency and a risk assessment of its potential impact on the purification process, the periodically monitored attributes could include microbial count, absence of coliforms, bacterial endotoxin levels, conductivity, TOC, pH, hardness, chlorine, silica, turbidity or silt density index, and others. These data could be useful in investigations and for operational adjustments to critical unit operation parameters and maintenance procedures, or for feedback to the potable water provider if unusual trends are observed.

PRETREATMENT AND PURIFICATION SYSTEM SAMPLING

The location and frequency of sampling from ports within the pretreatment and purification systems may be selected based on a risk analysis of unit operation purpose. The purpose of this sampling is primarily for PC, for example, to ensure maintenance of acceptable unit operation performance, to assess maintenance procedure efficacy, and to investigate the need for remedial action. Quality deviations in the early portions of the purification process can affect unit operation efficiency but usually do not impact the finished water quality or acceptable use.

PURIFIED WATER DISTRIBUTION SYSTEM SAMPLING

Purified Water distribution system sampling is intended to provide continuing assurance of ongoing PC and compliance with the user's finished water chemical and microbiological requirements. Generally, the locations for that sampling and the frequency of testing the specific attributes are a matter of process and quality control consistency, as well as risk tolerance in the event of a deviation.

Depending on the water system design, the chemical attributes of a water system tend to be relatively constant and more uniformly distributed than the microbiological attributes. Therefore, less frequent sampling at only selected locations could be justified for chemical testing based on familiarity with system design and the existence of historically consistent operational data. However, with some purification system designs, the chemical quality could change dramatically in a short period of time (such as from the exhaustion of deionization beds), so frequent or even continuous in-line/on-line monitoring of the chemical attributes would be advisable to be able to recognize and correct the cause of the problem before non-compliant water is produced and used.

For microbial testing, all use points and critical sample ports in a distribution system are typically sampled routinely, including those that are infrequently used by manufacturing. There is no prescribed sampling frequency for Purified Water system outlets, so typical outlet sampling frequencies vary from daily to monthly, with sampling occurring somewhere in the system at least at weekly intervals.

A risk analysis is suggested for determining the sampling plan for a Purified Water system. Factors in this analysis could include (but are not limited to) the test result history for the entire water system as well as specific outlets, the criticality of specific outlets to manufacturing, the usefulness of selected sample ports as indicators of ongoing system control, and the scope of impact on products and activities should an unfavorable test result occur. For the scope of impact, the less frequent the sampling, the more products and processes will be impacted by an unfavorable test result.

WATER FOR INJECTION DISTRIBUTION SYSTEM SAMPLING

The sampling plans for Water for Injection distribution systems (as well as any water system where some level of bacterial endotoxin control is needed) utilize the same general sampling approaches as do Purified Water systems. However, the regulatory expectations for Water for Injection distribution system sampling plans are more prescriptive because microbial control must be much more stringent as it is related to the bacterial endotoxin attribute. In general, water sampling for microbial and bacterial endotoxin testing is expected to occur daily somewhere in the system, with each outlet being sampled NLT weekly.

6.5 Non-Routine Sampling

Non-routine sampling can also be performed on the water system for episodic events or reasons for which the routine sampling plans are insufficient to capture the needed information. Examples include change control purposes such as evaluating potential changes to sampling, testing, maintenance procedures, or system design; data or event excursion investigation purposes; or simply for long-term informational purposes and establishing baselines for future investigational value. The purpose of the non-routine sampling dictates the sampling procedures to be used, the attributes to be tested, and the location and repeating occurrence (if any) of that testing. It should also be noted that such non-routine sampling may be done from sampling ports that may or may not be routinely tested. Sampling ports can be positioned

in a water system purely for investigational, non-routine sampling, and as such, they do not need to be part of a routine sampling plan.

7. CHEMICAL EVALUATIONS

7.1 Chemical Tests for Bulk Waters

The chemical attributes of Purified Water and Water for Injection that were in effect prior to *USP 23* were specified by a series of chemistry tests for various specific and nonspecific attributes with the intent of detecting chemical species indicative of incomplete or inadequate purification. Although these methods could have been considered barely adequate to control the quality of these waters, they nevertheless stood the test of time. This was partly because the operation of water systems was, and still is, based on on-line conductivity measurements and specifications generally thought to preclude the failure of these archaic chemistry attribute tests.

In 1996, USP moved away from these chemical attribute tests, switching to contemporary analytical technologies for the bulk waters *Purified Water* and *Water for Injection*. The intent was to upgrade the analytical technologies without tightening the quality requirements. The two contemporary analytical technologies employed were TOC and conductivity. The TOC test replaced the test for *Oxidizable Substances* that primarily targeted organic contaminants. A multi-staged *Conductivity* test that detects ionic (mostly inorganic) contaminants replaced, with the exception of the test for *Heavy Metals*, all of the inorganic chemical tests (i.e., *Ammonia*, *Calcium*, *Carbon Dioxide*, *Chloride*, *Sulfate*).

Replacing the heavy metals attribute was considered unnecessary because 1) the source water specifications (found in the U.S. EPA's NPDWR) for individual heavy metals were tighter than the approximate limit of detection of the *Heavy Metals* test for *USP XXII Water for Injection* and *Purified Water* (approximately 0.1 ppm), 2) contemporary water system construction materials do not leach heavy metal contaminants, and 3) test results for this attribute have uniformly been negative; there has not been a confirmed occurrence of a singular test failure (failure of only the *Heavy Metals* test with all other attributes passing) since the current heavy metal drinking water standards have been in place.

Total Solids and *pH* were the only tests not covered by conductivity testing. The test for *Total Solids* was considered redundant because the nonselective tests of conductivity and TOC could detect most chemical species other than silica, which could remain undetected in its colloidal form. Colloidal silica in *Purified Water* and *Water for Injection* is easily removed by most water pretreatment steps, and even if present in the water, it constitutes no medical or functional hazard except in extreme and rare situations. In such extreme situations, other attribute extremes are also likely to be detected. It is, however, the user's responsibility to ensure fitness for use. If silica is a significant component in the source water, and the purification unit operations could fail and selectively allow silica to be released into the finished water (in the absence of co-contaminants detectable by conductivity), then either silica-specific testing or a total-solids type testing should be utilized to monitor for and control this rare problem.

The *pH* attribute was eventually recognized to be redundant to the conductivity test (which included *pH* as an aspect of the test and specification); therefore, *pH* was discontinued as a separate attribute test.

The rationale used by USP to establish its *Purified Water* and *Water for Injection* conductivity specifications took into consideration the conductivity contributed by the two least-conductive former attributes of *Chloride* and *Ammonia*, thereby precluding their failure had those wet chemistry tests been performed. In essence, the *Stage 3* conductivity specifications (see *Water Conductivity* (645), *Bulk Water*) were established from the sum of the conductivities of the limit concentrations of chloride ions (from pH 5.0 to 6.2) and ammonia ions (from pH 6.3 to 7.0), plus the unavoidable contribution of other conductivity-contributing ions from water (H^+ and OH^-), dissolved atmospheric carbon dioxide (as HCO_3^-), and an electro-balancing quantity of either sodium (Na^+) or chlorine (Cl^-), depending on the pH-induced ionic imbalance (see *Table 1*). The *Stage 2* conductivity specification is the lowest value in this table, 2.1 $\mu S/cm$. The *Stage 1* specifications, designed primarily for on-line measurements, were derived by essentially summing the lowest values in the contributing ion columns for each of a series of tables similar to *Table 1*, created for each 5° increment between 0° and 100°. For example purposes, the italicized values in *Table 1*, the conductivity data table for 25°, were summed to yield a conservative value of 1.3 $\mu S/cm$, the *Stage 1* specification for a nontemperature-compensated, nonatmosphere-equilibrated water sample that actually had a measured temperature of 25°–29°. Each 5° increment in the table was similarly treated to yield the individual values listed in the table of *Stage 1* specifications (see *Water Conductivity* (645), *Bulk Water*).

Table 1. Contributing Ion Conductivities of the Chloride-Ammonia Model as a Function of pH
(in atmosphere-equilibrated water at 25°)

pH	Conductivity ($\mu S/cm$)						Combined Conductivities	Stage 3 Limit
	H^+	OH^-	HCO_3^-	Cl^-	Na^+	NH_4^+		
5.0	3.49	0	0.02	1.01	0.19	0	4.71	4.7
5.1	2.77	0	0.02	1.01	0.29	0	4.09	4.1
5.2	2.20	0	0.03	1.01	0.38	0	3.62	3.6
5.3	1.75	0	0.04	1.01	0.46	0	3.26	3.3
5.4	1.39	0	0.05	1.01	0.52	0	2.97	3.0
5.5	1.10	0	0.06	1.01	0.58	0	2.75	2.8
5.6	0.88	0	0.08	1.01	0.63	0	2.60	2.6
5.7	0.70	0	0.10	1.01	0.68	0	2.49	2.5
5.8	0.55	0	0.12	1.01	0.73	0	2.41	2.4
5.9	0.44	0	0.16	1.01	0.78	0	2.39	2.4
6.0	0.35	0	0.20	1.01	0.84	0	2.40	2.4
6.1	0.28	0	0.25	1.01	0.90	0	2.44	2.4
6.2	0.22	0	0.31	1.01	0.99	0	2.53	2.5
6.3	0.18	0	0.39	0.63	0	1.22	2.42	2.4
6.4	0.14	0.01	0.49	0.45	0	1.22	2.31	2.3
6.5	0.11	0.01	0.62	0.22	0	1.22	2.18	2.2
6.6	0.09	0.01	0.78	0	0.04	1.22	2.14	2.1
6.7	0.07	0.01	0.99	0	0.27	1.22	2.56	2.6
6.8	0.06	0.01	1.24	0	0.56	1.22	3.09	3.1
6.9	0.04	0.02	1.56	0	0.93	1.22	3.77	3.8
7.0	0.03	0.02	1.97	0	1.39	1.22	4.63	4.6

As stated above, this rather radical change to utilizing a conductivity attribute as well as the

inclusion of a TOC attribute allowed for on-line measurements. This was a major philosophical change and allowed industry to realize substantial savings. The TOC and conductivity tests can also be performed off-line in the laboratories using collected samples, although sample collection tends to introduce opportunities for adventitious contamination that can cause false high readings. The collection of on-line data is not, however, without challenges. The continuous readings tend to create voluminous amounts of data, where previously only a single data point was available. As stated under 6. *Sampling Considerations*, continuous in-process data are excellent for understanding how a water system performs during all of its various usage and maintenance events in real time, but this is too much data for QC purposes. Therefore, for example, one can use a justifiable portion of the data (at a designated daily time or at the time of batch manufacturing) or the highest value in a given period as a worst case representation of the overall water quality for that period. Data averaging is generally discouraged because of its ability to obscure short-lived extreme quality events.

7.2 Chemical Tests for Sterile Waters

Packaged/sterile waters present a particular dilemma relative to the attributes of conductivity and TOC. The package itself is the major source of chemicals (inorganics and organics) that leach over time into the packaged water and can easily be detected by the conductivity and TOC tests. The irony of organic leaching from plastic packaging is that before the advent of bulk water TOC testing, when the *Oxidizable Substances* test was the only "organic purity" test for both bulk and packaged/sterile water monographs in *USP*, the insensitivity of that test to many of the organic leachables from plastic and elastomeric packaging materials was largely unrecognized, allowing organic levels in packaged/sterile water to be quite high (possibly many times the TOC specification for bulk water).

Similarly, glass containers can also leach inorganics, such as sodium, which are easily detected by conductivity but poorly detected by the former wet chemistry attribute tests. Most of these leachables are considered harmless based on current perceptions and standards at the rather significant concentrations present. Nevertheless, they effectively degrade the quality of the high-purity waters placed into these packaging systems. Some packaging materials contain more leachables than others and may not be as suitable for holding water and maintaining its purity.

The attributes of conductivity and TOC tend to reveal more about the packaging leachables than they do about the water's original purity. These currently "allowed" leachables could render the sterile packaged versions of originally equivalent bulk water essentially unsuitable for many uses where the bulk waters are perfectly adequate.

Therefore, to better control the ionic packaging leachables, (645) is divided into two sections. The first is titled *Bulk Water*, which applies to *Purified Water*, *Water for Injection*, *Water for Hemodialysis*, and *Pure Steam*, and includes the three-stage conductivity testing instructions and specifications. The second section is titled *Sterile Water*, which applies to *Sterile Purified Water*, *Sterile Water for Injection*, *Sterile Water for Inhalation*, and *Sterile Water for Irrigation*. The *Sterile Water* section includes conductivity specifications similar to the *Stage 2* testing approach because it is intended as a laboratory test, and these sterile waters were made from bulk water that already complied with the three-stage conductivity test. In essence, packaging leachables are the primary target analytes of the conductivity specifications in the *Sterile Water* section of (645). The effect on potential leachables from different container sizes is the rationale for having two different specifications, one for small packages containing nominal

volumes of 10 mL or less and another for larger packages. These conductivity specifications are harmonized with the *European Pharmacopoeia* conductivity specifications for Sterile Water for Injection. All monographed waters, except *Bacteriostatic Water for Injection*, have a conductivity specification that directs the user to either the *Bulk Water* or the *Sterile Water* section of (645). For the sterile packaged water monographs, this water conductivity specification replaces the redundant wet chemistry limit tests intended for inorganic contaminants that had previously been specified in these monographs.

Controlling the organic purity of these sterile packaged waters, particularly those in plastic packaging, is more challenging. Although the TOC test can better detect these impurities and therefore can be better used to monitor and control these impurities than the current *Oxidizable Substances* test, the latter has a history of use for many decades and has the flexibility to test a variety of packaging types and volumes that are applicable to these sterile packaged waters. Nevertheless, TOC testing of these currently allowed sterile, plastic-packaged waters reveals substantial levels of plastic-derived organic leachables that render the water perhaps orders of magnitude less organically pure than is typically achieved with bulk waters. Therefore, usage of these packaged waters for analytical, manufacturing, and cleaning applications should only be exercised after the purity of the water for the application has been confirmed as suitable.

8. MICROBIAL EVALUATIONS

This section of the chapter presents a discussion about the types and sources of microorganisms and whether certain microbes are prone to colonize pharmaceutical water systems. This section also addresses microbiological examination of water samples, including a discussion on recovery methods.

8.1 Microorganism Types

Microorganisms are ubiquitous and their natural habitats are extremely diverse. Based on comparative ribosomal RNA sequencing, the phylogenetic tree of life consists of three domains: Bacteria and Archaea (both prokaryotes), and Eukarya (eukaryotes). Most microorganisms that contaminate pharmaceutical products are prokaryotic bacteria and eukaryotic fungi (yeasts and molds). These microbes are typical isolates from pharmaceutical environments, including the associated personnel, and a few are frank or opportunistic pathogens. Contamination with viruses is a concern in bioprocessing that uses animal cells.

ARCHAEANS

Microbes from the domain Archaea are phylogenetically related to prokaryotes but are distinct from bacteria. Many are extremophiles, with some species capable of growing at very high temperatures (hyperthermophiles) or in other extreme environments beyond the tolerance of any other life form. In general, most extremophiles are anaerobic or microaerophilic chemolithoautotrophs. Because of their unique habitats, metabolism, and nutritional requirements, Archaeans are not known to be frank or opportunistic pathogens, and they are not capable of colonizing a pharmaceutical water system.

BACTERIA

Bacteria are of immense importance because of their rapid growth, mutation rates, and ability to exist under diverse and adverse conditions. Some are very small and can pass through

0.2- μm rated filters. Others form spores, which are not part of their reproductive cycle. Bacterial spore formation is a complex developmental process that allows the organisms to produce a dormant and highly resistant cell in times of extreme stress. Bacterial endospores can survive high temperatures, strong UV irradiation, desiccation, chemical damage, and enzymatic destruction, which would normally kill vegetative bacteria.

Using a traditional cellular staining technique based on cell wall compositional differences, bacteria are categorized into Gram positive and Gram negative, although many sub-groups exist within each category based on genomic similarities and differences.

8.1.2.1 Gram positives: Gram-positive microbes are common in a pharmaceutical manufacturing environment but not in water systems. This is because Gram positives are generally not suited to surviving in a liquid environment that has the chemical purity of a pharmaceutical-grade water system. Gram positives include the spore-forming bacteria from the genus *Bacillus*, which are common soil and dust contaminants, and the non-sporulating bacteria from the genera *Staphylococcus*, *Streptococcus*, and *Micrococcus*, which normally colonize human skin and mucous membranes. Other types of Gram-positive bacterial contaminants include organisms from the genera *Corynebacterium*, *Mycobacterium*, *Arthrobacter*, *Propionibacterium*, *Streptomyces*, and *Actinomyces*. This latter group of microbes can be found in various natural habitats including the human skin and soil.

Although Gram-positive bacteria can be detected in pharmaceutical water samples, their recovery is often associated with faulty aseptic technique during sampling or testing, or associated with exogenous contamination sources. Although these non-aquatic microorganisms could be present in source water and could, in rare circumstances, make their way into the early stages of a water purification unit operation, Gram-positive bacteria are not known to colonize water systems. In addition, these microbes will likely be removed by one or more of the purification unit operations prior to the ultimate creation of the pharmaceutical-grade water.

8.1.2.2 Gram negatives: These types of bacteria are found in soil, water, plants, and animals. Gram-negative bacteria are of keen interest to pharmaceutical manufacturers, primarily due to their production of endotoxins, a topic discussed in 8.4 *Endotoxin*. Some Gram-negative bacteria prefer aquatic habitats and tend to colonize water systems and other wet environments as biofilms, a topic discussed in 8.2 *Biofilm Formation in Water Systems*.

8.1.2.3 Mycoplasma: Organisms from the genus *Mycoplasma* are the smallest of the bacteria. Unlike other bacteria, these organisms do not have a cell wall and many exist as intracellular or animal parasites. Mycoplasmas also require specific nutrients for survival, including cholesterol, and they cannot survive in a hypotonic environment such as pure water. Based on these facts, this type of bacteria is not a concern for pharmaceutical-grade water systems.

FUNGI

Fungi are mainly aerobic mesophilic microbes. They exist as unicellular (yeast) and multicellular filamentous (mold) organisms. Molds are often found in wet/moist but usually non-aquatic environments, such as soil and decaying vegetation. Examples of common environmental molds include those from the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, and *Alternaria*. As mold matures it develops spores, which, unlike bacterial spores, are part of their reproductive cycle and are less resistant to adverse conditions. Mold spores are easily spread through air and materials, and could contaminate grab water samples.

Yeasts are often associated with humans and vegetation. Examples of yeasts commonly found in pharmaceutical environments include the genera *Candida*, *Saccharomyces*, and *Rhodotorula*.

Some molds and yeasts are considered human pathogens and a few mold species produce mycotoxins. Neither yeasts nor molds are suited for colonization or survival in pharmaceutical water systems. Their recovery is often associated with faulty aseptic technique during sampling or testing, or associated with exogenous contamination sources. These non-aquatic microorganisms, if present in source water, could make their way into the early stages of a water purification system; however, they will likely be removed by one or more of the purification unit operations.

VIRUSES

A virus is a small infectious agent unlike eukaryotes and prokaryotes. This is because viruses have no metabolic abilities of their own. Viruses are genetic elements containing either DNA or RNA that replicate within host cells. Human pathogenic viruses, especially those of fecal origin, could be present in source water. However, they are easily neutralized by typical water purification treatments, such as chlorination. Therefore, it is unlikely that viruses will be present or will proliferate (due to the absence of host cells) in pharmaceutical-grade waters.

THERMOPHILES

Thermophiles are heat-loving organisms and can be either bacteria or molds. Thermophilic and hyperthermophilic aquatic microorganisms (see 8.1.1 *Archaeans*) require unique environmental and nutritional conditions to survive. These conditions do not exist in the high-purity water of pharmaceutical water systems, whether ambient or hot, to support their growth. Bacteria that are able to inhabit hot pharmaceutical water systems are invariably found in much cooler locations within these hot systems; for example, within infrequently used outlets, ambient subloops off of hot loops, use-point and sub-loop cooling heat exchangers, transfer hoses and connecting pipes, or dead legs. These bacterial contaminants are the same mesophilic (moderate temperature-loving) types found in ambient water systems and are not thermophiles. Based on these facts, thermophilic bacteria are not a concern for hot pharmaceutical-grade water systems.

8.2 Biofilm Formation in Water Systems

A biofilm is a three-dimensional structured community of sessile microbial cells embedded in a matrix of extracellular polymeric substances (EPS). Biofilms form when bacteria attach to surfaces in moist environments and produce a slimy, glue-like substance, the EPS matrix, while proliferating at that location. This slimy matrix facilitates biofilm adhesion to surfaces as well as the attachment of additional planktonic cells to form a microbial community.

The EPS matrix of biofilms that colonize water systems also facilitates adsorption and concentration of nutrients from the water and retains the metabolites and waste products produced by the embedded biofilm cells, which can serve as nutrients for other biofilm community members.

This EPS matrix is also largely responsible for biofilm's resistance to chemical sanitizers, which must penetrate completely through the matrix to contact and kill the biofilm cells within the matrix. Heat sanitization approaches do not generally have these EPS matrix penetration difficulties, so they are usually considered superior to chemicals in killing biofilms where materials of construction allow.

The three-dimensional structure of a well-developed biofilm, as well as the biofilm's creation and release of small, motile "pioneer cells" for further colonization, are facilitated through gene expression modulating "quorum sensing" chemicals released in tiny amounts by individual biofilm cells and concentrated to a functional level within this same EPS matrix. So, the EPS matrix of biofilms is primarily responsible for the biofilm's success in colonizing and proliferating in very low nutrient-containing high-purity water systems. The EPS matrix also explains the difficulty in killing and/or removing biofilms from water purification and distribution system surfaces.

BIOFILM-FORMING BACTERIA IN WATER SYSTEMS

Common microorganisms recovered from water system samples include Gram-negative bacteria from the genera *Pseudomonas*, *Ralstonia*, *Burkholderia*, *Stenotrophomonas*, *Comamonas*, *Methylobacterium*, and many other types of *Pseudomonas*-like organisms known collectively as pseudomonads. These types of microbes, found in soil and source water, tend to colonize all water system distribution and purification system surfaces including activated carbon beds, deionizing resin beds, RO systems, membrane filtration modules, connecting piping, hoses, and valves. If not controlled, they can compromise the functionality of purification steps in the system and spread downstream, possibly forming biofilms on the distribution system surfaces such as tanks, piping, valves, hoses, and other surfaces, from where they can be sheared or otherwise released into the finished water used in processes and products.

Some of the biofilm pseudomonads are opportunistic human pathogens and may possess resistance to commonly used pharmaceutical product preservatives, particularly when imbedded in EPS matrix flocs sheared from water system biofilms. Several pseudomonads are also capable of utilizing a wide variety of carbon sources as nutrients, allowing them to colonize austere, adventitious nutrient environments such as water systems. This nutritional diversity also makes them capable of growing to very high numbers in some pharmaceutical products and raw materials, thus leading to product adulteration and potential risk to patient health. Given that these bacteria are commonly found in aqueous environments, endotoxin control for Water for Injection systems (and some Purified Water systems) through biofilm control becomes critical.

NON-BIOFILM-FORMING BACTERIA IN WATER SYSTEMS

Other types of non-pseudomonad Gram-negative bacteria are generally non-aquatic by nature. They include coliforms from the genera *Escherichia*, *Salmonella*, *Shigella*, *Serratia*, *Proteus*, *Enterobacter*, and *Klebsiella*, which are used as indicators of fecal contamination. Although some of these bacteria can be human enteric pathogens, these non-pseudomonads are not suited to colonizing or surviving in pharmaceutical water systems owing to the water's chemical purity. In fact, non-pseudomonad enteric bacteria are extremely unlikely contaminants of pharmaceutical water systems unless local sewage and source water controls are not in place. Such controls are required in order to comply with the source water requirements for making USP-grade waters as described in their respective monographs.

8.3 Microorganism Sources

EXOGENOUS CONTAMINATION

Exogenous microbial contamination of bulk pharmaceutical water comes from numerous possible sources, including source water. At a minimum, source water should meet the microbial quality attributes of Drinking Water, which is the absence of fecal coliforms (*E. coli*). A wide variety of other types of microorganisms, chiefly Gram-negative bacteria, may be present in the incoming

water. If appropriate steps are not taken to reduce their numbers or eliminate them, these microorganisms may compromise subsequent water purification steps.

Exogenous microbial contamination can also arise from maintenance operations, equipment design, and the process of monitoring, including:

- Unprotected, faulty, or absent vent filters or rupture disks
- Backflow from interconnected equipment
- Non-sanitized distribution system openings for component replacements, inspections, repairs, and expansions
- Inadequate drain air-breaks
- Innate bioburden of activated carbon, ion-exchange resins, regenerant chemicals, and chlorine-neutralizing chemicals
- Inappropriate rinsing water quality after regeneration or sanitization
- Poor sanitization of use points, hard-piped equipment connectors, and other water transfer devices such as hoses
- Deficient techniques for use, sampling, and operation

The exogenous contaminants may not be normal aquatic bacteria but rather microorganisms of soil, air, or even human origin. The detection of non-aquatic microorganisms may be an indication of sampling or testing contamination or a system component failure, which should trigger investigation and remediation. Sufficient care should be given to sampling, testing, system design, and maintenance to minimize microbial contamination from exogenous sources.

ENDOGENOUS CONTAMINATION

Endogenous sources of microbial contamination can arise from unit operations in a water purification system that is not properly maintained and operated. Microorganisms present in source water may adsorb to carbon bed media, ion-exchange resins, filter membranes, and other equipment surfaces, and initiate the formation of biofilms.

Downstream colonization can occur when microorganisms are shed from existing biofilm-colonized surfaces and carried to other areas of the water system. Microorganisms may also attach to suspended particles such as carbon bed fines or fractured resin particles. When the microorganisms become planktonic, they serve as a source of contamination to subsequent purification equipment and to distribution systems.

Another source of endogenous microbial contamination is the distribution system itself. Microorganisms can colonize pipe surfaces, rough welds, misaligned flanges, valves, and dead legs, where they proliferate and form biofilms. Once formed, biofilms can become a continuous source of microbial contamination, which is very difficult to eradicate. Therefore, biofilm development must be managed by methods such as frequent cleaning and sanitization, as well as process and equipment design.

8.4 Endotoxin

Endotoxins are lipopolysaccharides found in and shed from the cell envelope that is external to the cell wall of Gram-negative bacteria. Some grades of pharmaceutical waters, such as those used in parenteral applications (e.g., *Water for Injection*, *Water for Hemodialysis*, and the sterilized packaged waters made from *Water for Injection*) strictly limit the amount of endotoxins that may be present because these compounds are pyrogenic.

SOURCES

Endotoxins may occur as collections of lipopolysaccharide molecules associated with living microorganisms, fragments of dead microorganisms, the EPS matrix surrounding biofilm bacteria, or free molecular clusters or micelles containing many lipopolysaccharide molecules. The monomeric form of the endotoxin molecule does not exist in high-purity water because of its amphiphilic nature. Endotoxins may be introduced into the system from the source water or may be released from cell surfaces of bacteria that colonize the water system. For example, a spike in endotoxin may occur following sanitization as a result of endotoxin release from killed cells. Endotoxin quantitation in water samples is not a good indicator of the level of biofilm development in a water system because of the multiplicity of endotoxin sources.

REMOVAL AND CONTROL

To control endotoxin levels in water systems, it is important to control all potential sources of contamination with Gram-negative bacteria as well as free endotoxin in the water. Contamination control includes the use of upstream unit operations to reduce bioburden from incoming water, as well as engineering controls (e.g., heat sanitization, equipment design, UV sanitizers, filters, material surface, and flow velocity) to minimize biofilm development on piping surfaces and to reduce re-inoculation of the system with free-floating bacteria.

Endotoxin remediation may be accomplished through the normal exclusion or removal action afforded by various unit operations within the treatment system. Examples of endotoxin removal steps in a water purification train include RO, deionization, ultrafilters, and endotoxin-adsorptive filters.

8.5 Test Methods

Microbes in water systems can be detected as exemplified in this section or by methods adapted from *Microbial Enumeration Tests* (61), *Tests for Specified Microorganisms* (62), or the current edition of *Standard Methods for the Examination of Water and Wastewater* by the American Public Health Association. This section describes classical culture approaches to bioburden testing, with a brief discussion on rapid microbiological methods.

Every water system has a unique microbiome. It is the user's responsibility to perform method validation studies to demonstrate the suitability of the chosen test media and incubation conditions for bioburden recovery. In general, users should select the method that recovers the highest planktonic microbial counts in the shortest time, thus allowing for timely investigations and remediation. Such studies are usually performed before or during system validation. In addition, the chosen method should be reassessed periodically, as the microbiome of a new water system gradually establishes a steady state relative to the system's routine maintenance, use, and sanitization procedures.

The steady state condition can take months or even years to be achieved, and can be affected by a change in source water quality, changes in finished water purity by using modified or increasingly inefficient purification processes, changes in finished water use patterns and volumes, changes in routine and preventative maintenance or sanitization procedures and frequencies, or any type of system intrusion (e.g., component replacement, removal, or addition).

MICROBIAL ENUMERATION CONSIDERATIONS

Most microbial contaminants in water systems are found primarily as biofilms on surfaces, with only a very small percentage of the microbiome suspended in the water, or planktonic, at any

given time. Although it would seem logical to directly monitor biofilm development on surfaces, current technology for surface evaluations in an operating water system makes this impractical in a GMP environment. Therefore, an indirect approach must be used: the detection and enumeration of planktonic microorganisms that have been released from biofilms. This planktonic microbiome will impact the processes or products where the water is used.

The detection and enumeration of the planktonic microbiome can be accomplished by collecting samples from water system outlets. Planktonic organisms are associated with the presence of biofilms as well as free-floating bacteria introduced into the system (pioneer cells), which may eventually form new biofilms. Therefore, by enumerating the microorganisms in water grab samples, the overall state of control over biofilm development can be assessed. This assessment has historically been accomplished with classical cultural techniques, which are viewed as the traditional method. However, nutritional limitations of the growth media may not satisfy growth requirements of organisms present in the water system that originated from a biofilm. As a result, traditional cultural methods may only detect a fraction of the biofilm bacteria present in the water sample. Other options are available, such as rapid microbiological methods.

There is no ideal cultural enumeration method that will detect all microorganisms in a water sample, although some media or incubation temperatures may be better than others. However, from a PC perspective, this limitation is acceptable because it is the relative changes in the trends for water sample microbial counts that indicate the state of PC.

THE CLASSICAL CULTURAL APPROACH

Classical cultural approaches for microbial testing of water include but are not limited to pour plates, spread plates, membrane filtration, and most probable number (MPN) tests. These methods are generally easy to perform, and provide excellent sample processing throughput. Method sensitivity can be increased via the use of larger sample sizes. This strategy is used in the membrane filtration method. Cultural approaches are further defined by the type of medium used in combination with the incubation temperature and duration. This combination should be selected according to the monitoring needs of a specific water system and its ability to recover the microorganisms of interest, i.e., those that could have a detrimental effect on the products manufactured or process uses, as well as those that reflect the microbial control status of the system.

8.5.2.1 Growth media: The traditional categorization is that there are two basic forms of media available: "high nutrient" and "low nutrient". Those media traditionally categorized as high-nutrient include Plate Count Agar (TGYA), Soybean Casein Digest Agar (SCDA or TSA), and m-HPC Agar (formerly m-SPC Agar). These media are intended for the general isolation and enumeration of heterotrophic or copiotrophic bacteria. Low-nutrient media, such as R2A Agar and NWRI Agar (HPCA), have a larger variety of nutrients than the high-nutrient media. These low-nutrient media were developed for use with potable water due their ability to recover a more nutritionally diverse population of microorganisms found in these environments. The use of R2A may not be the best choice for high-purity water systems. Even though high-purity water creates an oligotrophic environment, it has been shown empirically that in many high-purity compendial waters, the microbial count disparity between low- and high-nutrient media is dramatically less to nil, compared to potable water. Nevertheless, using the medium that has been demonstrated, through validation studies, to be the most optimal for the microbiome in a particular water system is essential.

8.5.2.2 Incubation conditions: Duration and temperature of incubation are also critical aspects of microbiological testing. Classical compendial methods (e.g., (61)) specify the use of high-nutrient media, typically incubated at 30°–35° for NLT 48 h. Given the types of microbes found in many water systems, incubation at lower temperatures (e.g., ranges of 20°–25° or 25°–30°) for longer periods (at least 4 days) could recover higher microbial counts than classical compendial methods. Low-nutrient media typically require longer incubation conditions (at least 5 days) because the lower nutrient concentrations promote slower growth. Even high-nutrient media can sometimes yield higher microbial recovery with longer and cooler incubation conditions. The decision to test a particular system using high- or low-nutrient media, higher or lower incubation temperatures, and shorter or longer incubation times should be based on comparative cultivation studies using the native microbiome of the water system. The decision to use media requiring longer incubation periods to recover higher counts should be also be balanced with the timeliness of results. Detection of marginally higher counts at the expense of a significantly longer incubation period may not be the best approach for monitoring water systems. Some cultural conditions using low-nutrient media lead to the development of microbial colonies that are much less differentiated in colonial appearance, an attribute that microbiologists rely on when selecting representative microbial types for further characterization. The nature of some of the slow growers and the extended incubation times needed for their development into visible colonies may also lead to those colonies becoming dysgonic and difficult to subculture. This could limit their further characterization, depending on the microbial identification technology used.

SUGGESTED CLASSICAL CULTURAL METHODS

The example methods presented in *Table 2* are optional and may or may not be optimal for recovering microorganisms, including those considered objectionable, or biofilms. Users should determine through experimentation which methods are best for in-process and QC monitoring, as well as for recovering specific microorganisms that could be found in the water system and might be objectionable for the intended water use.

Table 2. Example Culture Methods

<i>Drinking Water:</i>	Pour Plate Method or Membrane Filtration Method ^a
	Suggested Sample Volume—1.0 mL ^b
	Growth Medium—Plate Count Agar ^c
	Incubation Time—48-72 h ^d
	Incubation Temperature—30°-35° ^e
<i>Purified Water:</i>	Pour Plate Method or Membrane Filtration Method ^b
	Suggested Sample Volume—1.0 mL for pour plate or 100 mL for membrane filtration ^b
	Growth Medium—Plate Count Agar ^c
	Incubation Time—48-72 h ^d
	Incubation Temperature—30°-35° ^e
<i>Water for Injection:</i>	Membrane Filtration Method ^a
	Suggested Sample Volume—200 mL ^b
	Growth Medium—Plate Count Agar ^c
	Incubation Time—48-72 h ^d
	Incubation Temperature—30°-35° ^e
<p>^a A membrane filter with a rating of 0.45 µm is generally considered preferable to smaller porosity membranes.</p> <p>^b Sample size must be appropriate for the expected microbial count of the water in order to derive statistically valid colony counts.</p> <p>^c For optimum recovery, an alternative medium may be more appropriate (e.g., m-HPC, TSA/SCDA, R2A).</p> <p>^d For optimum recovery, alternative incubation times may be needed.</p> <p>^e For optimum recovery, alternative incubation temperatures may be needed.</p>	

For media growth promotion, use at a minimum *Pseudomonas aeruginosa* ATCC 9027 and *Bacillus subtilis* ATCC 6633. Additional organisms should be used to represent those that are considered objectionable and/or typically isolated from the water system (house isolates).

MICROBIAL IDENTIFICATION

In addition to the enumeration of the bioburden in the water, there is a need to identify and/or select for certain microbial species that could be detrimental to products or processes. Some bacteria may also be resistant to preservatives and other antimicrobial chemicals used in liquid and semi-solid products, thus leading to potential product spoilage. For example, *Pseudomonas aeruginosa* and *Burkholderia cepacia*, as well as some other pseudomonads, are known opportunistic pathogens. As such, it may be appropriate to consider these species as objectionable microorganisms for the type of water used to manufacture liquid and semi-solid products. There is a higher risk of infection if these organisms are found in products targeted for susceptible patient populations (e.g., the very young, the very old, and the immunocompromised) or products contacting highly susceptible tissues (e.g., inhaled products or some topical products). However, if the product where the water is used carries an absence

specification for a particular pathogenic species that is not capable of living in a high-purity water system, then these non-aquatic species should not be candidates for routine water testing.

For PC, it is valuable to know the microbial species present in the normal microbiome of a water system, even if they are not specifically objectionable. If a new species is detected, it may be an indication of a subtle process change or an exogenous intrusion. The identity of the microorganism may be a clue as to its origin and can help with implementation of corrective or preventive action. Therefore, it is industry practice to identify the microorganisms in samples that yield results exceeding established Alert and Action Levels. It may also be of value to periodically identify the normal microbiome in a water system, even if counts are below established Alert Levels. This information can provide perspective on the species recoveries from Alert and Action Level excursion samples, indicating whether they are new species or just higher levels of the normal microbiome. Water system isolates are often incorporated into a company culture collection for use in tests such as preservative challenge tests, microbial method validation/suitability testing, and media growth promotion.

RAPID MICROBIOLOGICAL METHODS

In recent years, new technologies that enhance microbial detection and the timeliness of test results have been adopted by pharmaceutical QC testing labs. Rapid Microbiological Methods (RMM) are divided into four categories: Growth-Based, Viability-Based, Artifact-Based, and Nucleic Acid-Based. Examples of RMM used for the evaluation of microbial quality of water systems include:

- Microscopic visual epifluorescence membrane counting techniques
- Automated laser scanning membrane counting approaches
- Early colony detection methods based on autofluorescence, ATP, bioluminescence, or vital staining
- Genetic-based detection/quantitation

Some of these methods provide greater precision, accuracy, and speed of microbial detection, compared to classical cultural approaches. These advantages are counterbalanced by limited sample processing throughput due to larger sample requirements and/or labor-intensive sample processing, or other instrument and sensitivity limitations.

In addition, some of these RMM kill the microbial cells to detect their presence. This precludes subsequent characterization, or identification, which are often necessary for system control and QC testing. Consequently, culturing approaches have traditionally been preferred over some of the rapid microbiological technologies because cultures offer a balance of desirable test attributes and post-test capabilities.

9. ALERT AND ACTION LEVELS AND SPECIFICATIONS

9.1 Introduction

Establishment of Alert and Action Levels for any manufacturing process facilitates appropriate and timely control. In the case of a pharmaceutical water system, the key PC parameters can be specific chemical, physical, and microbiological attributes of the water produced. Typically, most chemical attributes can be determined in real time or in the lab within a few min after sample collection. Physical attributes such as the pressure drop across a filter, temperature,

and flow rate—which are sometimes considered critical for operation or sanitization of the water system—must be measured *in situ* during operation. Obtaining timely microbial data is more challenging compared to chemical and physical attributes, often taking several days. This limits the ability to control microbial attributes in a timely manner, and therefore requires a more challenging evaluation of the test results and conservative implementation of PC levels. This section provides guidance on the establishment and use of Alert and Action Levels, as well as Specifications to assess the suitability of the water and the water system for use in production.

9.2 Examples of Critical Parameter Measurements

Examples of measurements and parameters that are important to water system processes and products are described below. The list, which is not intended to be exhaustive or required, contains some examples of parameters that could be measured to demonstrate that the system is in a state of control.

Examples of measurements that could be critical to the purification or sanitization process include:

- Temperature, for thermally sanitized systems
- % Rejection of an RO system
- Endotoxin levels of feed water to a distillation system
- Chlorine presence immediately prior to an RO system

Examples of measurements that could be critical to the water distribution process include:

- Return/end-of-loop line pressure, to forewarn of a negative pressure from simultaneous use of too many outlets
- Beginning and end-of-loop flow rate, to forewarn of an excessively high flow rate at the UV lamps if too many outlets are open
- Flow rate, to ensure that sufficient water is available for operations

Examples of measurements that could be critical to final water quality include:

- Conductivity
- TOC
- Endotoxin—for Water for Injection systems
- Particulate matter
- Bioburden
- Ozone or other chemicals—for chemically sanitized systems

9.3 Purpose of the Measurements

Although the purpose of each measurement varies, the results can be used to provide system performance feedback, often immediately, serving as ongoing PC and product quality indicators. At the same time, the results provide information necessary for making decisions regarding the immediate processing and usability of the water (see 6.1 *Purposes and Procedures*). However, some attributes may not be monitored continuously or may have a long delay in data availability (e.g., microbial data). Regardless, both real-time data and data with longer cycle times can be used to properly establish Alert and Action Levels, which can serve as an early warning or indication of a potentially approaching quality shift.

As PC indicators, Alert and Action Levels are trigger points for the potential need for investigation and/or remedial action, to prevent a system from deviating from normal conditions

and producing water unsuitable for its intended use. This "intended use" minimum quality is sometimes referred to as a "Specification" or "Limit", and may include limits for conductivity and TOC listed in water monographs, or other specifications required for these waters that have been defined by the user internally.

In all cases, the validity of the data should be verified to ensure that the data are accurate and consistently representative of the water quality in the system, regardless of whether the sample was collected from a sampling port or use point. The resulting data must not be unduly biased, positively or negatively, due to the sampling method, the environment in the vicinity of the sampling location, the test procedure, instrumentation, or other artifacts that could obscure or misrepresent the true quality of the water intended by the purpose of the sampling, i.e., for PC or for QC.

9.4 Defining Alert and Action Levels and Specifications

Data generated from routine water system monitoring should be trended to ensure that the system operates in a state of chemical and microbiological control. To assist with the evaluation of system performance, companies should establish in-process control levels based on historical data or a fraction of the water Specifications (as long as this latter approach yields values with relevance to process performance).

When establishing Alert and Action Levels and Specifications, a two- or three-tier approach is typically used. In a three-tier approach, the typical structure is to establish in-process controls using *Alert Level*, *Action Level*, and *Specification*. Alert and Action Levels are used as proactive approaches to system management prior to exceeding Specifications. The criteria for defining and reacting to adverse trends should be set by the user. These levels should be set at values that allow companies to take action to prevent the system from producing water that is unfit for use. Water Specifications or Limits represent the suitability for use of the water.

In a two-tier approach, a combination of the above terminology is used, depending on the parameter to be monitored. For example, if the attribute does have a monograph specification, the two tiers are Alert Level (or Action Level) and Specification. If the attribute does not have a limit/specification, the two tiers are usually Alert Level and Action Level.

A single-tier approach is possible, but this is risky and difficult to manage. With this approach, where the water/system is either acceptable or not acceptable, the single-tier method does not allow for any adjustment, correction, or investigation prior to stopping production.

However, certain sampling locations, such as sampling ports that are not used for manufacturing products or processes, do not represent the finished water quality where a Specification could be applied. In these locations, a two-tier approach (Alert and Action Levels only) could be applied. In some sampling locations, a single PC level might possibly be appropriate, depending on the attribute.

ALERT LEVEL

An Alert Level for a measurement or parameter should be derived from the normal operating range of the water system. Specifically, Alert Levels are based on the historical operating performance under production conditions, and then are established at levels that are just beyond the majority of the normal historical data. The Alert Level for a parameter is often a single value or a range of values, such as:

- Higher than typical conductivity or TOC

- Higher than typical microbial count
- Higher than typical endotoxin level
- Low temperature during thermal sanitization
- pH range control prior to an RO
- Ozone concentration in a storage tank

Various methods, tools, and statistical approaches are available for establishing Alert Levels, and the user needs to determine the approaches that work for their application. Some numerical examples are two or three standard deviations Ω (or more) in excess of the mean value, or some percentage above the mean value but below a Specification. An event-based example could be the appearance of a new microorganism or a non-zero microbial count where zero is the norm.

When an Alert Level is exceeded, this indicates that a process or product may have drifted from its normal operating condition or range. Alert Level excursions represent a warning and do not necessarily require a corrective action. However, Alert Level excursions may warrant notification of personnel involved in water system operation, as well as the QA personnel. Alert Level excursions may also lead to additional monitoring, with more intense scrutiny of the resulting and neighboring data as well as other process indicators.

ACTION LEVEL

An Action Level is also based on the same historical data, but the levels are established at values (or ranges) that exceed the Alert Levels. The values/ranges are determined using the same types of numerical or event-based tools as the Alert Levels, but at different values.

In a three-tier approach, it is good practice to select an Action Level that is more than the Alert Level, but less than the Specification to allow the user to make corrective actions before the water would go out of compliance.

Exceeding a quantitative Action Level indicates that the process has allowed the product quality or other critical parameter to drift outside of its normal operating range. An Action Level can also be event-based. In addition to exceeding quantitative Action Levels, some examples of event-based Action Level excursions include, but are not limited to:

- Exceeding an Alert Level repeatedly
- Exceeding an Alert Level in multiple locations simultaneously
- The recovery of specific objectionable microorganisms
- A repeating non-zero microbial count where zero is the norm

If an Action Level is exceeded, this should prompt immediate notification of both QA staff and the personnel involved in water system operations and use, so that corrective actions can be taken to restore the system back to its normal operating range. Such remedial actions should also include investigative efforts to understand what happened and eliminate or reduce the probability of recurrence. Depending on the nature of the Action Level excursion, it may be necessary to evaluate its impact on the water uses during the period between the previous acceptable test result and the next acceptable test result.

SPECIAL ALERT AND ACTION LEVEL SITUATIONS

In new or significantly altered water systems, where there is limited or no historical data from which to derive trends, it is common to establish initial Alert and Action Levels based on equipment design capabilities. These initial levels should be within the process and product

Specifications where water is used. It is also common for new water systems, especially ambient water systems, to undergo changes, both chemically and microbiologically, over time as various unit operations (such as RO membranes) exhibit the effects of aging. This type of system aging effect is most common during the first year of use. As the system ages, a steady state microbial population (microorganism types and levels) may develop due to the collective effects of system design, source water, maintenance, and operation, including the frequency of re-bedding, backwashing, regeneration, and sanitization. This established or mature microbial population may be higher than the one detected when the water system was new. Therefore, there is cause for the impurity levels to increase over this maturation period and eventually stabilize.

Some water systems are so well controlled microbially—such as continuously or intermittently hot Water for Injection distribution systems—that microbial counts and endotoxin levels are essentially nil or below the limit of reasonable detectability. This common scenario often coincides with a very low Specification that is poorly quantifiable due to imprecision (as much as two-fold variability) of the test methods that may be near their limits of detection. In such systems, quantitative data trending has little value, and therefore, quantitative PC levels also have little value. The non-zero values in such systems could be due to sporadic sampling issues and not indicative of a water system PC deviation; however, if these non-zero values occur repeatedly, they could be indicative of process problems. So, an alternative approach for establishing Alert and Action Levels with these data could be the use of the incident rate of non-zero values, with the occasional single non-zero “hit” perhaps being an Alert Level (regardless of its quantitative value), and multiple or sequential “hits” being an Action Level. Depending on the attribute, perhaps single hits may not even warrant being considered an Alert Level, so only a multiple-hit situation would be considered actionable. It is up to the user to decide on their approach for system control, i.e., whether to use one, two, or three levels of controls for a given water system and sampling location, and whether to establish Alert and Action Levels as quantitative or qualitative hit-frequency values.

SPECIFICATIONS

Water Specifications or Limits are set based on direct potential product and/or process impact and they represent the suitability for use of the water. The various bulk water monographs contain tests for *Conductivity*, *TOC*, and *Bacterial Endotoxins* (for Water for Injection). Aside from the monographs for *Water for Hemodialysis* and multiple sterile waters, microbial specifications for the bulk waters are intentionally not included in their monograph tests. The need for microbial specifications for bulk waters (*Purified Water* and Water for Injection) depends on the water use(s), some of which may require strict control (e.g., very low bioburden, absence of objectionable organisms, or low ionic strength) while others may require no specification due to the lack of impact. For example, microbial specifications are appropriate and typically expected for water that is used in product formulations and final equipment rinses. However, where the water is used for analytical reagent preparation or for cleaning processes that conclude with a final antimicrobial heat drying or solvent rinsing step, the microbial quality of the water is likely less of a concern. The decision to establish microbial Specifications for bulk pharmaceutical waters should be based on a formal risk assessment of its uses and justified by scientific rationale.

It is very important to understand the chemical and microbial quality of the water in its final form as it is delivered from a water system to the locations where it is used in manufacturing activities and other points of use. The quality of the water within the water system could be

compromised if it picks up chemical or microbial contaminants during its delivery from the system to the points of use. These points of use, where cumulative contamination could be present, are the locations where compliance with all the water Specifications is mandated.

As discussed above, compliance with chemical Specifications can be confirmed periodically between uses, immediately prior to use, or even while the water is being utilized in product manufacturing. While the use of RMM may provide for timely microbial data, the use of conventional cultivative microbiological testing usually delays confirmation of microbial compliance until after the water has been used. However, for some applications, this logistical limitation should not eliminate the need for establishing microbial Specifications for this very important raw material.

The manufacturing risk imposed by these logistics accentuates the value of validated microbial control for a water system. It also emphasizes the value of unbiased microbial monitoring of samples collected from pertinent locations, with evaluation of the resulting data against well-chosen, preferably trend-derived Alert and Action Levels, which can facilitate remedial PC to preclude Specification excursions.

Users should establish their own quantitative microbial Specifications suited to their water uses. But these values should not be greater than 100 cfu/mL for *Purified Water* or 10 cfu/100 mL for *Water for Injection* unless specifically justified, because these values generally represent the highest microbial levels for pharmaceutical water that are still suitable for manufacturing use.

A Specification excursion should prompt an out-of-specification (OOS) investigation. The investigation is performed to determine 1) the root cause of the excursion so that CAPA may be taken for remediation purposes, and 2) assess the impact on affected processes and finished products where the water was used. Product disposition decisions must be made and are dependent on factors which could include:

- Role of water in the product or in-process material
- Chemical or microbial nature of the attribute whose Specification value was exceeded
- Level of product contamination by the water
- Presence of objectionable microorganisms
- Any downstream processing of affected in-process materials that could mitigate the OOS attribute
- Physical and chemical properties of the finished product where the water was used that could mitigate the OOS attribute
- Product administration routes and potentially sensitive/susceptible users

SOURCE WATER CONTROL

The chemical and microbial attributes of the starting source water are important to the ability of the water system to remove or reduce these impurities to meet the finished water Specifications (see 2. *Source Water Considerations*). Using the example microbial enumeration methods in *Table 2*, a reasonable maximum bacterial Action Level for source water is 500 cfu/mL. This number is derived from U.S. EPA NPDWR where it is used as an Action Level for the water authority indicating the need for improving disinfection and water filtration to avoid the penetration of viral, bacterial, and protozoal pathogens into the finished Drinking Water. It is not, however, a U.S. EPA heterotrophic plate count Specification or Maximum Contaminant Level (MCL) for Drinking Water.

Nevertheless, of particular importance could be the microbial and chemical quality of this

starting water because the water is often delivered to the facility at a great distance from its source and in a condition over which the user has little or no control. High microbial and chemical levels in source water may indicate a municipal potable water system upset, a change in the supply or original water source, a broken water main, or inadequate disinfection, and therefore, potentially contaminated water with objectionable or new microorganisms or coincidental chemical contaminants.

Considering the potential concern about objectionable microorganisms and chemical contaminants in the source water, contacting the water provider about the problem should be an immediate first step. In-house remedial actions could also be needed, including performance of additional testing on the incoming water (as well as the finished water in some cases) or pretreating the water with additional microbial and chemical purification operations (see 5.1 *Unit Operations Considerations*).

■ 2S (USP39)

BRIEFING

⟨1239⟩ **Vaccines for Human Use—Viral Vaccines.** This new general information chapter addresses a need for the description of best practices in the development, production, and testing of viral vaccines for human use. This chapter will serve as a useful addition to an existing family of vaccine-related chapters, which includes *Vaccines for Human Use—Polysaccharide and Glycoconjugate Vaccines* ⟨1234⟩, *Vaccines for Human Use—General Considerations* ⟨1235⟩, and *Vaccines for Human Use—Bacterial Vaccines* ⟨1238⟩ thus far. The General Chapters—Biological Analysis Expert Committee presents ⟨1239⟩, which describes the different types of viral vaccines with their advantages and challenges. Sections of ⟨1239⟩ also address the raw materials, primary cell lines, seed lot systems and cell banks, culture and harvest, purification, intermediates, drug substance, drug product, and lot release tests for viral vaccines.

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Comment deadline: November 30, 2015

Add the following:

■ ⟨1239⟩ VACCINES FOR HUMAN USE—VIRAL VACCINES

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1. INTRODUCTION

Vaccines for the prevention of at least 15 human viral diseases have been commercialized in the United States (*Table 1*); others have been licensed outside the U.S. Together, these vaccines have formed a primary public health measure for the prevention of these viral diseases worldwide. Other viral vaccines are in development and in clinical trials. For the purposes of this chapter, a viral vaccine is a vaccine that prevents a viral disease; this includes products derived from cultured viruses and those derived from recombinant expression of viral antigens. General considerations for both viral and bacterial vaccines are provided in *Vaccines for Human Use—General Considerations* (1235).

Table 1. Examples of Human Diseases Preventable with Viral Vaccines

Live Attenuated Virus Vaccines	Inactivated Virus Vaccines
<ul style="list-style-type: none"> • Adenovirus types 4 and 7 • Influenza A and B • Japanese encephalitis • Measles • Mumps • Poliomyelitis types 1, 2, and 3 • Rotavirus • Rubella • Smallpox • Yellow fever • Varicella-zoster (separately, chickenpox, and shingles) 	<ul style="list-style-type: none"> • Hepatitis A • Poliomyelitis types 1, 2, and 3 • Rabies
	Subunit Virus Vaccines
	<ul style="list-style-type: none"> • Influenza A and B
	Recombinant Virus-Like Particle Vaccines
	<ul style="list-style-type: none"> • Hepatitis B • Human papillomavirus types 6, 11, 16, and 18

1.1 General Categories of Viral Vaccines

There are currently four general categories of viral vaccines that have received regulatory approval: live attenuated, inactivated, virus-derived subunit vaccines, and virus-like particle (VLP) vaccines using recombinant proteins. Other strategies, including DNA-based vaccines, are in development. The characteristics, advantages, and challenges of commercially available viral vaccines are listed in *Table 2*.

Table 2. Characteristics, Advantages, and Challenges of Different Types of Viral Vaccines

Live Attenuated Viral Vaccines		
Characteristics	Advantages	Challenges
<ul style="list-style-type: none"> • Able to replicate in the host • Attenuated in pathogenicity • Elicit both antibodies and cell-mediated immunity 	<ul style="list-style-type: none"> • May elicit broader immune responses than other vaccine types (i.e., both humoral and cellular) • May require fewer doses • Highly effective • Protection is generally longer lasting 	<ul style="list-style-type: none"> • Limited understanding of mechanism and stability of attenuation • Ensuring stability • Analysis • Manufacture • Possible pathogenic reversion • Possible transmission to another person via "shedding" • Controlling reactogenicity
Inactivated, Subunit, and Recombinant VLP Viral Vaccines		
Characteristics	Advantages	Challenges
<ul style="list-style-type: none"> • Unable to replicate in the host • Not pathogenic • Elicit mostly humoral immune responses 	<ul style="list-style-type: none"> • Cannot multiply or revert to pathogenicity • Generally less reactogenic • Not transmissible to another person • More stable than live viral vaccines 	<ul style="list-style-type: none"> • Ensuring sufficient immunogenic potency • May require adjuvant to enhance immune responses • Stimulating cell-mediated immune responses • May require multiple doses • Variable efficacy

Viral vaccines may be based on live viruses that have been attenuated to reduce or eliminate their ability to cause disease. Attenuation, the loss of virulence, results from reassortment or from mutations induced by passage of the virus in semi-permissive or atypical animal cells (or animals), or under altered growth conditions (e.g., at temperatures lower than the host's body temperature), or both. Variants (mutants) of the virus exist that induce reduced disease pathology in their original host and are selected. Such variants, nevertheless, are still able to replicate and are still fully immunogenic. The development of these vaccines is not

standardized, and the passage and propagation is varied dependent on each virus. The virus is often passaged in alternate hosts for varying periods and then tested in the desired host after several passages to determine whether the vaccine is sufficiently attenuated pathogenically but still immunogenic enough to elicit protective responses (i.e., to avoid over-attenuation leading to loss of immunogenicity).

Because live vaccines are grown in animal cells (e.g., primary chick embryo cells) or human cells (e.g., MRC-5, WI-38), contamination by extraneous viruses and other adventitious agents already present in these substrates or introduced during manufacturing (e.g., raw material contamination) is a potential risk. This risk of contamination requires a control strategy for manufacturing and testing that will ensure vaccine purity and safety.

1.2 Inactivated Viral Vaccines

Inactivated viral vaccines are produced by culturing and isolating a virus and then inactivating it. Inactivated viral vaccines may or may not be purified after harvest and before inactivation. Although many such vaccines have been successful in controlling viral diseases, not all viral proteins are equally immunogenic, and an unbalanced presentation (greater number of less immunogenic proteins) can dampen the immune response to protective antigens. Modification of the primary protective antigens can induce atypical immune responses that may enhance disease upon natural infection. This phenomenon was observed for formalin-inactivated measles virus and respiratory syncytial virus in early vaccines developed in the 1960s. Because of this experience, the use of live attenuated measles vaccines is now recommended.

1.3 Subunit Vaccines

Subunit vaccines are composed of viral components, which may be extracted from cultured virus or produced via recombinant technology. Traditional inactivated influenza vaccine components (influenza A and B) are grown in embryonated eggs, and the hemagglutinin (HA) and neuraminidase (NA) antigens are purified following membrane solubilization (splitting).

1.4 Virus-like Particle Vaccines

VLP vaccines, in which recombinant viral antigens are assembled (or self-assembled) into particles or are incorporated into a lipid vesicle, have been developed for hepatitis B and human papillomavirus (HPV) infections. The most common recombinant platforms are yeast and insect cell lines. Although they are more immunogenic than the monomeric antigen, all currently manufactured VLP vaccines based on recombinant protein technology require the addition of adjuvants (e.g., aluminum salts, monophosphoryl lipid A) to optimize immunogenicity. These vaccines have advantages and challenges similar to those of inactivated viral vaccines.

2. RAW MATERIALS

Raw materials can directly affect the identity, strength, purity, and quality of viral vaccines. A consistent manufacturing process is critically dependent on the use of consistent raw materials. This applies during seed banking, fermentation, harvest, purification, and formulation

(see <1235>). Unlike bacterial cultures, which can often be maintained productively with relatively few and simple culture medium components, cultures of eukaryotic animal and insect cells require a large number of components to sustain cell growth and viral replication; basal media often contain over 60 ingredients. Culture ingredients must be selected carefully, especially for enveloped viruses, as many of these can only be partially purified without sustaining loss of infectivity or antigenicity. In such cases, the suitability of raw material for human administration can be a key criterion. A formal risk analysis of all raw materials should be performed to assess any risk of low-level contamination by unwanted entities.

Expectations for the types and composition of media to be used for cell culture in preparing viral vaccines are discussed in <1235>. Low molecular weight components (approximately 1000 Da or less) are usually synthesized chemically, although some can be derived from animal- or plant-sourced raw materials. These small molecule components should conform to applicable compendial requirements where they exist, although some may require further qualification via specific tests (e.g., *Bacterial Endotoxins Test* <85>). Use testing often takes the form of a cell-growth promotion test. Virus-growth promotion tests are more difficult to configure, because detection of virus-neutralizing antibodies is more straightforward in a serial-dilution format against a reference serum. Eukaryotic cells in culture typically require additives, such as cytokines, growth factors, attachment factors, lipid carriers, and other beneficial components as well (see *Growth Factors and Cytokines Used in Cell Therapy Manufacturing* <92>). These can sometimes be supplied individually from recombinant sources, but other times animal blood serum (e.g., fetal bovine serum) with or without protein hydrolysates is added to the culture medium as a simple solution for these requirements.

For animal-derived raw materials in particular, it is important to guard against entry of adventitious agents into the process. Many vaccine viruses cannot be purified away from environmental contaminants, and therefore prevention of entry is required to ensure the safety of the product during the manufacturing operation. Animal raw materials are controlled in several ways. The origin of the herd(s) or flock(s) used are documented, and sometimes controlled or isolated and monitored, to ensure the absence of disease. The material is harvested from the animals under controlled conditions using standardized, documented practices. Preferably, these conditions and practices are specifically designed for pharmaceutical use to ensure that the raw material is not inadvertently contaminated with other viruses or microbes. Therefore, the material is processed and stored appropriately to avoid contamination. In addition, it is usually treated to eliminate any microbe that may have been introduced from the source or during processing. These treatments may include terminal sterile filtration, UV or gamma irradiation, detergents, heat, or a combination thereof.

Quantitative risk analysis with careful documentation should be performed on all of these factors that impact viral safety to ensure an appropriate level of product safety. For specific guidance on bovine serum quality attributes, sourcing, and testing, see *Bovine Serum* <1024> and *Fetal Bovine Serum—Quality Attributes and Functionality Tests* <90>.

3. EGGS AND PRIMARY CELL LINES USED IN VIRAL VACCINE PRODUCTION

3.1 Maintenance of Flocks

For *in ovo* cultured vaccines, the eggs are derived from isolated flocks of birds that are established for several generations and tested to ensure absence of common transmissible

pathogens before, during, and after use for specific-pathogen-free (SPF) egg laying. Potential contaminants of the avian flocks (including those that infect nonhuman species) are routinely tested for and monitored. Flock origin and husbandry, isolation and management practices, health history, and testing should be clearly documented.

3.2 Handling of Eggs

The number of eggs used for vaccine production is variable, depending on the validated process in the manufacturers' license. Upon arrival at the facility, the eggs are first candled, i.e., held up to light to view the embryo to determine embryo viability and lack of contamination for egg selection. The eggs are then sanitized and injected with virus, which is allowed to propagate until harvest. Embryo age, candling procedures, sanitization methods, and injection and incubation methods are all critical manipulations in the manufacturing process and should be clearly documented.

3.3 Primary Avian Embryo Cell Lines

For vaccines produced in primary avian embryo cells, the above practices should be followed and described to the step of sanitization. Embryo retrieval, tissue dissection, and dissociation methods before *in vitro* culture should be conducted under highly controlled environmental conditions and with reagents of appropriate quality; the methods and raw materials should be described. For these cultures, a substantial portion of cells is held aside uninfected for several weeks after the virus culture has been completed to ensure detection of any slow-growing microbes or viral agents. These uninfected control cell cultures are propagated and handled following the same process used for the virus-inoculated cells to help ensure that the cells used for vaccine production are free of adventitious agents.

If feasible, control cells should be evaluated for the presence of adventitious agents in conjunction with product testing using the same tests that are performed on the production cultures. This time period may be 14 days or more, when needed to allow for detection of potential adventitious agents that may be latent, endogenous, or poorly replicating. The susceptibility of the cell substrate to infection by adventitious agents of potential concern can influence the tests needed to confirm absence of contamination. Tests on the production control cultures must be sensitive and specific enough to detect cytopathicity, hemadsorption, and the presence of retroviruses. Retroviruses may be either endogenous (i.e., encoded within the cell substrate genome) or exogenously acquired, such as avian leucosis viruses. Testing of the cell substrate should address the potential that infectious retrovirus could contaminate the product.

3.4 Primary Cell Lines from Other Animal Sources

Primary cell cultures from tissues can be cultured *in vitro* and used for vaccine production. Animals used to generate the primary cells or cell bank must be screened for potential viruses or have their serum tested to detect specific antibodies (see *Virology Test Methods* (1237) for specific guidance). Use of controlled colonies, herds, or flocks is recommended to monitor for specific pathogens. The use of primary cells requires additional testing from each manufacturing run to ensure the absence of contamination by infectious agents. To support the reduction of animal use, diploid and continuous cell lines are recommended. Continuous cell lines can be characterized extensively, and the culture conditions can be optimized and standardized by use

of a cell banking system.

4. SEED LOT SYSTEMS AND CELL BANKS

For the production of vaccines from cultured virus, two cell banks are required: one for the cell substrates and another for the viral seed lots. General information on the development, characterization, testing, and production of cell banks and the related documentation requirements is provided in (1235). In cases where recombinant technology is used to develop the seed, the applicable guidance regarding information that should be supplied regarding the characterization and expression of such recombinant vectors can be found in *Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products* (1048).

4.1 Cell Substrate Master Cell Bank

The prerequisite for viral vaccine manufacture is the establishment of cell cultures to be used for viral production. Many types of cell substrates are used for the production of viral vaccines, including banked cells that have been cryopreserved and characterized extensively. The latter include normal diploid cells, continuous aneuploid cells, and cells that have been genetically modified to allow virus propagation. These cells may be from mammalian, avian, or insect species. At their origin or during the course of culture for banking, they may have been exposed to raw materials and environments that create quality risk. The testing scheme takes into account all of these factors. Cell banks should be established under current Good Manufacturing Practices (cGMP). See also *Vaccines for Human Use—Bacterial Vaccines* (1238) for additional information.

The Master Cell Bank (MCB) is generally made from a cell line or primary source of cells that has been prepared for banking by expanding cultures in a progressively greater number of vessels. The propagation of cells is carried out whenever possible without the use of antimicrobials (in particular, use of penicillin and other β lactam compounds in the culture media should be avoided). The source of cells, from isolation through to development of the MCB, should be well known, and the propagation should be conducted with appropriate controls on the environment, raw materials, and methods, with thorough documentation. Donor tissue, species, and medical history should be described where relevant. All propagations of the final cell line should be documented, including, as applicable, the method of subculture, any use of animal-derived material, a record of subcultivations, and information on storage conditions. The documentation must include the constituents of the culture medium, in particular materials of human or animal origin such as serum, enzymes, protein hydrolysates, or other living cells.

Cell lines derived from human origin should include relevant information from the original donor, such as tissue or organ of origin, ethnic background, geographical origin, age, sex, and if available, health status or medical history including any exposure to pathogenic agents. If the tissue is of fetal origin, the age of the fetus and reasons for tissue harvest should be documented along with the information above, if known, for the parents. All information must be documented, because these parameters may influence in vitro lifespan and cell bank testing requirements. The same procedure should be followed for the establishment of animal cell lines. Additional procedures used to produce the cell bank (e.g., physical or chemical procedures, added nucleotide sequences, genetic manipulation, cloning, or selection) should be documented.

4.2 Characterization of the Master Cell Bank

Extensive testing is carried out on the MCB to confirm the identity, purity, and suitability of the cell substrate for vaccine production. The cell bank must be free of cells from other cell lines present in the manufacturing facility, as well as adventitious agents and endogenous viral contamination. For general considerations concerning adventitious agents testing, see *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* (1050), and for vaccine specifics see (1235). The end-of-production cells are also characterized to demonstrate the stability of characteristics and confirm the absence of extraneous agents that might be present in the MCB.

The stability (including genetic stability) of master and working cell banks in real time should be demonstrated, as described in (1048). Stability of cell-line viability under the cryopreservative storage conditions must be demonstrated using real-time testing; see *Cryopreservation of Cells* (1044) for guidance on cryopreservation of cell lines. Frozen cell bank preparations should be tested for consistency of recovery by determining the viability of cells from vials representative of the beginning, middle, and end of the cryopreservation process. The location of the cells in cryovials or ampules in the vapor or liquid phase of liquid nitrogen must be documented. It is recommended to have at least two separate controlled storage areas for the vials to avoid loss due to facility/equipment failures. Use of qualified shipping containers and manipulation of all vials should be controlled and documented to ensure the consistency of cell bank inventory. A working cell bank (WCB) is generally derived from the MCB, and these two cell banks should be prepared in a similar manner if possible. However, the MCB and WCB preparations may differ from those used in the production process, or from clinical trial materials or commercial supply. The preparation procedures for all cell culture processes must be documented, along with details on process changes. Comparability of product quality must be demonstrated when changes between WCBs occur. This ensures the consistency and long-term supply for vaccine production. Identity testing of the cell substrate is required for each WCB to ensure lineage to the MCB. In cases where recombinant vectors are used, construct purity should be verified from MCB to WCB.

Manufacturers should describe their strategy for providing a continuous supply of cells. The lot size and anticipated use rate of the cell banks for production should be documented, including the details for the subculture and population-doubling level. The qualification of master and working cell banks should be established to ensure an adequate supply of equivalent cells able to support production over the lifespan of the product. A flow chart can be used to illustrate the preparation of the cell banking system.

4.3 Viral Seed Lots

A master virus seed bank preparation is established under cGMP conditions in a manner similar to the preparation of the cell banking process, as described in (1235). Details on the derivation, including the sourcing of all biological materials used—plasmids, animal raw materials (e.g., fetal bovine serum), donor isolate and strain—must be recorded carefully, and reagents must be of appropriate quality. Tests for identity, sterility, mycoplasma, and adventitious agents should be performed on the basis of established compendial and regulatory requirements. Specific tests for additional agents that could potentially be present must also be carried out on the master virus seed preparation (risk based analysis approach) depending on the historical manipulation of the virus isolate (raw materials, agents, and biological reagents that the seed was in

contact with). The master virus seed bank is used to produce a working virus seed bank. The subsequent testing of the working virus seed preparation is dependent on the extent of testing carried out on the master seed, as well as the process and raw materials used to produce the working virus seed preparation.

4.4 Storage

To ensure consistent production of the viral vaccine product, a well-defined storage system is required. Virus seed preparations are frozen and stored in at least two separate controlled areas to avoid loss due to facility/equipment failure. Manipulation of all vials must be controlled and documented to ensure the consistency of virus seed stock inventory. Virus seeds must be stored at -60° or below.

The stability of the master and working virus seed preparations under the frozen storage conditions must be demonstrated using real-time testing. Virus replication (infectivity testing) can be determined during routine production of clinical trial material. Trending the infectivity titer will alert the manufacturer to any stability issues and the need to produce a new working virus seed preparation. The master virus seed preparation should also be monitored in real time during the preparation of new working virus seed preparations. In the event that the master viral seed is not required for an extended period of time, a stability protocol with defined testing intervals should be implemented. If the infectious titers of the working virus seed are not decreasing, and it was produced in a manner similar to the master virus seed preparation, testing of the master virus seed preparation may be reduced or stopped to conserve the supply.

4.5 Recombinant Cell Lines

To date, the production of recombinant viral antigens has used yeast and insect cell lines. The requirements, as for any other recombinant expression system, are set out in (1235). For cell lines containing exogenously assembled expression constructs (recombinant cell lines), characterization of nucleotide and amino acid sequences should be performed. Extensive characterization of cell banks should use the most relevant qualified technology. The chapter (1048) provides advice on the characterization of the engineered cell line and expression construct.

5. CULTURE AND HARVEST

5.1 *In ovo* Cultivation

Culture of viruses *in ovo* is fairly straightforward. Common egg culture variables include: the amount of virus inoculated per egg, the mode of inoculation, harvest timing, and sometimes incubation conditions such as temperature, depending on the virus strain. These factors have to be optimized for each individual serotype and master/working seed (for influenza viruses in particular), and the impact of all of these factors should be investigated and documented. Flock genetics, nutrition, and age can all affect *in ovo* virus propagation, as can egg sanitization procedures that may compromise the allantoic membrane cells. These factors should also be investigated and documented; however, for influenza, these will not usually be optimized for each season's production campaign.

5.2 Establishment of Primary Cell Cultures

Procedures for recovery of primary cells should be described from organ recovery through dispersed cell suspension, with attention paid to reproducibility of the cell substrate's properties in culture. This analysis may involve subsequent growth reproducibility, or if minimal growth is involved, it should be aimed at reproducibility of metabolism and viral receptivity/productivity. In cases where whole organism harvest is involved (e.g., whole avian embryo dissociation), only a fraction of the resulting cells may be susceptible to viral infection. Other cells from the harvest provide supporting matrix and other factors and can potentially inhibit virus replication. Preferably, a modern process would be used to characterize the spectrum of cells harvested and their differentiation state under production culture conditions, thereby ensuring the reproducibility of these factors. Under conditions suitable for viral replication, little or no growth is expected of the cells.

5.3 Cell Culture

Culture for viral vaccine production generally consists of two phases: a cell growth phase and a virus production phase. These processes are often complicated due to the diversity of cell types and their general dependence on a surface for growth. Virus properties—including replication cycle speed, yield of virus from the cells, and stability of infectious virus or antigen under culture conditions—also exert major influences on process technology, configuration, and operation in the manufacturing setting. Reproducibility of the cell processing steps is critical, and therefore good documentation of each manipulation ensures consistency of a key parameter for product manufacturing.

The virus culture process begins with the establishment of a receptive cell mass of sufficient size to produce a batch, which is typically about 10^5 to 10^6 doses of vaccine. Generally, about 10^6 – 10^8 cells are cryopreserved in one or more aliquots, whereas about 10^8 – 10^{12} cells may be required for a production batch. Historically, processes have also used secondary or tertiary cultures, in which primary tissues are grown for several cell divisions to increase the biomass size and batch size, to minimize the amount of primary tissue required per batch, or both. Recovery of cells from cryopreservation involves thawing the cells and diluting out the cryoprotective (banking) medium such that maximum cell viability is preserved and reproducible cell growth is ensured. Practices should be documented and justified on the basis of development experiments; typical parameters investigated are thaw rates and temperatures, dilution rates, and dilution factors.

A protocol must be devised that results in reproducible cell numbers in a state conducive to virus production. This requires passaging the cells, and for attachment-dependent cells, providing a larger surface area for growth. Cell expansion output:input ratios for a passage are typically 2:1–20:1, depending on the cell type and growth medium. In certain contexts, cells require a minimum threshold concentration to provide each other with growth factors, and this requirement may reduce the passage ratio. Other cell types, or media that provide these growth factors, can allow much lower inocula and larger ratios.

Two typical objectives for expansion passaging reproducibility are 1) ensure that cells experience a minimal "lag phase" (sometimes caused by the need for sufficient production and accumulation of growth factors and cellular attachment factors), and 2) ensure that cells do not grow to the point of stagnation/death (often caused by nutrient exhaustion, or by crowding on the surface that causes "contact inhibition" of growth). Either of these issues can

lead to a lag phase in the next passage. Early cell expansion is usually performed in a separate production area to avoid the potential for cross-contamination. The earliest, small-volume passages are often conducted in open laboratory vessels (e.g., flasks, roller bottles); however, it is highly desirable to use closed containers to facilitate aseptic handling of containers and to minimize or eliminate operator exposure risk. Control of the environment and aseptic handling practices are written into the procedures for use during development and are maintained during manufacturing.

The final cell expansion is typically an integral part of virus production, with goals of achieving the final biomass required for production and producing cells that are highly productive for virus. In batch or fed-batch cultures, a "cell density effect" may exist, whereby growth to higher cell concentrations correlates with lower virus productivity on a per-cell basis. This can be caused by many factors and should be characterized to ensure that the process operates in a robust and predictable manner.

5.4 Virus Culture

The final cell expansion and virus culture are typically a biphasic process in which the cells are grown in the final production vessel(s) and then are inoculated with virus. The viral inoculation often coincides with medium replacement or addition, as well as alteration of other culture parameters such as temperature. If cell growth is conducted in a serum-containing medium, the medium for viral production is usually switched to a serum-free medium to minimize serum carryover into the harvest. If the virus is secreted into the medium and cannot be purified rigorously after harvest, extensive washing may be required to reduce residual serum to acceptable levels (see 21 CFR § 610.15 *Constituent materials*). Documentation of washing effectiveness should be developed. Replacement of serum may be required for maximal virus growth, and this may require the addition of other protein sources such as albumin, transferrin, or insulin.

Virus infection results are known to be sensitive to cell concentration; history; time of harvest; future cell substrate growth trajectory at the point of infection; and the "multiplicity of infection" (MOI), which is the number of infectious virus particles inoculated per cell. These factors affect the quantities of infectious virus and total virus and can govern the quality of a vaccine via the ratio of infectious to total virus, coupled with the degradation products that may have reduced immunogenicity. In addition to assessing titers over the duration of infection, it is useful to characterize virus stability under culture conditions with cell-free culture broth samples, because this can illuminate whether titer changes are due to changes in synthesis or degradation. High MOIs over repeated passages are generally undesirable, especially for RNA viruses, because of the propagation of defective genomes that are trans-complemented by co-infection of a cell by another viral genome. Genetic stability of the vaccine virus should be well characterized. During development, the infection parameters need extensive characterization against all of the key quality attributes for the vaccine to ensure a robust process; thus, these parameters should be described.

Virus disposition upon replication is a significant determinant of the infection process configuration. Enveloped viruses are typically 1) secreted into the culture medium as they acquire their lipid envelope or 2) as secretory vesicles fuse with the plasma membrane. For these viruses, multiple batch-wise harvests of the culture supernatant are common, and re-feeding the culture upon harvest can promote higher yields. Perfusion processes are not often used because of product dilution and the difficulty, as well as added expense, of downstream concentration. For lytic viruses, destruction of the cells allows only for a single harvest;

depending on the time elapsed from infection to lysis, feeding of the culture may maximize yields. Other viruses remain intracellular, and processes for these may allow for extensive feeding to accumulate virus product. If the cell integrity is not compromised, washing to remove undesirable medium constituents may be performed at the end of the culture. Many parameters of the culture environment also affect virus growth and stability, and it is expected that all relevant process parameters will be controlled to meet product quality expectations, as well as their acceptable ranges ("operating space") as defined in *Guidance for Industry: Q8(R2) Pharmaceutical Development*. It is important to note that there is biological variability in cell growth; transient viral infections occur and are inherently variable; virus-host cell interactions are ill defined; and the analytics for potency and other parameters are highly variable and should be defined. Consequently, despite efforts to characterize viral vaccines and their processes, it is not uncommon to experience root mean square (RMS) variances of 50%–100% in process output, even for a well-controlled process, unlike some other biological medicines.

Influenza vaccine produced using a baculovirus expression system, with the viruses grown in insect cell lines, is manufactured in much the same way as virus in any other cell line. Cells are grown in serum-free medium composed of chemically defined lipids, vitamins, amino acids, and mineral salts. Recombinant HAs are extracted from the cells with octylphenol ethoxylate and purified by column chromatography. Harvest time is an important parameter. All processing of the cell banks and baculovirus seed lots, as well as subsequent cell cultures, is done under aseptic conditions in an area where no other cells are being handled. Several single harvests may be pooled before testing. No antibiotics are added at the time of harvesting or at any later stage of manufacturing. Control cells are tested for absence of arboviruses in addition to other adventitious agents.

5.5 Culture Process Technologies and Facility

Because of safety concerns regarding the use of tumorigenic cell types, the cells used for viral vaccine culture are almost uniformly dependent on an attachment surface for growth. The few anchorage-independent exceptions [e.g., Madin-Darby canine kidney (MDCK) cells for influenza] will become more commonly used as regulatory paradigms for tumorigenic and designer cells are developed. The attachment requirement creates a need to supply not only soluble nutrients but also an attachment surface for the common cell types (e.g., MRC-5 and Vero). Given the large variability in virus yields and production volumes, surface area requirements can differ substantially. This has created a situation in which a variety of technologies are used for cultivation. For small-scale operations, simple adaptation of laboratory vessels may suffice, and scale-up consists of scale-out, i.e., increasing the number of vessels. Beyond a certain point, aseptic risk precludes further scale-out. Intermediate scales of culture involve the use of specialized culture vessels, along with robotics where scale-out strategies are still used. Many of these technologies use disposable vessels and reactors with a focus on 1) minimizing the risk of contamination, and 2) the compatibility of product contact surfaces with the cells and virus. An extractable/leachable assessment should be performed for each disposable system used (see *The Biocompatibility of Materials Used in Drug Containers, Medical Devices, and Implants* (1031)).

Large-scale production is performed in engineered bioreactor systems that use agitation technology similar to that for bacterial vaccines and other biologicals. In the most common reactors using agitation, attachment-dependent cells use "microcarrier" technology, which consists of small beads of about 150- μm diameter that are easily suspended by agitation and

provide a very large surface area in a given volume of culture. Other configurations with packed or static beds are also used for these systems. The vessels used can be made of either disposable plastics or stainless steel. Compatibility of the material with the medium and the cells, and the absence of leaching, should be established for either type of vessel, as well as for fittings and probes used in monitoring and controlling the cultures. For nondisposable equipment, cleaning and sterilization procedures must be established, validated, and well documented. For reactors with loose or suspended carriers, the carriers should be readily excluded from the product stream and should not shed pieces or chemicals that are not suitable for human administration.

For all systems, the environment should be designed appropriately, reflecting the need for aseptic manipulations if they are required for either culturing or bioreactor set-up. In addition, the potential for spills or breakage and the risk of operator exposure need to be taken into account, especially when culturing wild-type virus strains. In a multi-product facility, changeover procedures that minimize the potential for cross-contamination, and validation of inactivation should be established and documented. Operator training and facility management procedures must be established for handling cultures and avoiding contamination of the product, the facility, and the operators themselves.

Note that production of recombinant viral antigens in yeast or insect cell lines does not involve infection of the growing cell, and the requirements are essentially the same as those for other recombinant proteins, described in the relevant compendial guidance for recombinant protein products.

6. PURIFICATION

A general overview of purification for viral-derived vaccines is presented in (1235). For live attenuated and inactivated virus vaccines, the desired antigen is a virus entity that is amplified from a working virus seed, in a complex biological substrate, and generally with minimal downstream processing to prepare the drug substance. Although inactivated virus vaccines also require virus seed expansion, virus inactivation and purification are necessarily more complex to ensure complete removal of infectious virus, removal of inactivating agents, and maintenance of the desired antigenic properties.

Many factors should be considered in developing a downstream purification strategy, with the understanding that all processing steps will result in some loss of viable virus. Downstream processing steps are designed to: 1) remove cellular debris (host cell protein and DNA) from the vaccine harvest; 2) remove impurities or reduce their levels; 3) concentrate virus; 4) add a virus stabilizer; and 5) filter sterilize the virus preparation to meet a specified level of adventitious agent removal (limited in the case of live-virus vaccines).

6.1 Live Attenuated and Inactivated Viral Vaccines

After removal of cellular debris, further purification can be accomplished by using zonal centrifugation on a density gradient or by column chromatography. Nucleic acid in Vero cell-produced vaccines can be reduced by treatment with suitable endonucleases, and removed by ultrafiltration using a 50 kDa membrane or by ion-exchange chromatography. The resulting drug product may be stabilized (via cryoprotectant or lyophilization formulation), sterile filtered (using a 0.22- μm pore size filter), or both, and should be stored frozen at less than -60° .

7. VIRAL INACTIVATION

Because of the pathogenic nature of the viruses (e.g., hepatitis A, Japanese encephalitis, rabies, and poliomyelitis), many viral antigens must be inactivated while maintaining their antigenic properties. Inactivation may involve use of chemicals, detergents/surfactants, or irradiation, or various combinations thereof. Removal or reduction of the inactivating chemicals/detergents/surfactants from the antigen to acceptable levels may be achieved by centrifugation, chromatography, ultrafiltration, diafiltration, or combinations thereof.

7.1 Subunit Vaccines

Although the seasonal influenza vaccines (influenza A and B) are prepared either in embryonated eggs or via cell culture, the derived virus is processed using different manufacturing methods to make the drug substance. The drug substances can be a live attenuated virus, a detergent-solubilized "split vaccine", or a subunit vaccine resulting from purification of the surface glycoproteins (HA and NA) from the internal nucleocapsids. To produce the split virus and subunit vaccines, the whole virus is subjected to disruption with a surfactant, which solubilizes the viral membrane. For subunit vaccines, the internal subviral core of the virus is separated from the surface proteins on the basis of their differing sedimentation rates. With split-virus vaccines, the choice and use of surfactant ensures that the subviral core itself is disassembled. Diafiltration is normally used to remove the surfactant and other media components from the product, and then the process is terminated with a sterile filtration step to produce a monovalent bulk solution.

7.2 Recombinant VLP Vaccines

Recombinant proteins that are components of VLP vaccines, such as hepatitis B and HPV, undergo more rigorous purification processes. As with inactivated viral vaccines, the purification methods must maintain the desired antigenic properties. Overall, the purification schemes are similar to those used for other viral vaccines. After fermentation, the cells are harvested and purified by downstream processing. This may involve chromatography, precipitation, microfiltration, ultrafiltration, or other techniques that will be specific for the vaccine being produced by each manufacturer. After purification, the VLPs may be mixed or adsorbed onto an adjuvant to increase the immune response.

Characterization of the recombinant monomeric antigens follows the typical requirements for recombinant proteins and would include peptide mapping coupled to mass spectrometry to assess the integrity of the primary structure, as well as SDS-PAGE and other appropriate assays. Characterization of the intact VLPs will focus on assessing the size distribution of the particles using techniques such as transmission electron microscopy (TEM) or cryo-electron microscopy (cryoEM) and dynamic light scattering (DLS).

Testing for adsorbed VLPs (MBAP) includes differential scanning calorimetry (DSC), immunogenicity in animals, and a sandwich ELISA *in vitro* relative potency (IVRP) assay used to measure the antigenicity of the drug substance.

7.3 In-Process Controls

Manufacturers identify critical process parameters and perform appropriate tests to monitor the

purification process. Among the latter are filter integrity tests, microbial enumeration tests (see *Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests* (61)), bacterial endotoxin tests (see (85)), and other suitable tests for known process residues of concern. Process residuals include raw materials, culture medium components (e.g., serum and antibiotics), cell substrates (e.g., proteins and DNA), buffer components (EDTA), and other materials (endonucleases, protamine sulfate, detergents/surfactants) used in purification. In addition to in-process control tests, manufacturers may perform in-process monitoring tests to monitor total protein, specific antigens, or ancillary materials at various steps in the purification process of intermediates. These in-process monitoring tests are carried out to gather product and process knowledge to evaluate consistency in the manufacturing process.

To demonstrate process performance and reliability, manufacturers should characterize inherent process-related impurities (e.g., protein, DNA, and lipids). Process validation studies may be carried out to demonstrate sufficient and consistent removal of residuals to an acceptable level such that routine testing is no longer required. If material must be sterile, analysts can perform *Sterility Tests* (71).

8. INTERMEDIATES

Intermediates are defined as the unformulated active (immunogenic) drug substances that are further processed before final formulation and can be stored for long periods of time ((1235)). Examples of intermediates for U.S. licensed viral vaccines include: live attenuated (e.g., influenza, measles, mumps, rubella, and yellow fever); inactivated whole (e.g., rabies); subunit (e.g., influenza); and VLP (e.g., hepatitis B and HPV). If intermediates are to be stored, stability release tests should be performed before proceeding to the next steps. Hold times for intermediates should be validated through a formal stability study/program. Stored intermediates are often suitable for more detailed characterization studies than are harvest bulks or final products. Some release tests are routinely performed before the intermediates are converted to the final bulk; this varies depending on the individual vaccines.

8.2 Tests for Intermediates

UNPROCESSED BULK HARVEST

In general, viral safety lot release testing is done at the bulk harvest stage before any purification. This is true regardless of whether the assay detects infectious virus or viral components. The presence of host cell DNA may need to be assessed in the case of biologics manufactured in animal cells. A hold step may be introduced at this processing step, for example to combine two or more single virus harvests for further processing.

Table 3. Typical In-Process Testing of the Unprocessed Cell Harvest

Assay	Purpose	Method
Adventitious agent	Verify absence of viral contamination	Tissue culture with cytopathic effect (CPE) and hemadsorption end points
Mycoplasma	Detect mycoplasma	Cell culture, polymerase chain reaction (PCR), growth, or fluorescent staining as end points (<i>Mycoplasma Tests</i> (63))
Sterility	Find evidence of microbial or fungal contamination	(71)
Potency	Measure quantity of infectious virus	Tissue culture with plaque or Tissue Culture Infective Dose (TCID ₅₀) end points; RNA quantification

In-process adventitious agent inactivation steps are not included for live viral vaccines, because these steps could compromise the live nature of the vaccine itself. As a result, it may not be possible to validate clearance of any adventitious agents. For this reason, comprehensive testing for adventitious agents and qualification and control of the vaccine source materials are essential as part of vaccine safety control. Bioburden is monitored according to (61).

Other product-specific testing may be appropriate. For example, for live influenza vaccines, each lot of viral harvest is tested for the cold-adapted phenotype (can grow at 25°), the temperature-sensitive phenotype (restricted replication at 37°–39°), and the attenuation phenotype (restricted replication in the upper and lower respiratory tract of ferrets). Each lot of viral harvest is also tested extensively by in vitro and in vivo methods for adventitious and contaminating agents. Viral harvests from the three strains (H₁N₁, H₃N₂, and B) are subsequently blended and diluted as required to the desired potency to produce bulk vaccine.

Table 4. Tests for Inactivated Virus Bulks

Assay	Purpose	Method
Completeness of inactivation	Demonstrate inactivation of virus	Hemagglutination assay or infectivity assay
Bioburden	Find evidence of microbial or fungal contamination	(61)
Antigen content	Quantify viral dose	Single-radial-immunodiffusion assays (SRID) or other immunological method

Table 5. Typical Tests for Post-Purification Live Viral Vaccine

Assay	Purpose	Method
Adventitious agents	Detect adventitious viral contamination	Product-enhanced reverse transcriptase assay (PERT) or dual-template reverse transcriptase assay
Identity	Identify product	Electrophenotyping, plaque neutralization, RNA hybridization, PCR
Residual DNA	Measure the level of residual DNA	2-(<i>N</i> -Bis-(3-dimethylaminopropyl)-amino)-4-(2,3-dihydro-3-methyl-(benzo-1,3-thiazol-2-yl)-methylidene)-1-phenyl-quinolinium fluorescent DNA binding dye or PCR
Potency	Measure quantity of infectious virus	Tissue culture with plaque or TCID ₅₀ end points; RNA quantitation
Sterility	Ensure absence of viable microorganisms	<71>

Table 6. Tests for Recombinant Viral Antigen Intermediates

Assay	Purpose	Method
Total protein	Measure antigen content	Appropriate protein assay
Antigen content and identification	Determine content, identity, molecular weight	Radioimmunoassay (RIA), ELISA, immunoblot, SRID, and reducing SDS-PAGE
Percent intact monomer for VLPs	Characterization	Purity assay
VLP size and structure	Characterization	Dynamic light scattering (<i>Spectrophotometry and Light-Scattering</i> (851))
Aluminum content for VLP	Purity	Appropriate assay
Identity	Identity	Immunological assay or PCR
Host cell- and vector-derived DNA	Purity	PCR, fluorescent DNA-binding dyes
Residual host cell proteins	Purity	Appropriate assay
Purity	Purity	LC or SDS-PAGE
Degree of adsorption if adsorbed	Characterization	Separation, then content of adjuvant by appropriate method (e.g., GC for 3-O-desacyl-4'-monophosphoryl lipid A)
Residual cesium, if cesium used in gradient purification	Safety	Appropriate assay
pH	Characterization	<i>pH</i> (791)
Endotoxin content	Safety	(85)
Bioburden or sterility	Safety	(61) (71)

9. DRUG SUBSTANCE

The drug substance is the final bulk antigen following the manufacturing process that is ready for the addition of other ingredients, such as adjuvants, and others to produce the finished dosage formulation. The resulting material, which may be sterile filtered or aseptically processed and then tested for sterility, becomes the vaccine drug substance.

Drug substances can be stored in containers at temperatures (e.g., below -60°) that ensure their stability before further processing. Hold times for drug substances should be validated through a formal stability study/program. Testing of the drug substance must be performed to ensure its identity and purity. Product- and process-related impurities are monitored to confirm that their levels meet the requested specifications. Because viral vaccine drug substances generally have high potency, and subsequent dilution to the drug product potency may be significant, measurements of process impurities are best obtained from the drug substance rather than from the final container.

Examples of release tests for the drug substance include:

- Appearance
- Identity of antigen
- Potency/activity, or quantity of epitope or antigen
- Infectious titer (live attenuated viral vaccines)
- Protein integrity and purity (e.g., by SDS-PAGE)
- Monomer content in VLP vaccines
- Pyrogenicity or endotoxin content
- Sterility
- Mycobacteria
- Mycoplasma/spiroplasma

Table 7. Typical Release Tests for Different Types of Viral Vaccine Drug Substance

Live Attenuated Viral Vaccines	Inactivated Viral Vaccines	Subunit or Recombinant VLP Vaccines
Appearance	Appearance	Appearance
Identity	Identity	Identity of antigens
Potency	Immunogenicity	Antigen or epitope quantity
Infectious titer	Completeness of inactivation	Protein content
Adventitious agents such as mycobacteria, mycoplasma, spiroplasma, arbovirus, porcine or bovine viruses	Pyrogenicity or endotoxin content	Particle size (VLPs, virosomes) and size heterogeneity
Host cell proteins or surrogates	Host cell proteins or surrogates	Pyrogenicity or endotoxin content
Reverse Transcriptase (RT) assay	Residual inactivation reagents	Host cell proteins or surrogates
Endotoxin	Aluminum content (if adjuvanted)	Adjuvant content (if adjuvanted)
Sterility	Sterility	Sterility
Bovine serum albumin	Process residuals	Process residuals

In addition to the examples listed above, other methodologies can be applied for identity and purity evaluation. For instance, specific impurities that must be measured are agreed upon between manufacturers and the licensing authority during the licensure process.

9.1 Final Container

General requirements for vaccine products are provided in <1235>. This includes requirements for labeling and assessing the stability of vaccines.

Mechanically, the processes of formulation and filling for viral vaccines have changed little over time. Formulation is usually a simple dilution of high-potency drug substance to the appropriate strength for filling, taking into account yield losses expected through subsequent temperature exposures (including freeze-thaw cycles), and where applicable, lyophilization yield losses. Some inactivated, subunit, and recombinant VLP vaccines are produced as formulations that are adjuvanted, alum adsorbed, or both. The aluminum adsorption is commonly done as part of drug substance processing, with dilution into more aluminum to maintain constancy of adjuvant dose. It is important to note that production of drug product lots involves filling containers into the tens or hundreds of thousands, and special attention must be paid to ensuring homogeneity of product over very many vessels with volumes ranging from 0.1 to 10 mL. For live products, thermal degradation can occur over the course of the filling operation, which should be considered in process design. For products containing aluminum suspensions, homogeneity of the suspension is a significant concern. For all viral vaccines, product is "overfilled", both with respect to volume—to ensure the ability to withdraw a full dose—and with respect to potency—to ensure a statistically acceptable probability that each container receives the

declared potency at the end of expiry, given the variability of the process and assays used.

10. DRUG PRODUCT AND LOT RELEASE TESTS

Drug product is defined in 21 CFR § 210.3(b)(4) as "a finished dosage form, for example, tablet, capsule, solution, etc., that contains an active drug ingredient generally, but not necessarily, in association with inactive ingredients. The term also includes a finished dosage form that does not contain an active ingredient but is intended to be used as placebo." In compliance with 21 CFR § 600 *Biological Products: General*, and in particular, 21 CFR § 601.2, *Applications for biologics licenses; procedures for filing*, the manufacturer "shall submit data derived from nonclinical laboratory and clinical studies which demonstrate that the manufactured product meets prescribed requirements of safety, purity, and potency...". Documentation of the viral vaccine drug product includes: components used in manufacture; composition of the drug product; specification for each component; manufacturing and packaging procedure and in-process controls for the drug product; and specifications necessary to ensure identity, strength, quality, purity, and potency in accordance with 21 CFR § 211, *Current Good Manufacturing Practice for Finished Pharmaceuticals*.

Chapter (1235) summarizes the lot release procedure in accordance with 21 CFR § 610.1 and 21 CFR § 610.2. Samples and protocols for viral vaccines containing all appropriate tests are submitted to the Food and Drug Administration (FDA) for review, testing, or both. If the FDA determines that the lot meets the standards of safety, purity, and potency required for the particular vaccine as contained in its license, the lot is approved for release, distribution, and marketing. The lot release protocol for each viral vaccine includes the specific potency test, as well as common tests such as visual inspection of final vials, safety, sterility, and purity testing for each lot.

10.1 Potency Assays

General requirements for potency tests are described in (1235). Many types of potency-indicating tests exist for viral vaccines, and the selected potency test depends on the type of vaccine.

For the attenuated viral vaccines, the possible potency assays may be:

- A quantitative plaque formation assay, which assesses the ability of the virus to infect cells
- An end-point dilution assay (TCID₅₀)
- A virus neutralization assay
- A quantitative PCR assay, which specifically quantifies the amount of viral nucleic acid present in the sample

For inactivated, subunit, or recombinant VLP vaccines, the potency assay may be:

- An immunogenicity assay, which determines the ability of the vaccine to induce a specific antibody response
- A neutralization assay, which assesses the ability of the vaccine to induce a specific

antibody response which neutralizes the (infectivity/pathogenicity) of the target virus

- A mass-based assay, which quantifies the amount of antigen that is present in the vaccine
- An antigen quantification assay, typically using a monoclonal antibody to quantify the protective epitope present in the vaccine

10.2 Common Tests

Tests for general safety, sterility, purity, residual moisture, identity, and constituent materials are as described in <1235>, except as described below. Modifications to the requirements for a general safety test for inactivated influenza virus vaccine are described in 21 CFR § 610.11a and indicate a test for endotoxin. Viral and rickettsial vaccines and antigens are generally exempted from a requirement for a pyrogenicity test.

10.3 Manufacturing Residuals

Expectations for the control of manufacturing residuals, including surfactants and those chemicals used in the inactivation process(es) are described in <1235>. The chemicals are reduced to a minimum, often by dilutions included in the manufacturing process. Formaldehyde and other chemicals used to manufacture vaccines must be minimized. Limits are set on their residual content in the final vaccine, and content specifications are defined in the approved product license application.

Requirements to control residual material from cell substrates are described in <1235>, including the steps that should be taken to prevent potential complications for hyperresponsive recipients of these vaccines.

10.4 Preservatives

The chapter <1235> provides information on the acceptable use of antimicrobial preservatives, the types of preservatives used, the minimization of thimerosal content, and the production of thimerosal-free vaccines. Limits and content specifications are set for each vaccine in the product license.

10.5 Excipients

Formulation chemicals for viral vaccines are often introduced during drug substance production, during the infection phase, or at harvest to enhance process productivity, particularly for enveloped live virus vaccines. Non-enveloped viruses or vaccines with extensive purification may introduce formulation chemicals only at the end of the purification process. A notable exception is formulation for combination viral vaccines, where the individual viruses may need stabilizers that are different from the global optimum for the mixture. In these cases, the final formulation of chemicals may be introduced late in the process to preserve maximum potency. Formulation components may be included to minimize oxidation, to compete for other reactions that would damage the product, to provide pH buffering or tonicity, and in the case of lyophilized formulations, to replace water that would otherwise bind to proteins or provide other functions in the lyophilization process (e.g., cake formation/integrity). Chemically defined components are desirable, and all components should meet appropriate pharmacopeial

standards. In some instances, macromolecules such as albumin or dextran or fragments thereof (such as hydrolyzed gelatin) may be included in formulations to function as highly effective stabilizing agents. For those that originate from animal or plant sources or for new excipients, risk assessment should be conducted on the origin and processing of the materials to ensure freedom from infectious agents, and for other biological safety aspects (see *Excipient Biological Safety Evaluation Guidelines* (1074)). Where pharmacopeial monographs exist, they should conform. In addition to stabilizing agents, viral vaccines may contain preservatives, especially if the final container contains multiple doses. Preservatives should meet antimicrobial effectiveness testing requirements in the formulation (see *Antimicrobial Effectiveness Testing* (51)). It is common for preservatives to reduce the sensitivity of drug product sterility testing, and this needs to be addressed during sample qualification for release testing to ensure adequate safety (see *Validation of Microbial Recovery from Pharmacopeial Articles* (1227)). In addition, some formulations may be incompatible with other in vitro or in vivo safety testing and may require alterations of conditions or sample pretreatments to achieve valid test results.

10.6 Adjuvants

Aluminum compounds including aluminum potassium sulfate (alum), aluminum phosphate, aluminum hydroxide, and combinations of these compounds are currently approved adjuvants for vaccine use. Chapter (1235) outlines sections of the 21 CFR § 610.15 regulation governing the use of aluminum and the allowed amounts. Aluminum is quantitated using colorimetric, titrimetric, or emission or atomic absorption spectroscopic methods of analysis (see *Aluminum* (206)). The degree of adsorption of protein components to the adjuvant should be determined. The adjuvant monophosphoryl lipid A (MPL) is also licensed for vaccine use. It is quantified either through degradation to fatty acid methyl esters (FAMES) and quantification by GC, or through quantification of the glucosamine content.

10.7 Alternative Tests

See (1235).

11. OTHER REQUIREMENTS

Retention samples are held by the manufacturer for at least 6 months after the expiration date, in accordance with 21 CFR § 600.213. Enough material from each lot of each product is held for examination and testing for safety and potency.

Records are maintained concurrently with each step in the manufacture and distribution of product such that at any time, successive steps of manufacture and distribution may be traced (see 21 CFR § 600.12).

For storage conditions, shelf life, and expiry date see 21 CFR § 610.50 and 21 CFR § 610.53.

11.1 Container

The final container for the drug product is typically a vial with a rubber stopper, or a pre-filled syringe with a glass barrel and rubber plunger. Vial/syringe material is typically USP Type I borosilicate glass (*Containers—Glass* (660)), treated to prevent delamination (spalling) resulting from heat sterilization or with certain formulations, or to prevent vaccine adsorption. Adsorption

is prevented by selecting appropriate liquid formulation components, which may include proteins or mild non-ionic detergents; they also prevent virus aggregation and promote a more consistent product. Plastic containers have been introduced recently. Stopper and plunger components are typically butyl rubber compounds, which can be polytetrafluoroethylene or silicone coated to minimize vaccine adsorption, leaching of extractable components, or both. Sterilization methods should be characterized to ensure that they do not alter the container material properties with respect to vaccine interactions; for example, moisture absorbed during steam sterilization may add moisture over time to lyophilized vaccines, and ionizing radiation may alter the surface chemistry of polymer compounds. All containers and product-contact components should meet USP Class VI extractables testing (see <1031>) for the formulation chemistry and sterilization methods of interest.

11.2 Labeling

The requirements for container labeling and package labeling are described in <1235>. Regarding the information insert (label), vaccine product labeling is regulated in compliance with 21 CFR § 201 and 21 CFR § 610. Requirements have been set for both container labeling and package labeling.

11.3 Shelf Life, Expiry Dates, and Storage Conditions

The requirement to conduct a formal stability study to ensure that a product remains within specification, in line with 21 CFR § 211.166, 21 CFR § 610.50, and 21 CFR § 610.53, is described in <1235>. Additional guidance and detail on the general principles of stability testing for biologics can be found in *Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products* <1049>. ■2S (USP39)

BRIEFING

<1602> **Spacers and Valved Holding Chambers Used with Inhalation Aerosols—Characterization Tests**, PF 40(1) [Jan.–Feb. 2014]. This proposed new USP general information chapter addresses the testing of spacers and valved holding chambers (VHCs), which are widely prescribed for use with inhalation aerosols. It is a companion chapter to *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* <601> and has been revised on the basis of comments received. Regulatory agencies worldwide have increasingly recognized that spacers and VHCs modify inhalation aerosols [commonly known as pressurized metered-dose inhalers (pMDIs)] plumes to the extent that quality data should be required to evaluate the performance of specific add-on device(s) and products from pMDI drug product users. The existing methods for evaluating inhalation aerosols rely on sampling at a constant flow rate; however, spacers and VHCs are typically prescribed for patients who delay inhalation following pMDI actuation (poor pMDI technique). This chapter addresses aspects of spacer and VHC use through development of patient-use procedures. Much of the content of this chapter is based on CAN/CSA/Z264.1-02 “Spacers and Holding Chambers for Use with Metered-Dose Inhalers” published by the Canadian Standards Association. The information given herein is not to be construed as guidance for batch release testing. Where measures of performance of a spacer/VHC are given, they are indicative in nature based on experience associated with the development of the predecessor Canadian Group standard and are therefore not intended as specifications. The rationale for development

of <1602> was published in a *Stimuli* article in *PF* 37(4) [July–Aug. 2011].

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCDF: K. Zaidi.)

Correspondence Number—C145100

Comment deadline: November 30, 2015

Add the following:

▪ **<1602> SPACERS AND VALVED HOLDING CHAMBERS USED WITH INHALATION AEROSOLS—CHARACTERIZATION TESTS**

1. INTRODUCTION

- 1.1 Background
- 1.2 Purpose
- 1.3 Rationale
- 1.4 Recommendations
- 1.5 Definitions of Key Terms Relating to This Chapter
- 1.6 Choice of Drug Product with Which to Test

2. TEST METHOD SELECTION

- 2.1 Spacer/VHC Configurations
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3. MEASUREMENT OF APSD

- 3.1 No Delay between the pMDI Actuation and Sampling Onset
- 3.2 Delay between the pMDI Actuation and Sampling Onset

4. MASS OF DRUG DELIVERED FROM A SPACER/VHC WHILE SIMULATING PATIENT TIDAL BREATHING

- 4.1 Without Facemask
- 4.2 With Facemask

1. INTRODUCTION

1.1 Background

Spacers and valved holding chambers (VHCs) are widely used in conjunction with inhalation aerosols [commonly known as pressurized metered-dose inhalers (pMDIs)]. When used correctly, VHCs assist with the administration of inhalation aerosols to patients who have poor coordination of pMDI actuation and inhalation, and VHCs reduce oro-pharyngeal deposition of

the drug. Patients using these devices breathe tidally when inhaling their medication, regardless of any delay, and this is particularly true for young and elderly users. Also, spacers and VHCs often come with a facemask instead of the mouthpiece normally supplied for inhalation aerosols. These add-on devices interface with the actuator/mouthpiece of the pMDI and provide additional volume for the aerosol plume to develop. In this chapter, these aspects are addressed by providing patient-use appropriate procedures that have been evaluated extensively.

Although existing methods for evaluating inhalation aerosols rely on sampling at a constant flow rate, spacers and VHCs are typically prescribed for patients who have poor inhalation technique, i.e., they delay inhalation after actuating the pMDI. This chapter describes tests that are intended to provide information on how the spacer/VHC modifies the aerosol emitted by the pMDI.

1.2 Purpose

The purpose of *Spacers and Valved Holding Chambers Used with Inhalation Aerosols—Characterization Tests* (1602) is to define standardized methods for characterizing the in vitro performance of a given pMDI drug product with a specific spacer and VHC, and this chapter may be used to develop specifications that could be used in a product quality control environment. It is recognized that spacer/VHC configurations will modify the emitted dose delivered compared with the dose indicated on the label by the manufacturer of the pMDI product, chiefly by removing almost all of the portion of the dose contained in particles too large to penetrate to the airways of the lungs. This chapter does not advise on the outcome of such changes, rather the interpretation of data obtained using the methods described should be developed by the user after discussion with the appropriate receiving organization.

1.3 Rationale

Following the process described in the predecessor Canadian Group standard (CAN/CSA/Z 264.1-02: 2008; Canadian Standards Association, Mississauga, Ontario, Canada), (1602) includes separate testing by a multi-stage cascade impactor to determine aerosol aerodynamic particle size distribution (APSD) and tests mimicking fully coordinated and fully uncoordinated use, with tidal breathing simulated to establish emitted mass (EM) with the pMDI-spacer/VHC coupled to a breathing simulator. It is now possible to combine both APSD and EM measurements by means of a mixing induction port (Copley Scientific Ltd., Nottingham, UK, or RDD-Online, Richmond, VA, USA). However, the more conservative approach adopted herein reflects the absence of method validation data for multiple marketed drug products delivered by pMDI. Spacers and VHCs modify the APSD substantially from what is emitted by the pMDI when used alone. In particular, almost all of the mass fraction of drug associated with particles having ballistic trajectories, by virtue of propellant expansion upon actuation, are contained within the add-on device. The user should refer to the Canadian Group standard for further interpretation of changes in APSD brought about by the presence of a spacer or VHC.

1.4 Recommendations

The use of the induction port identified in *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* (601) is recommended for compliance with the pharmacopeial standard. However, it is acknowledged that alternative designs of the

induction port, as yet to be included as pharmacopeial standards, offer unique testing opportunities. These are either anatomically correct or idealized to have aerosol transport properties similar to an anatomically correct induction port. Given the widespread use of spacers/VHCs for infants and small children, it is important to note that many of these anatomically appropriate induction ports are scaled in terms of such potential users (e.g., the "Alberta" idealized induction ports, developed at the University of Edmonton, Canada, and available from Copley Scientific Ltd., Nottingham, UK). The user is free to adopt such an induction port but should specify the induction port design and age group with which the induction port will be used.

The evaluation of facemask performance requires a model face of the appropriate age range specified in the labeling for the add-on device (i.e., infant, small child, or adult). This arrangement is necessary because there is no other way to accurately simulate the magnitude of the dead space or test for the possibility of leak pathways between facemask and face, both of which are known to influence efficiency of the drug delivery. An adult face model is available from Copley Scientific Ltd, Nottingham, UK, but there are currently no commercially available infant or small-child face models. Therefore, because the use of a face model might be an appropriate way to test these add-on devices, the user will need to either acquire a model from one of the research laboratories where age-appropriate face models have been developed and validated, or develop and validate their own design. Whichever pathway is chosen, a description of the model, in particular the dimensions of the face where the facemask comes into contact, should be provided in the data report to the recipient.

1.5 Definitions of Key Terms Relating to This Chapter

Spacers: Open tubes that do not have a valve at the exit nearest the patient interface (either the mouthpiece or facemask) to retain the aerosol before the user can inhale (see *Figure 1*). Spacers simply increase the distance between the pMDI mouthpiece and the mouth of the patient. If the patient exhales instead of inhaling, the medication will be blown out of the spacer and lost to aerosol collection.

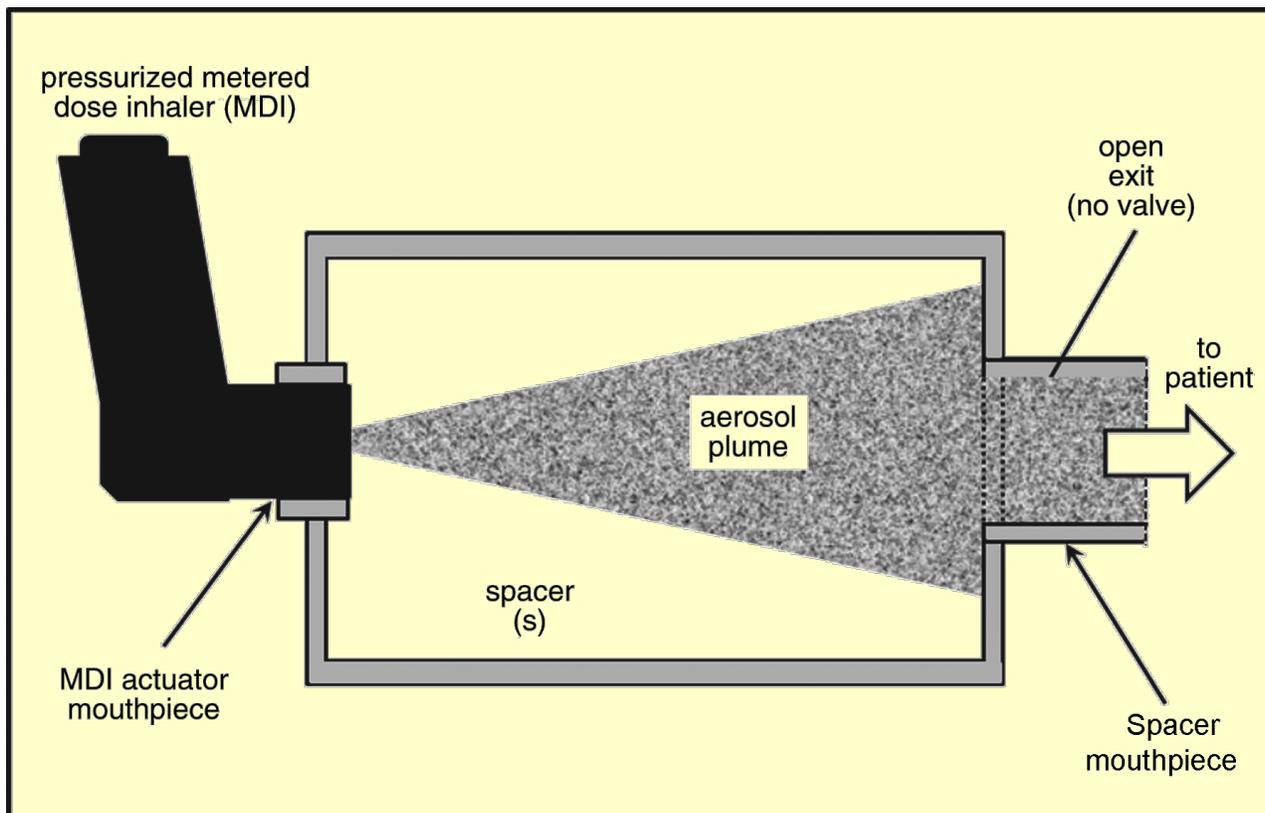


Figure 1. Open-tube spacer with no mechanism for aerosol conservation during exhalation.

VHCs: Contain at least one valve that opens to allow the patient to inhale aerosol on inspiration. This inhalation valve remains closed at other times during each breathing cycle (see *Figure 2*). Some VHCs may also contain an exhalation valve that is open only during exhalation to direct the exhaled flow away from the inhalation valve.

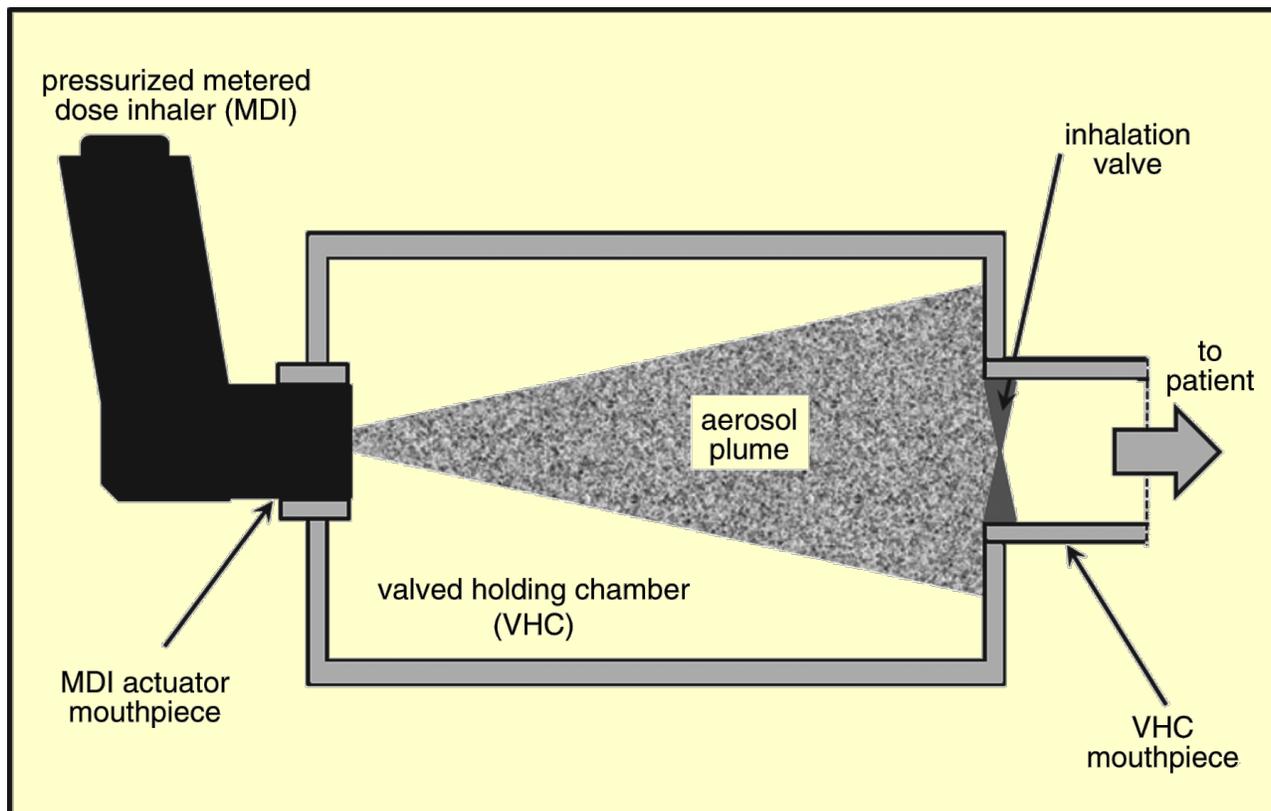


Figure 2. Conventional VHC with valve opening upon inhalation, accepting pMDI with forward-firing actuator mouthpiece.

Integral actuator: A companion piece with some spacers and VHCs that accepts a pMDI canister removed from its actuator-mouthpiece. This so-called “reverse firing” feature enables the design to be modified such that the built-in actuator faces away from rather than toward the user. In the example illustrated in *Figure 3*, the spacer operates in this way. It has some of the characteristics of a VHC, in that an air dam is created by closure of the valve located distally from the user on exhalation into the chamber. This type of spacer can therefore be evaluated as if it were a VHC, because the aerosol released on pMDI actuation is conserved during exhalation.

Note—With all types of spacers/VHCs, the aerosol characteristics in terms of the APSD may be different from those of the pMDI alone.

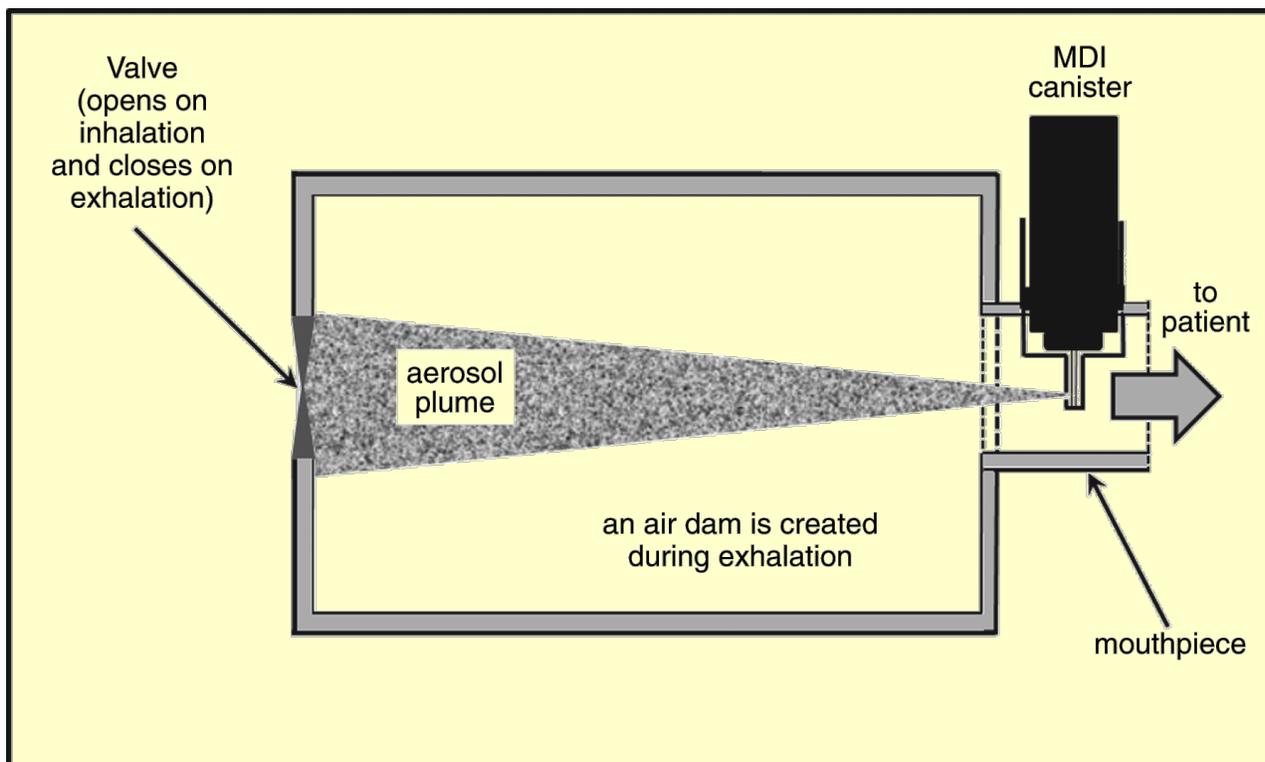


Figure 3. Holding chamber with “reverse-firing” integral actuator for pMDI canister.

1.6 Choice of Drug Product with Which to Test

It is understood that testing will take place with each single active-component drug product or with each strength of multi-strength drug products for which the spacer/VHC is indicated for use on the prescribing information for the add-on device, or as requested by the recipient of the data.

2. TEST METHOD SELECTION

2.1 Spacer/VHC Configurations

Four spacer/VHC configurations have been identified that require different test methods to complete the evaluation process (see *Table 1*).

Table 1. Identification of Test Conditions based on Configuration of the Spacer/VHC

Condition	Spacer	VHC	Facemask	Mouthpiece
A	+ ^a	- ^b	-	+
B	+	+	+	-
C	-	+	-	+
D	-	+	+	-

^a + denotes that this item was part of the condition.

^b - denotes that this item was not part of the condition.

In condition A, the device being tested is a spacer with a mouthpiece. Likewise, in condition B,

the device under test is a spacer with a facemask. In condition *C*, the device is a VHC with a mouthpiece, and in condition *D* the device is a VHC with a facemask. The evaluation of devices without patient interface (i.e., those intended for use by patients on mechanical ventilation) is outside the scope of this chapter.

PATHWAY A

Pathway A (see *Figure 4*) is selected for a spacer with a mouthpiece and comprises two tests: 1) APSD measurement with no delay (see *Part 1A* testing); and 2) EM by breathing simulation—fully coordinated (see *Part 2A* testing). Testing with a delay would be inappropriate for this configuration because the medication cannot be retained by the spacer during the delay interval.

PATHWAY B

Pathway B (see *Figure 4*) is selected for a VHC with a mouthpiece and comprises four tests: 1) APSD measurement with no delay (see *Part 1A* testing); 2) APSD measurement with delay (see *Part 1B* testing); 3) EM by breathing simulation—fully coordinated (see *Part 2A* testing); and 4) EM by breathing simulation—fully uncoordinated (see *Part 2B* testing).

PATHWAY C

Pathway C (see *Figure 4*) is selected for a spacer with a facemask and comprises one test with the facemask in place: 1) EM by breathing simulation—fully coordinated. Note that this class of device may also be evaluated for APSD measurement with no delay (see *Pathway A, Figure 4*) by removing the facemask and coupling the spacer on-axis to the induction port entry with a suitable connector. Testing with delay would be inappropriate for this configuration, because the medication cannot be retained by the spacer during the delay interval.

PATHWAY D

Pathway D (see *Figure 4*) is selected for a VHC with a facemask and comprises two tests with the facemask in place: 1) EM by breathing simulation—fully coordinated; and 2) EM by breathing simulation—fully uncoordinated. Note that this class of device may also be evaluated for APSD measurement with and without delay (see *Pathway B, Figure 4*) by removing the facemask and coupling the VHC on-axis to the induction port entry with a suitable connector.

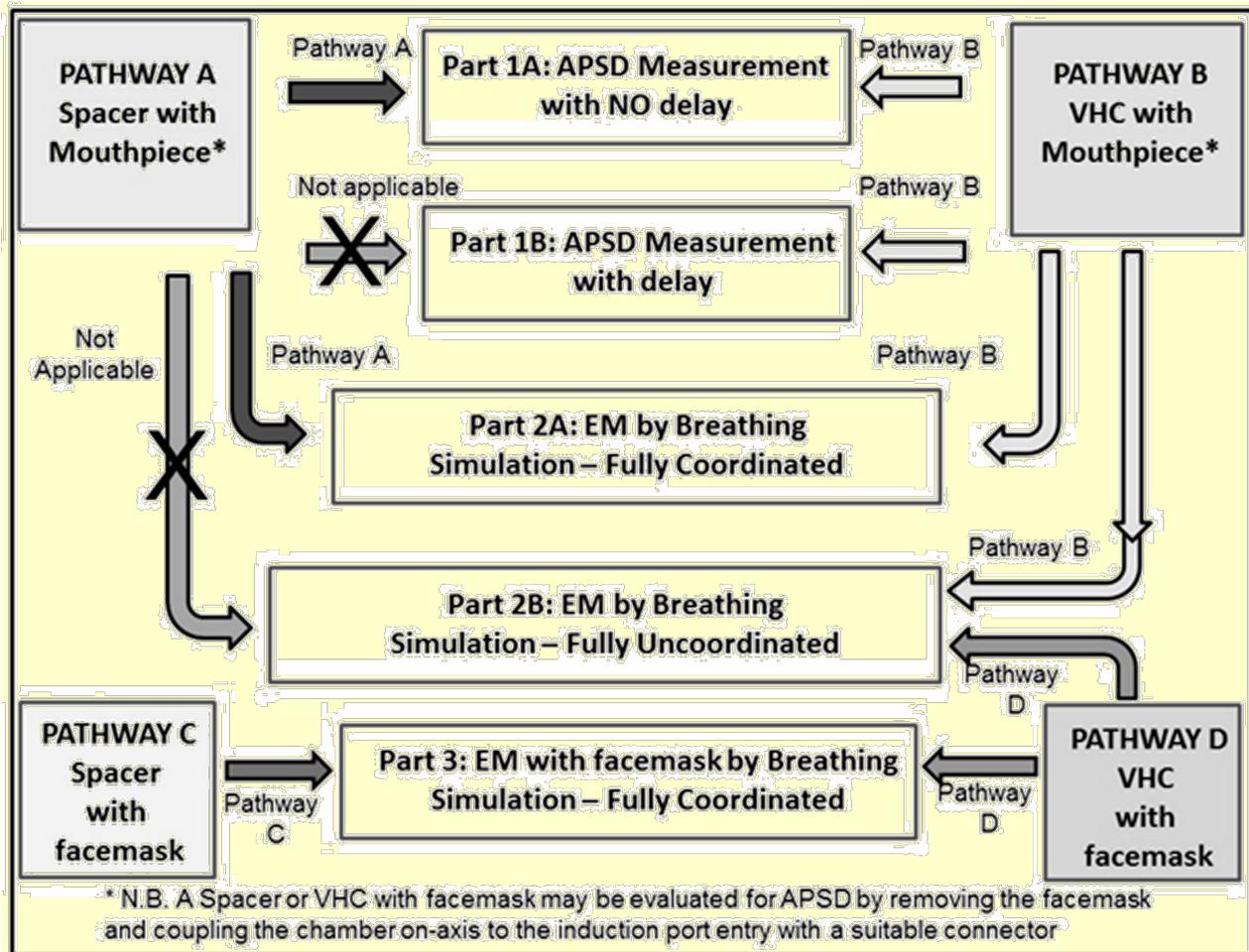


Figure 4. Decision tree for test selection by add-on device type.

2.2 Comments on Test Methods

The tests in *Parts 1A and 1B* (see *Table 2*) are an extension of the procedures described in (601). However, sampling at a constant flow rate via a cascade impactor does not do more than enable the assessment of the APSD of the emitted aerosol. In the later parts of chapter *Products for Nebulization—Characterization Tests* (1601), tests are described in which tidal breathing is simulated to assess spacer/VHC performance in terms of the delivered dose (equivalent to EM) in situations that are more representative of patient use. In *Part 1A* (see *Table 2*), the purpose is to enable the determination of metrics for which a direct comparison can be made with and without the add-on device. This methodology is the same as that described using either *Apparatus 1* or *Apparatus 6* in (601) and is therefore not repeated here. In *Part 1B* (see *Table 2*), delayed sampling is introduced because add-on devices, especially VHCs, are widely prescribed for individuals who, for whatever reason, are unable to coordinate pMDI actuation with the onset of inhalation as described for the pMDI alone in “patient information” and “instructions for use”.

Table 2. In Vitro Tests for Spacers and VHCs

Test	Delay at Constant Flow Rate	Breathing Simulation	Suitability	Clinical Relevance
Part 1A: Measurement of APSD with 4-L sample volume	None	Not applicable	Spacers and VHCs	Baseline performance compared with pMDI alone
Part 1B: Measurement of APSD with 4-L sample volume	For example 2, 5, or 10 s ^a	Not applicable	VHCs only	Simulation of a delay by a poorly coordinated user
Part 2: Measurement of EM with sufficient number of inhalations following guidance in the patient instructions to ensure that the drug is delivered from the VHC as intended ^b	Not applicable	Filter collection of aerosol simulating tidal breathing in coordinated and uncoordinated use	VHCs only	Simulation of optimum use (fully coordinated with pMDI actuation) and fully uncoordinated use
Part 3: Measurement of EM delivered by facemask with sufficient number of inhalations following guidance in the patient instructions to ensure that the drug is delivered from the VHC as intended ^b	Not applicable	Filter collection of aerosol behind lips or at nares of model, simulating tidal breathing in coordinated use	VHCs only	Simulation of use with facemask applied with a 1.6- kg force ^c
<p>^a Other delay intervals may also be used as required, if validated and justified.</p> <p>^b Representative breathing patterns for different patient ages are listed in <i>Table 2</i>; other patterns may be used as required, if validated and justified.</p> <p>^c 1.6-kg applied force has been shown to be clinically appropriate, but the force used may differ from this value if justified.</p>				

The tests in *Part 2* (see *Table 2*) evaluate the performance of the inhalation valve (and exhalation valve, if equipped) of the add-on device. This evaluation is accomplished by simulating tidal breathing, with parameters that are appropriate for the intended user group (e.g., infant, small child, or adult). This testing approach creates the continually varying flow conditions that are expected in use. Note that in the determination of EM, a sufficient number of inhalations should be taken, following the advice provided by the manufacturer of the VHC, to ensure that the intended amount of drug is delivered.

All of the above tests are undertaken with the facemask removed from the spacer/VHC equipped with this type of patient interface. This modification is allowed so that the device can be readily interfaced by fitting the facemask adapter directly to the entry of the induction port, without the complication of a direct facemask-to-induction port connection, whose internal

dead space would likely be both ill defined and unrepresentative of the “in-use” condition. Spacers/VHCs using a facemask as the patient interface should be evaluated ideally with the facemask in place (see *Rationale*). The desirable approach is such that in vitro testing makes use of a model face and is representative of the age range for which the spacer/VHC is intended. There are currently no commercially available model faces; however, the peer-reviewed literature contains several articles providing the necessary technical information to enable suitable age-appropriate model(s) to be created for the purpose of testing spacer/VHCs. It is also important that the surfaces of the face coming into contact with the facemask have mechanical characteristics (i.e., deformability) that are representative of the skin and underlying soft tissues when the facemask is applied to the face with an appropriate force. In *Part 3* (see *Table 2*), the spacer/VHC is evaluated by a breathing simulation with the facemask fitted, mimicking similar fully coordinated use as defined in *Part 2* (see *Table 2*). By comparing the EM of the drug with and without the use of a facemask, it is possible to assess the influence of the facemask.

Note—Not all of the tests described in this chapter are applicable to open-tube spacers because such devices require fully coordinated use; otherwise, drug delivery will be significantly reduced. *Figure 4* shows the decision tree to be followed so that the tests chosen are appropriate to the type of add-on device being evaluated.

Table 2 further defines the test, method, applicable device, and clinical relevance of the procedures that are described in this chapter. Example delay intervals of 2, 5, and 10 s are noted in the table, although other delay intervals may be substituted or added as required and justified. For the testing in *Part 3* (see *Figure 4*), it is recognized that currently there is no standardized and commercially available series of models representing the most widely recognized age categories for which this interface may be developed, i.e., infant, small child, and adult. However, several face models have been described in the peer-reviewed literature or are in late-stage development and validation. During the interim period, before validated commercially available models become available, the user is advised to select one of these models or develop their own, providing justification for the selection as required. Additionally, *Table 3* shows representative tidal-breathing patterns for the various patient age categories from neonate to adult.

Table 3. Representative Tidal Breathing Patterns

Parameter	Pediatric			Adult	
	Neonate	Infant	Small Child	Normal 1 ^a	Normal 2 ^a
Tidal volume (mL)	25	50	155	770	500
Frequency (min ⁻¹)	40	30	25	12	13
Duty cycle (%) ^{b,c}	25	25	33	33	33
Minute volume (mL)	1000	1500	3875	9240	6500

^a *Normal 1* represents a resting adult of average build; *Normal 2* represents an active adult of large build.

^b Duty cycle = (inspiratory time/total time for a single inspiratory/expiratory cycle) × 100 for regular tidal breathing.

^c *I/E* ratio may also be used as an alternative to duty cycle, where *I/E* = inspiratory time/expiratory time.

Note—With the permission of the Canadian Standards Association (operating as the CSA Group), material is reproduced from CSA Group standard CAN/CSA-Z264.1-02 (R2011), "Spacers and Holding Chambers for Use with Metered-Dose Inhalers" which is copyrighted by CSA Group, 5060 Spectrum Way, Suite 100, Mississauga ON, L4W 5N6. This material is not the complete and official position of the CSA Group on the referenced subject, which is represented solely by the standard in its entirety. Although use of the material has been authorized, CSA is not responsible for the manner in which the data are presented, nor for any interpretations thereof. For more information or to purchase standards from the CSA Group, please visit <http://shop.csa.ca/> or call 1-800-463-6727.

3. MEASUREMENT OF APSD

3.1 No Delay between the pMDI Actuation and Sampling Onset

BACKGROUND

In *Part 1A* (see *Figure 4*), the measurement of APSD from the spacer or VHC with no delay following actuation of the inhaler is defined as an important test of the optimum performance of the device in the hands of the patient. This test also provides information with which to compare the in vitro performance of the pMDI with and without the add-on device present.

TEST PROTOCOLS

If the spacer/VHC is intended for adult use, and comparison is being made with the pMDI alone, follow the procedure given for *Apparatus 1* or *Apparatus 6*, as required, in (601). *Apparatus 1* is the Andersen 8-stage "non-viable" cascade impactor without preseparator, and *Apparatus 6* is the Next Generation Impactor, also without preseparator. Alternatively, the model 150 Marple-Miller impactor (MSP Corp., St. Paul, MN), which has the same stage cut-point sizes at 30 L/min as those of *Apparatus 2*, may be used.

If the spacer/VHC is intended for neonates, infants, or small children, it may be appropriate to use an alternative apparatus that operates at the reduced flow rate(s) more appropriate for these classes of patients. Examples are *Apparatus 6* operated at 15.0 L/min or the pediatric

version of *Apparatus 2* (model 150P Marple-Miller impactor) that functions at either 4.9 or 12.0 L/min.

Conduct the evaluation with the mouthpiece of the spacer or VHC, if so equipped, connected to the test apparatus. If the spacer or VHC is equipped with a facemask, remove the facemask and perform the evaluation with the add-on device connected to the test apparatus by means of the facemask adapter.

In the group of VHCs to be tested, remove each device from its packaging (if supplied), and prepare it for the test by prewashing in accordance with the manufacturer's instructions, if so indicated. If no instructions for preparation are provided, test the device out of the package without prewashing, and note this information in the test report.

Connect the spacer or VHC via the mouthpiece or facemask adapter to the entry to the induction port described in (601). This connection can be made either with a short piece of flexible tubing or with a purpose-built coupling that ensures on-axis alignment with the entry to the induction port. If flexible tubing is used for this connection, ensure that the add-on device is supported in such a way that its long axis aligns with the axis of the induction port entry. Care should be taken to ensure that the coupling, however constructed, does not result in a significant loss of the drug and/or change in measured APSD.

Verify that the coupling is leak tight by first connecting the calibrated gas flow meter to the entry of the induction port and then verifying that the flow rate specified for the test is within $\pm 5\%$ of the desired flow rate. This is achieved by applying a vacuum to the test apparatus by means of an appropriate regulation valve located between the cascade impactor and vacuum source. Remove the flow meter from the induction port and connect the spacer/VHC as described above. Verify that the volumetric flow rate determined by attaching the same flow meter to the pMDI mouthpiece acceptance port of the spacer/VHC is NLT 95% of that obtained at the first flow rate measurement at the entry of the induction port. Turn off the source of vacuum to this apparatus after this check has been completed, and remove the flow meter, insuring the connection between the spacer/VHC and induction port is not disturbed.

The practice of actuating the pMDI with the spacer or VHC initially disconnected from the induction port and then rapidly connecting the add-on device after pMDI actuation should be discouraged, because there is always a small delay interval and medication will be lost in an uncontrolled manner from an open-tube spacer if this procedure is followed.

Follow the directions in the patient information and instructions for use for the pMDI to prime it before inserting into the pMDI adapter of the spacer/VHC. In the case of an add-on device equipped with an integral actuator, after priming the pMDI canister in its mouthpiece adapter as supplied, remove the canister from the adapter, and clean the external valve stem with a suitable wipe. Insert the canister valve stem into the receptacle following the *Instructions for Use* for the particular add-on device, taking care to avoid premature actuation.

In cases where more than one actuation of the pMDI is needed to collect sufficient mass of drug product, follow the directions in the patient information and instructions for use; if no directions are provided, allow a minimum of 30 s between actuations. Turn on the vacuum to the cascade impactor before delivering any actuations. It is important to deliver the minimum number of actuations to obtain an adequate sample of the size-fractionated drug mass for measurements to be made to the same degree of precision as would be the case if the pMDI was evaluated alone.

After the pMDI actuation and aerosol sampling are completed, recover the drug from each component of the cascade impactor, including the induction port, and also from within the spacer/VHC by using a validated method appropriate to the product being measured. Carefully remove the backup filter of the cascade impactor, whose purpose is to capture the drug mass

passing the last impaction stage, from its location, and place it in a suitable container. For each sample, add a known volume of a suitable solvent to the container, and agitate to dissolve the collected drug. Transfer a sample to a cuvette or vial for drug assay using a syringe equipped with an in-line filter to retain any suspended material. Care must be taken with the recovery of a drug retained by the add-on device, because a greater volume of solvent may be needed, compared with volumes needed for recovery from each impaction plate/cup of the impactor. Perform separate assays to measure the mass of drug recovered from each component, using a validated procedure.

Repeat the measurement with the required number of devices and replicates per device.

Typically, five separate spacers or VHCs are each tested once, but other testing protocols may be adopted as required, if validated and justified. These protocols may include replicate measurements per device to obtain information about intra- as well as inter-device performance. Calculate the mean value for each metric described below, together with its standard deviation (SD). Determine the coefficient of variation (CoV) as a percentage, based on the formula:

$$\text{CoV} = [(\text{SD}/\text{mean}) \times 100]$$

Measurements

Total mass of drug collected: This procedure is not a test of the product or add-on device but serves as a system suitability test to ensure that the results are valid. The total mass of drug collected comprises the sum of the drug mass in all of the components, including the pMDI mouthpiece and from within the spacer/VHC [material balance (MB)] divided by the number of actuations of the pMDI. Note that typically up to 5% of the target-delivered label claim may be retained by the cascade impaction apparatus as inter-stage wall losses.

The total mass of drug emitted: The total mass of drug emitted from the spacer/VHC with zero seconds delay [delivered dose, equivalent to emitted mass (EM)] is determined from the sum of the values for the mass of drug collecting in all of the components of the test apparatus divided by the number of actuations of the pMDI. Spacers and VHCs are intended to reduce the mass of coarse particles inhaled by the patient; therefore, an important part of data interpretation of the APSD should involve separate assessments of the pertinent subfractions relevant to the inhalation aerosol product. The precise size limits for these subfractions will be agreed with the recipient of the test data. However, to provide initial guidance on this matter, it is suggested that the following be considered as a minimum:

- Determination of the mass of drug retained by the spacer/VHC together with the mass recovered from the induction port
- Stage-by-stage profile of the remaining EM

Further interpretation of these data is at the discretion of the user. The performance of the spacer/VHC is compared with that of the metered-dose pMDI alone, following the procedure described in (601).

3.2 Delay between the pMDI Actuation and Sampling Onset

[Note—This test is inappropriate for spacers.]

BACKGROUND

This test requires the adaptation of USP's *Apparatus 1* and *Apparatus 6* to accept a means by

which the delay interval can be realized. The actuation of the pMDI into the VHC on test with its mouthpiece or facemask adapter disconnected from the entry to the induction port, followed by connection to the port with the vacuum applied to the apparatus immediately after the delay interval has elapsed, is difficult to perform accurately and may result in leakage or an inaccurate realization of the delay interval. For these reasons, an apparatus of the type described in *Figure 5* is recommended, because it enables the VHC mouthpiece/facemask adapter to remain in position throughout the measurement. The methodology for delay testing is based on the apparatus illustrated, but other equipment offering similar capability may be used if validated.

TEST PROTOCOLS

The vertically mounted shutter plate comprises a circular opening in its upper half and is mounted at setup such that the gap between the VHC mouthpiece/facemask adapter and induction port entry is closed, as illustrated (see *Figure 5*). The induction port and VHC adapter fit tightly into the mounting block. In this position, when vacuum is applied to the test apparatus, air is sampled by the apparatus at the desired flow rate via a bypass channel on the side of the adapter facing the induction port. This arrangement avoids the need to start the flow through the apparatus after the delay interval has elapsed, so that the cascade impactor is always operated at a constant flow rate.

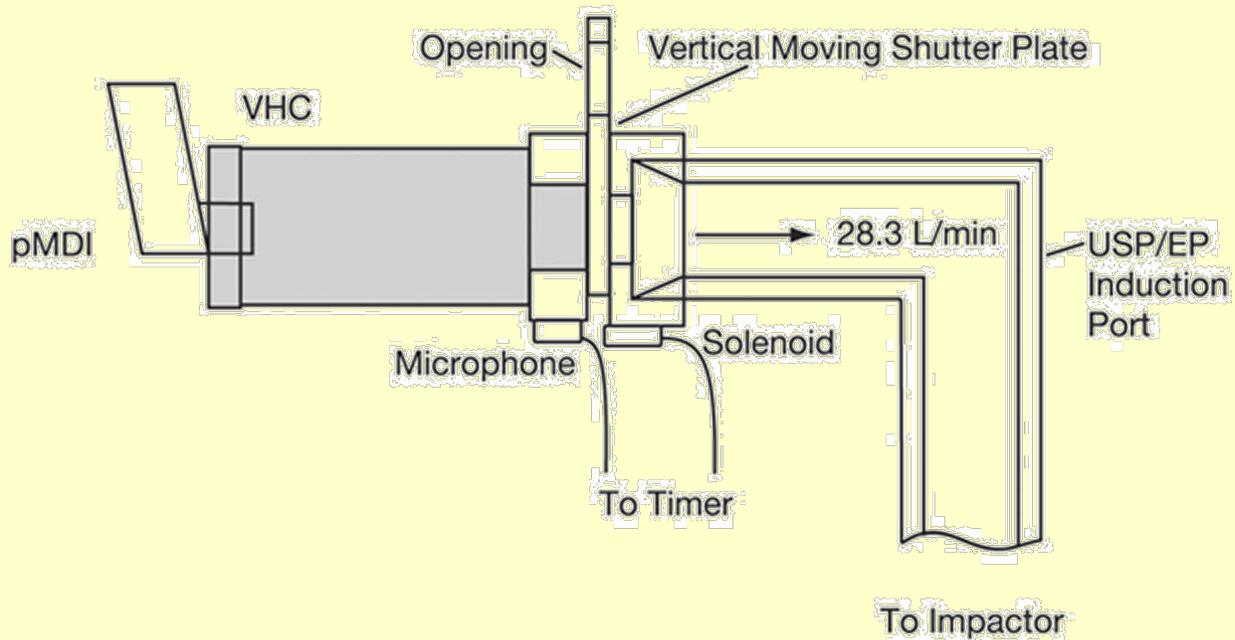


Figure 5. "Delay" apparatus shown with a USP/EP induction port and 8-stage Andersen cascade impactor.

In the configuration described in *Figure 5*, when the pMDI is actuated, a microphone located on the adapter block detects the sound emitted at actuation of the pMDI, starting a timer that operates a solenoid valve that retracts a pin immediately after the preset delay interval has expired. This process permits gravity to operate on the shutter, which drops to the lower position in which the aperture is aligned with the VHC mouthpiece/facemask adapter and induction port entrance. The aerosol retained within the VHC is sampled as soon as the shutter moves to the "open" position. This procedure avoids the risk of capturing any "blow-by" aerosol

that might escape the VHC as the propellant expands immediately following pMDI actuation but would not be inhaled by a user. If blow-by is observed, it should be noted in the test report. In the configuration shown in *Figure 5*, the adapter introduces <5 mL of additional volume to the aerosol pathway from the VHC to the filter, and the minimum delay interval achievable is 1 s. Example delay intervals of 2, 5, and 10 s have been noted earlier in this chapter, although other delay interval(s) may be substituted or added if required and justified.

Securely attach the exit port from the delay apparatus to the entry of the induction port. Connect the VHC via the patient interface (mouthpiece or facemask adapter) to the entry port of the delay apparatus and verify that the coupling is leak tight. This check is most easily done by connecting a calibrated gas flow meter to the port for the pMDI (pMDI adapter) on the VHC and verifying that the volumetric flow rate is NLT 95% of that obtained with the same flow meter connected to the entry of the induction port. This should be done before attaching the delay apparatus, with the vacuum source applied to the test apparatus to achieve the appropriate nominal flow rate. Turn off the vacuum source to this apparatus after this check has been completed, and remove the flow meter. Reconnect the VHC, and turn on the vacuum source again.

Before actuating the pMDI for the first time into the VHC being tested, ensure that the delay apparatus is set such that the shutter is in the "up" or "closed" position. Select the desired delay interval for the timer.

Follow the directions in the patient information and instructions for use before inserting the actuator/mouthpiece of the pMDI into the pMDI adapter of the VHC before the first actuation into the test apparatus. If the spacer/VHC is designed with an integral actuator, after priming the pMDI canister in its mouthpiece adapter as supplied, remove the canister from the adapter and clean the external valve stem with a suitable wipe. Insert the canister valve stem into the receptacle following the instructions for the particular add-on device, taking care to avoid premature actuation.

In cases where more than one actuation of the pMDI is needed to collect sufficient mass of drug product, follow the directions in the patient instructions, or if no direction is provided, allow a minimum of 30 s between actuations. Deliver the minimum number of actuations needed to obtain an adequate sample of the size-fractionated drug mass so that measurements can be made to the same degree of precision as if the pMDI was evaluated alone. Reposition the shutter of the delay apparatus in the up or closed position, and check that the timer is set for the desired delay after each pMDI actuation.

After the sampling part of the measurement is completed, recover the drug from each component of the cascade impactor, including the induction port, and from within the spacer/VHC by using a validated method appropriate to the specific product. In the configuration shown in *Figure 5*, the surface area of the shutter that is exposed to aerosol particles is minimal. Therefore, recovery of drug from this component needs to be done only for the most accurate work; in which case, the inner surfaces of the aperture in the shutter plate and the exit port are washed with recovery solvent. Care should be taken with the recovery of drug retained by the VHC, because a greater volume of solvent than is needed for recovery from each impaction plate/cup of the impactor may be needed to be sure that drug is quantitatively recovered. Assay for the mass of drug recovered from each component separately by using a validated procedure.

Repeat the measurement with the required number of devices and replicates per device.

Typically, five separate spacers or VHCs are each tested once, but other designs, including replicate measurements per device, may be adopted as required, if validated and justified.

Calculate the mean values for each metric described below, together with its SD. Determine the

coefficient of variation (CoV) as a percentage, based on the formula:

$$\text{CoV} = [(\text{SD}/\text{mean}) \times 100]$$

Measurements

Total mass of drug collected: The total mass of drug collected in all of the components, including the pMDI mouthpiece and from within the spacer/VHC (material balance) is divided by the number of actuations of the pMDI. The component of the dose that may escape past the inhalation valve of the VHC on pMDI actuation (due to momentary pressurization of the interior of the chamber) is termed blow-by, and it may not be captured for the assay. If this phenomenon is observed, it must be noted. [Note—Quantification of blow-by is not a practical proposition because the act of collecting the aerosol will inevitably apply back-pressure to the valve, reducing or eliminating the phenomenon altogether.]

The performance of the spacer/VHC tested with delay is compared with that of the spacer/VHC tested with no delay, following the procedure described in (601).

VHCs are intended to reduce the mass of coarse particles inhaled by the patient, so an important part of data interpretation of the APSD should involve separate assessments of the pertinent subfractions relevant to the inhalation aerosol product. The precise size limits for these subfractions will be agreed with the recipient of the test data. However, to provide initial guidance on this matter, it is suggested that the following be considered as a minimum:

- Determination of the mass of drug retained by the spacer/VHC together with the mass recovered from the induction port
- Stage-by-stage profile of the remaining EM

Further interpretation of these data is at the discretion of the user.

4. MASS OF DRUG DELIVERED FROM A SPACER/VHC WHILE SIMULATING PATIENT TIDAL BREATHING

4.1 Without Facemask

Cascade impactors used in the assessment of APSD are designed to operate at fixed flow rates. However well the add-on device may perform during an in vitro test of function using such an apparatus, the operation of critical moving components (i.e., inhalation and exhalation valves of VHCs) is not evaluated in the way that these components would perform when the VHC is used by the patient. An additional test that simulates tidal breathing is therefore included, because this type of respiratory pattern is most commonly encountered with patients using an add-on device, particularly neonates, infants, and children who are incapable of a forced inhalation maneuver. Several representative, age-related breathing patterns are listed in *Table 2*.

There is no breathing simulator recognized in (601). However, (1601) provides for the use of a commercially available breathing simulator that is able to generate the same breathing profiles as those specified in *Table 2*. The methodology provided assumes that equipment meeting this standard is available. *Figure 6* illustrates schematically a setup that has been effective for these measurements, but other configurations may be used as required, if validated and justified.

The filter system used to collect the aerosol at the mouthpiece/facemask adapter of the spacer/VHC should be a suitably validated, low-resistance filter capable of quantitatively

collecting the aerosol and enabling recovery of the drug substance with use of an appropriate solvent. If the filter is contained in its own housing, the dead volume of the filter casing shall not exceed 10% of the tidal volume used in the breath simulation. This restriction will likely make it necessary to use filters without a separate housing when performing tests that simulate neonatal or infant use.

The first part of the test is suitable for both spacers and VHCs, because it simulates the delivery of medication mimicking a fully coordinated user actuating the pMDI at the onset of inhalation. However, the second part of the test is only suitable for VHCs because the pMDI is actuated at the onset of exhalation to simulate a fully uncoordinated user.

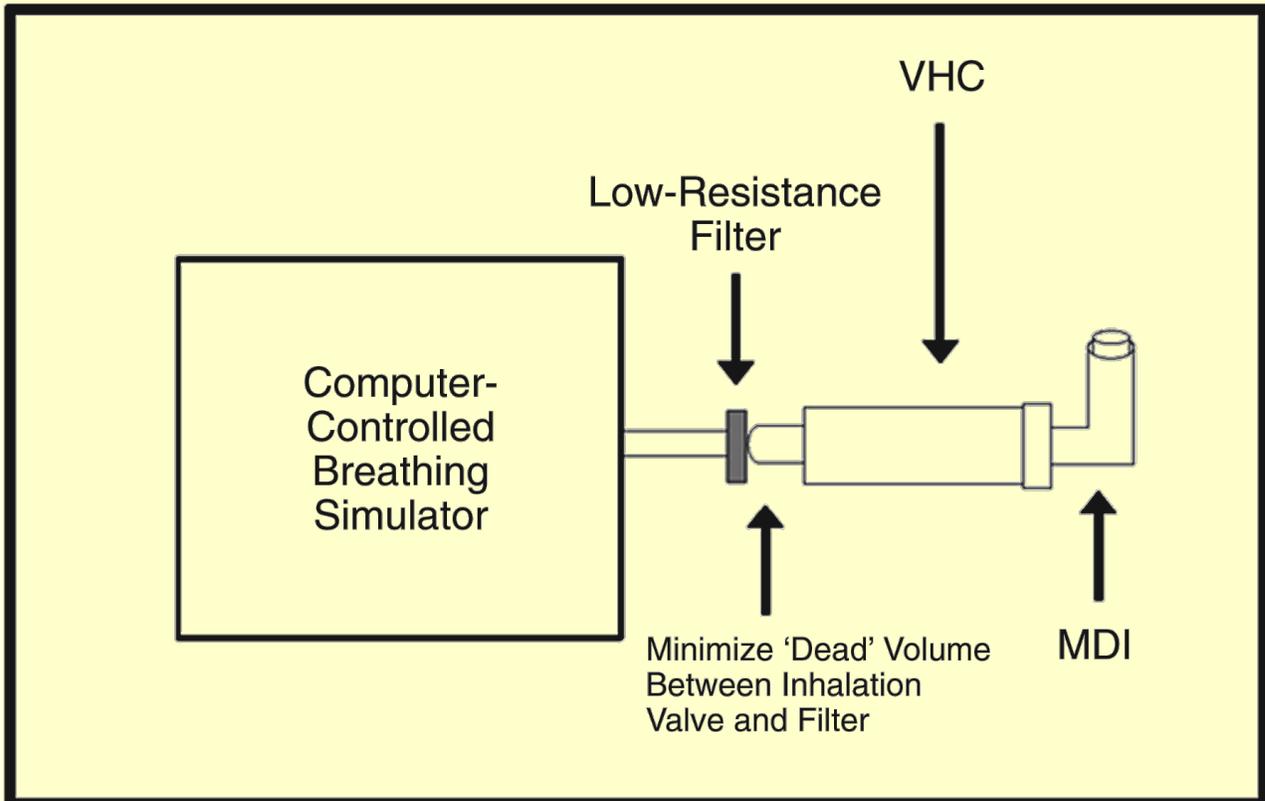


Figure 6. VHC evaluation by breathing simulator.

TEST, PART 1

Set the breathing simulator to the required breathing pattern (identified from *Table 2*) in accordance with the manufacturer's instructions. Alternative breathing patterns may be used if required. Ensure that the simulator has been calibrated before use so that the actual volumes and frequency are within $\pm 5\%$ of the indicated value during each breathing cycle. Calibration syringes are available from various suppliers of mechanical ventilation equipment to verify volumes. To verify frequency, the method varies among breathing simulator manufacturers, and therefore the advice of the particular supplier/manufacturer should be sought if the method is not explicitly stated in the operating instructions for the apparatus.

Ensure that the selected flow waveform from the *Breathing simulator* (Figure 6) is stable before proceeding. Remove each device in the group of spacers or VHCs to be tested from its packaging (if supplied), and prepare for the test by prewashing in accordance with the manufacturer's instructions, if indicated. If no instructions for preparation are provided, test the devices out of package without prewashing and note this fact in the test report.

Connect the mouthpiece or facemask adapter of the spacer/VHC to the appropriate port of the

breathing simulator by means of a short length of flexible hose. Locate the aerosol filter as close as possible to the mouthpiece of the spacer/VHC using an adapter or other means that will enable an airtight seal to be formed with the minimum amount of space (dead volume) between the device and the filter. Ensure that the adapter does not restrict the path of the aerosol.

Filtrete™ (3M Corp, St. Paul, MN) is a suitable electret filter medium that readily releases collected drug quantitatively upon addition of a suitable solvent during the assay procedure. However, other suitable filter media may be used provided that the tester is satisfied that the filter is capable of collecting the entire emitted dose and that drug recovery for assay is quantitative.

Follow the directions in the patient information and instructions for use for the pMDI to prime it before inserting into the pMDI adapter of the spacer/VHC before the first actuation into the test apparatus. In the case of an add-on device equipped with an integral actuator, after priming the pMDI canister in its mouthpiece adapter as supplied, remove the canister from the adapter, and clean the external valve stem with a suitable wipe. Insert the canister valve stem into the receptacle by following the instructions for use for the particular add-on device, taking care to avoid premature actuation. This test typically should require only one actuation of the pMDI into the spacer/VHC per determination. However, for certain highly potent products delivering low unit mass of drug per actuation, more than one actuation of the pMDI may be needed to collect a sufficient mass of drug product. Under such circumstances, follow the directions in the patient information and instructions for use for the pMDI, or allow a minimum of 30 s between actuations if no direction is provided. It is important to deliver the minimum number of actuations to obtain an adequate sample of the drug mass for measurements to be made with acceptable precision.

For the first part of the test, perform a single actuation timed to coincide with the beginning of an inhalation. Allow sampling to occur for five additional breathing cycles. If additional actuations are required to improve analytical sensitivity, ensure that the sampling time is of sufficient length for the spacer/VHC to be emptied of remaining aerosol before the next actuation is delivered, and do not disconnect the spacer/VHC from the breathing simulator between actuations.

Remove the filter carefully from its location, and place it in a suitable container. Add a known volume of a suitable solvent to the container and agitate to dissolve the collected drug. Transfer a sample to a cuvette or vial for drug assay using a syringe equipped with an in-line filter to retain any suspended material. Recover the mass of drug from the filter by using a validated procedure appropriate to the specific product, and assay for the mass of drug collected by using a validated procedure. Calculate the total mass of drug per actuation; this is the emitted mass in the "fully coordinated" condition (EM_c). Repeat the measurement with the required number of devices and replicates per device.

TEST, PART 2

[Note—This part of the test is inappropriate for spacers.] For the second part of the test, either clean the VHC between measurements, or evaluate a new VHC out of its packaging, as required and justified. Report whether cleaning or replacement of the VHC was carried out. Repeat the procedure above in *Test, Part 1* with the same VHC, this time actuating the pMDI timed to coincide with the onset of exhalation. Calculate the total mass of drug per actuation; this is the EM in the "fully uncoordinated" condition (EM_{uc}). Repeat the measurement with the required number of devices and replicates per device.

Construct a table of data showing the mass of drug collected on the filter for each individual measurement of EM_c and EM_{uc} . Calculate the mean and SD for each group of measurements. In the example shown in Table 4, there are three separate VHCs (VHC-1, VHC-2, and VHC-3), each tested three times (R1, R2, and R3) for each condition.

Table 4. Emitted Mass of Drug with VHC Evaluated by Tidal Breathing Simulator

Condition	VHC-1			VHC-2			VHC-3			Group	
	R1	R2	R3	R1	R2	R3	R1	R2	R3	Mean	SD
Coordinated	EM_{c1-1}	EM_{c1-2}	EM_{c1-3}	EM_{c2-1}	EM_{c2-2}	EM_{c2-3}	EM_{c3-1}	EM_{c3-2}	EM_{c3-3}	\overline{EM}_c	SD_c
Uncoordinated	EM_{uc1-1}	EM_{uc1-2}	EM_{uc1-3}	EM_{uc2-1}	EM_{uc2-2}	EM_{uc2-3}	EM_{uc3-1}	EM_{uc3-2}	EM_{uc3-3}	\overline{EM}_{uc}	SD_{uc}

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However, other designs may be used following discussion between the user and recipient of the data.

Compare the magnitudes of

$$\overline{EM}_{uc}$$

and

$$\overline{EM}_c$$

4.2 With Facemask

The purpose of this test is to provide confirmation that the emitted mass from a spacer/VHC equipped with a facemask is comparable to that obtained in the fully coordinated simulation

with the facemask removed. Facemasks are widely prescribed for infants, small children, and adults who lack coordination to use a mouthpiece-based product. The evaluation of spacers/VHCs supplied with a facemask, rather than a mouthpiece, requires additional attention beyond the methods described previously, because the facemask itself has a major part to play in the transport of the drug-containing aerosol particles from the add-on device to the patient (*Figure 7*). It is therefore important that the facemask be tested in situ as part of the add-on device, rather than separately. A critical component of the test apparatus is the representation of a human face appropriate to the age range for which the add-on device is intended (e.g., neonate, infant, small child, or adult). This is an active topic of research and there are, as of yet, no standardized models that can be defined as apparatus in a way that is similar to what is done for cascade impactors in the aerodynamic particle size analysis of pMDI-generated aerosols, although an adult face model is available commercially (Copley Scientific Ltd., Nottingham, UK). A review of face models [see Mitchell, JP. Appropriate face models for evaluating drug delivery in infants and small children: the current situation and prospects for future advances. *J Aerosol Med.* 2008;21(1):97–111] provides a summary of the prerequisites for a model face suitable for spacer/VHC facemask testing.

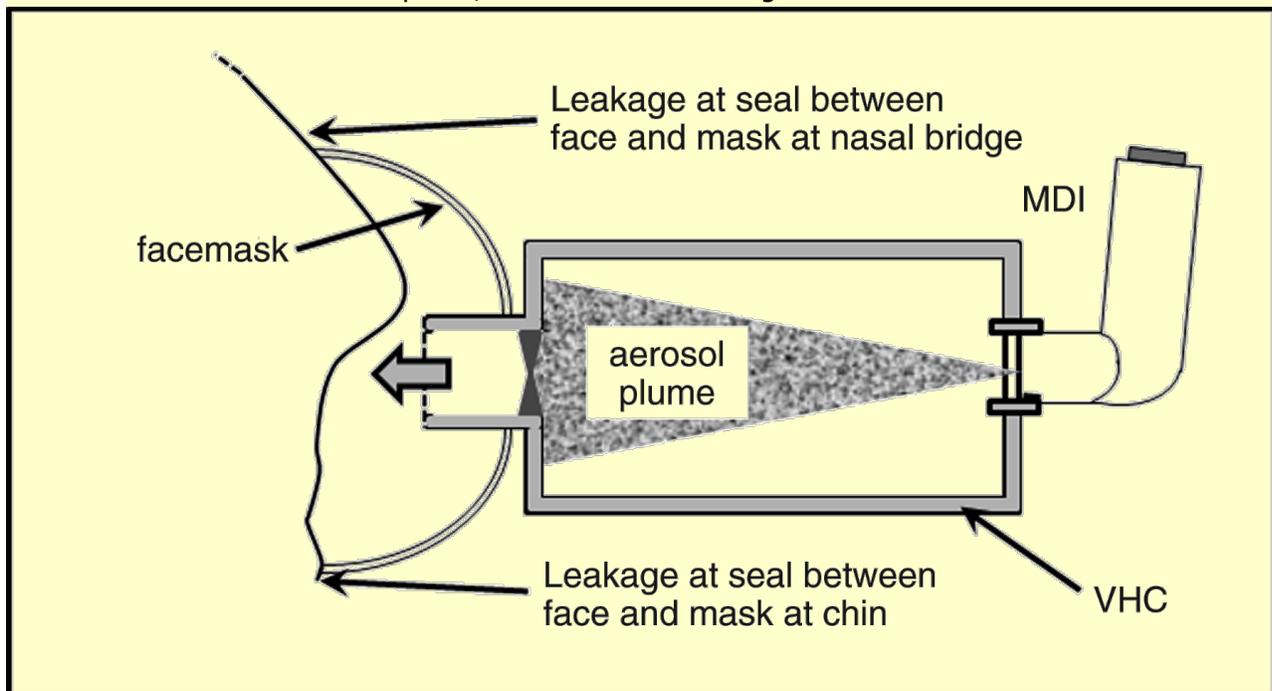


Figure 7. VHC with facemask applied to face.

Prerequisites for face models are the following:

- Appropriate facial dimensions for the intended user age range
- Ability to apply the facemask with the predicted amount of dead space when it is applied with a clinically appropriate force to the model
- Physiologically accurate soft facial tissue modeling around the chin, cheeks, and nose where the facemask makes contact
- Means of correctly mounting the spacer/VHC so that the facemask is oriented with the correct alignment to the face, as would occur when in use by a patient

[Note—Some models may include anatomically accurate realization of the upper airway (nasal or oropharynx), with the aerosol collection filter located at the distal port of the model

representing the entry to the lungs.] For the sake of simplicity and to realize a measure of spacer/VHC performance comparable to the measure obtained with facemask removed in *Part 2* (see *Figure 4*), it is assumed that the model face simulates open-mouth breathing without an anatomically accurate upper airway, and that the aerosol collection filter is located in a cavity immediately behind the lips of the model. The mass of drug is reported as the emitted mass (EM_{facemask}).

Select a face model appropriate to the intended age range for the spacer/VHC-facemask, and mount the face model in an appropriate fixture. The fixture should enable the facemask to be located at an appropriate angle to the face model, such that an effective seal between the facemask and face model is created with a clinically appropriate force. This is typically a loading force of 1.6 kg but may differ from this value if justified by the design of the facemask. The arrangement shown schematically in *Figure 8* is one way of achieving the desired result, but other approaches may be adopted as required, if validated and justified.

Locate and secure the filter media in the cavity behind the lips of the face model. Filtrete™ (3M Corp, St. Paul, MN) is a suitable Electret filter medium that readily releases collected drug quantitatively upon addition of a suitable solvent during the assay procedure. However, another suitable filter medium may be used, provided that the tester is satisfied that it is capable of collecting the entire emitted dose, and that drug recovery for the assay is quantitative.

Verify that a seal has been obtained between the facemask and face model. This is conveniently done by connecting the outlet (distal) port of the face model, using a short length of flexible hose, to an in-line calibrated gas flow meter whose distal port is in turn connected to the vacuum source via a regulating valve capable of setting the flow rate to a suitable value, typically 30 L/min. Connect a similar flow meter to the port for the pMDI (pMDI adapter) on the spacer/VHC, and verify that the volumetric flow rate is NLT 90% of that obtained with the in-line flow meter between face model and regulating valve. Turn off the vacuum source after this check has been completed, and disconnect the flexible hose from the in-line flow meter. Remove the upstream flow meter from the pMDI adapter of the spacer/VHC.

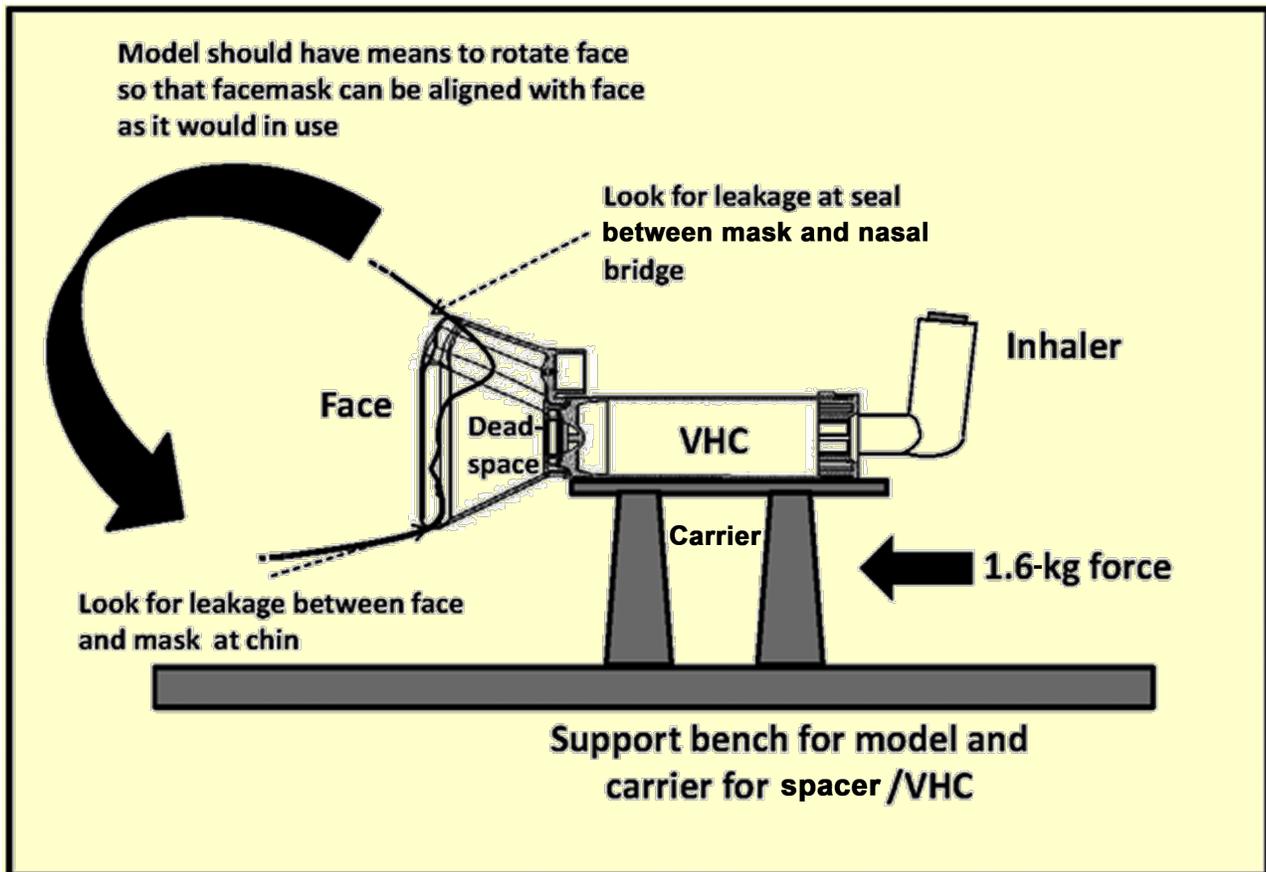


Figure 8. Mount for face model with alignment fixture including carriage for spacer/VHC; a fixed (i.e., 1.6-kg) weight acts on the pulley below the VHC and draws the carriage toward the face with the required force.

Connect the outlet (distal) port of the face model to the appropriate port of the breathing simulator using a short length of flexible hose. Set the breathing simulator to the required breathing pattern (identified from *Table 2*) in accordance with the manufacturer's instructions. Alternative breathing patterns may be used if required. Ensure that the simulator has been calibrated before use so that the actual volumes and frequency are within $\pm 5\%$ of the indicated value during each breathing cycle. Calibration syringes are available from various suppliers of mechanical ventilation equipment to verify volumes. To verify frequency, the method varies among breathing simulator manufacturers, and therefore the advice of the particular supplier/manufacturer should be sought if the method is not explicitly stated in the operating instructions for the apparatus. Ensure that the selected flow waveform from the breathing simulator is stable before proceeding.

Follow the directions in the patient information and instructions for use for the pMDI to prime it before inserting it into the pMDI adapter of the spacer/VHC before the first actuation into the test apparatus. In the case of an add-on device equipped with an integral actuator, after priming the pMDI canister in its mouthpiece adapter as supplied, remove the canister from the adapter, and clean the external valve stem with a suitable wipe. Insert the canister valve stem into the receptacle according to the instructions for use for the particular add-on device, taking care to avoid premature actuation. Typically, it should require only one actuation of the pMDI into the spacer/VHC per determination. However, for certain highly potent products delivering a low-unit mass of drug per actuation, more than one actuation of the pMDI may be

needed to collect a sufficient mass of drug product. Under such circumstances, follow the directions in the patient instructions, or allow a minimum of 30 s between actuations if no direction is provided. Deliver the minimum number of actuations needed to obtain an adequate sample of the drug so that mass measurements can be made with acceptable precision. Perform a single actuation, timed to coincide with the beginning of an inhalation. Allow sampling to occur for five additional breathing cycles. If additional actuations are required to improve analytical sensitivity, ensure that the sampling time is of sufficient duration for the spacer/VHC to be emptied of remaining aerosol before the next actuation is delivered, and do not disconnect the spacer/VHC from the breathing simulator between actuations.

Following the tests, remove the filter carefully from its location, and place it in a suitable container. Add a known volume of a suitable solvent to the container and agitate to dissolve the collected drug. Transfer a sample to a cuvette or vial for drug assay using a syringe equipped with an in-line filter to retain any suspended material. Recover the mass of drug from the filter by using a validated method appropriate to the specific product, and assay for the mass of drug collected by using a validated procedure. Calculate the total mass of drug per actuation; this is the emitted mass in the “fully coordinated” condition with facemask fitted to the add-on device (EM_{c-fm}). Repeat the measurement with the required number of devices and replicates per device.

Construct a table of data showing the mass of drug collected on the filter for each individual measurement of EM_{c-fm} . Transfer the values of EM_c from the test undertaken in *Part 2* (see *Figure 4*) in which the facemask adapter was removed for comparison of emitted mass, simulating fully coordinated use by a breathing simulator. Calculate the mean and SD for each group of measurements. In the example shown in *Table 5*, there are three separate VHCs (VHC-1, VHC-2, and VHC-3), each tested three times (R1, R2, and R3) for each condition. However, other designs may be used following discussion between the user and recipient of the data.

Table 5. Measures of Emitted Mass of Drug with VHC with and without Facemask Attached Evaluated by Tidal Breathing Simulator

Facemask	VHC-1			VHC-2			VHC-3			Group	
	R1	R2	R3	R1	R2	R3	R1	R2	R3	Mean	SD
Present	$EM_{c-fm1-1}$	$EM_{c-fm1-2}$	$EM_{c-fm1-3}$	$EM_{c-fm2-1}$	$EM_{c-fm2-2}$	$EM_{c-fm2-3}$	$EM_{c-fm3-1}$	$EM_{c-fm3-2}$	$EM_{c-fm3-3}$	\overline{EM}_{c-fm}	SD_{c-fm}
Facemask removed	EM_{c1-1}	EM_{c1-2}	EM_{c1-3}	EM_{c2-1}	EM_{c2-2}	EM_{c2-3}	EM_{c3-1}	EM_{c3-2}	EM_{c3-3}	\overline{EM}_c	SD_c

Compare the magnitudes of

EM_{c–fm}

and

EM_c

■ 2S (USP39)

BRIEFING

⟨1782⟩ **Vibrational Circular Dichroism Spectroscopy—Theory and Practice.** The General Chapters—Chemical Analysis Expert Committee proposes this new general chapter to provide information on the theory and practice of vibrational circular dichroism (VCD), in support of another proposed chapter in this issue of *PF* titled *Vibrational Circular Dichroism Spectroscopy* (782). A *Stimuli* article in *PF* 39(4) titled *Vibrational Circular Dichroism as a New Technology for Determining the Absolute Configuration, Conformation, and Enantiomeric Purity of Chiral Pharmaceutical Ingredients* provides insight into the rationale for adopting the technology for absolute configuration and enantiomeric purity determination.

(GCCA: K. Zaidi.)

Correspondence Number—C149025

Comment deadline: November 30, 2015

Add the following:

■ **⟨1782⟩ VIBRATIONAL CIRCULAR DICHROISM SPECTROSCOPY—THEORY AND PRACTICE**

1. INTRODUCTION
2. DEFINITION OF VIBRATIONAL CIRCULAR DICHROISM
3. VCD INSTRUMENTATION
4. MEASUREMENT OF VCD SPECTRA
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1. INTRODUCTION

Chirality is a ubiquitous aspect of the world of three spatial dimensions. Molecules that possess sufficient structural complexity so that their mirror-image structures are nonsuperimposable are termed chiral. For chiral pharmaceutical molecules, two important structural measures critical to their physical characterization are absolute configuration (AC) and enantiomeric excess (EE), also termed enantiomeric purity. The AC of a chiral molecule specifies its three-dimensional structure in space and distinguishes it from its mirror-image structure. Structures related by mirror symmetry are known as "enantiomers". The AC of a particular enantiomer is critical to its action as a pharmaceutical agent because mirror-image structures, i.e., enantiomers, have different therapeutic effects, both desired and undesired. The EE of a sample specifies, usually as an excess percentage, the relative amounts of enantiomers. An EE of 100% is a pure enantiomer (100% enantiomeric purity), 0% is a racemic mixture with equal amounts of enantiomers and no excess, and -100% specifies a pure sample of the opposite enantiomer with respect to the EE definition.

The principal chapter that addresses molecular chirality in the *USP* is *Optical Rotation* (781). The optical rotation (OR) of a sample is the measure of the angle of rotation of a plane of polarized light by a chiral sample at a particular wavelength, typically in the visible or near-UV region. The AC and EE of any sample are determined by a measure of its OR if the measurement has been previously calibrated using a sample of known AC and EE. The sign of the OR determines the larger enantiomer amount, and the magnitude of the OR determines the EE, where 100% EE corresponds to the maximum OR possible for the chiral molecule under consideration. Although OR is a simple, well-established method for determining AC and EE relative to their known calibrations, not all molecules have measurable OR, particularly with limited sample quantities in which the presence of chiral impurities cannot be determined by an OR measurement. The OR is simply a number with no structural information about the sample molecule.

In the past several decades, the AC of an unknown chiral molecule has been determined using

the Bijvoet method of single-crystal X-ray crystallography. The Bijvoet method requires a single high-purity crystal of the molecule with a single chiral phase. Analysis by this method is not always possible, e.g., for noncrystallizable liquids or insoluble solids, and in many cases analysis using the Bijvoet method requires lengthy efforts and time to achieve crystallization.

This general information chapter presents a technique for the determination of AC that relies on vibrational circular dichroism (VCD), a procedure that is now widely used throughout the pharmaceutical industry for chiral molecules for which the AC is unknown. The technique involves comparing the measured VCD spectrum for a chiral molecule to the quantum chemistry calculation for the same molecule. If the measured and calculated VCD spectra show agreement on the principal features and their signs, this means that the AC of the physical sample is the same as the AC of the structure used in the calculation. If the signs are opposite, the AC of the sample is the opposite of that used in the calculation.

As described below, statistical methods have been developed for assessing the degree of confidence that the AC has been correctly determined by the VCD method. Usually the AC of a chiral molecule is specified by connecting its structural chirality, labeled with *R* or *S* for chiral centers or *P* or *M* for chiral axes, to the sign of its measured OR as (+) or (-). Once the AC of a molecule has been determined by VCD, for example, (*S*)-(-)- α -pinene, its VCD spectrum can become a USP Reference Standard of the AC of this molecule, and the AC of any subsequent sample of this molecule can be determined by comparison to its VCD Reference Standard. An important advantage of determining AC by VCD is the spectral richness of a VCD spectrum that supports the simultaneous determination of the structural identity of the molecule and its absolute chirality. In contrast to OR, every chiral molecule has a VCD spectrum, because all molecules have infrared (IR) absorption bands, each one of which acts as a chromophore for a VCD spectrum. VCD also is sensitive to molecular conformations. As demonstrated below, information about the conformation of a chiral molecule is obtained as a bonus from the VCD determination of AC, but AC is the principal informational content of chiral pharmaceutical molecules. An extensive body of literature describing the AC of biological molecules has been created over the past 30 years and can be used as a basis for determining secondary and higher-order structural states of biological drug substances. Stereo-specific methods using VCD can be developed to characterize the production, formulation, and stability of biopharmaceutical products.

This chapter also presents a method for the determination of EE using VCD. EE determination with VCD takes advantage of the fact that the magnitude of a VCD spectrum, measured as the circular polarization absorbance difference for a constant parent IR absorbance spectrum, is directly proportional to EE with no offset. Thus, the maximum relative size of a VCD spectrum is obtained for an EE of 100%, is zero for an EE of 0% (racemic mixture), and is maximum with opposite signs (all positive VCD bands are negative and all negative VCD bands are positive) for an EE of -100%. The advantage of VCD EE determination is that the EE of any molecular sample can be determined by a single VCD measurement once the relative size of VCD for 100% EE (or any %EE value) is known for that molecule. Separation of enantiomers with chiral chromatography is not needed.

2. DEFINITION OF VIBRATIONAL CIRCULAR DICHROISM

VCD is defined as:

$$\Delta A = A_L - A_R$$

ΔA = the difference in the IR absorbance

A_L = sample absorbance A for left circularly polarized (LCP) radiation

A_R = sample absorbance A for right circularly polarized (RCP) radiation

Unpolarized IR absorbance intensity is defined as the average of LCP and RCP intensities:

$$A = (A_L + A_R)/2$$

The IR intensities of enantiomers are identical, whereas enantiomers have equal and opposite-signed VCD intensities.

3. VCD INSTRUMENTATION

The measurement of a VCD spectrum and its parent mid-IR spectrum is based on Fourier transform-IR (FT-IR) absorption spectroscopy. The optical-electronic layout for the measurement of VCD using an FT-VCD spectrometer is illustrated in *Figure 1*. The output beam of an FT-IR spectrometer is optically filtered and then linearly polarized before passage through a photoelastic modulator (PEM). The zinc selenide (ZnSe) PEM, typically used for VCD measurement, changes the polarization state of the beam between LCP and RCP at a frequency of 37 kilocycles/s (37 kHz). A sample solution in a standard IR cell is placed in the beam, and a liquid nitrogen-cooled detector of mercury-cadmium telluride (MCT) detects the transmitted beam. The detector produces signals in two frequency ranges: 1) I_{DC} , in the range of 1–2 kHz, represents the FT-IR interferogram; and 2) I_{AC} , centered at 37 kHz, represents the FT-VCD interferogram. The FT-IR interferogram may be directly Fourier transformed to yield the IR spectrum. After synchronous demodulation (using a lock-in amplifier or purely numerical processing) with a reference at 37 kHz, the VCD interferogram can be Fourier transformed to yield the VCD spectrum. Both the IR and VCD spectra are presented in dimensionless absorbance units, A , and hence the ratio of the VCD to the IR intensity for any band in the spectrum yields the dimensionless anisotropy ratio for that vibrational transition. *Figure 1* illustrates the minimum setup for VCD measurement, but technically advanced instruments use two PEMs to increase baseline stability and two sources to increase signal quality or reduce sample measurement time. Measurement of VCD in other spectral regions, for example, the hydrogen-stretching or near-IR, can be carried out with appropriate changes in sources, polarizers, filters, PEM, cells, and detectors.

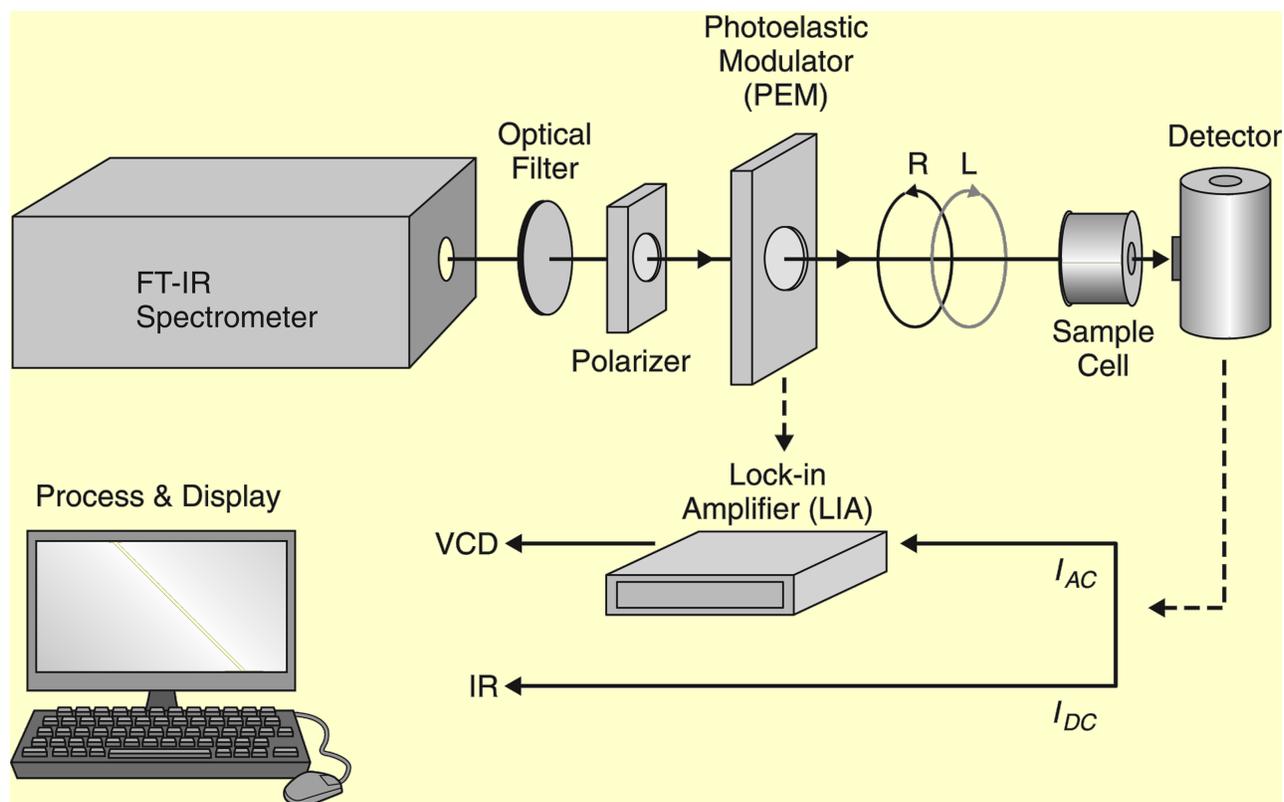
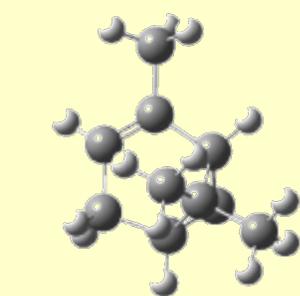


Figure 1. Diagram illustrating the typical features of an FT-VCD spectrometer for the measurement of the IR and VCD spectra of a chiral sample.

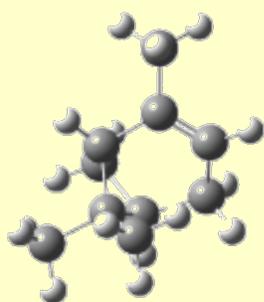
4. MEASUREMENT OF VCD SPECTRA

Figure 2 shows the IR and VCD spectra for (+)-*R*- and (–)-*S*- α -pinene as a neat liquid measured at 4 cm^{-1} spectral resolution for 1 h. Also shown are the stereo-structures of the two opposite enantiomers (+)-*R*- and (–)-*S*- α -pinene. This figure shows that the IR spectra are nearly identical (superimposed dash and solid traces) for these enantiomers and that IR is therefore blind to chirality. By contrast, the corresponding VCD spectra have intensities that are equal in magnitude with opposite signs for each band in the spectrum. The two stereo-structures for α -pinene are presented in a form that makes clear their mirror symmetry, and it follows that mirror-symmetric pairs of chiral molecules have mirror-symmetric VCD spectra about the zero baseline. The VCD baseline for these spectra is offset slightly above zero and can be baseline corrected as discussed below. Inspection of the intensity scales for the IR and VCD spectra reveals that the VCD intensities are approximately four orders of magnitude smaller than the corresponding IR intensities. Finally, each band in the IR spectrum has a corresponding VCD band. The richness of the resolved spectral bands in the VCD spectrum, each one representing what has traditionally been called a chromophore in electronic CD (ECD), gives VCD its exceptional combination of structural richness and stereochemical specificity.

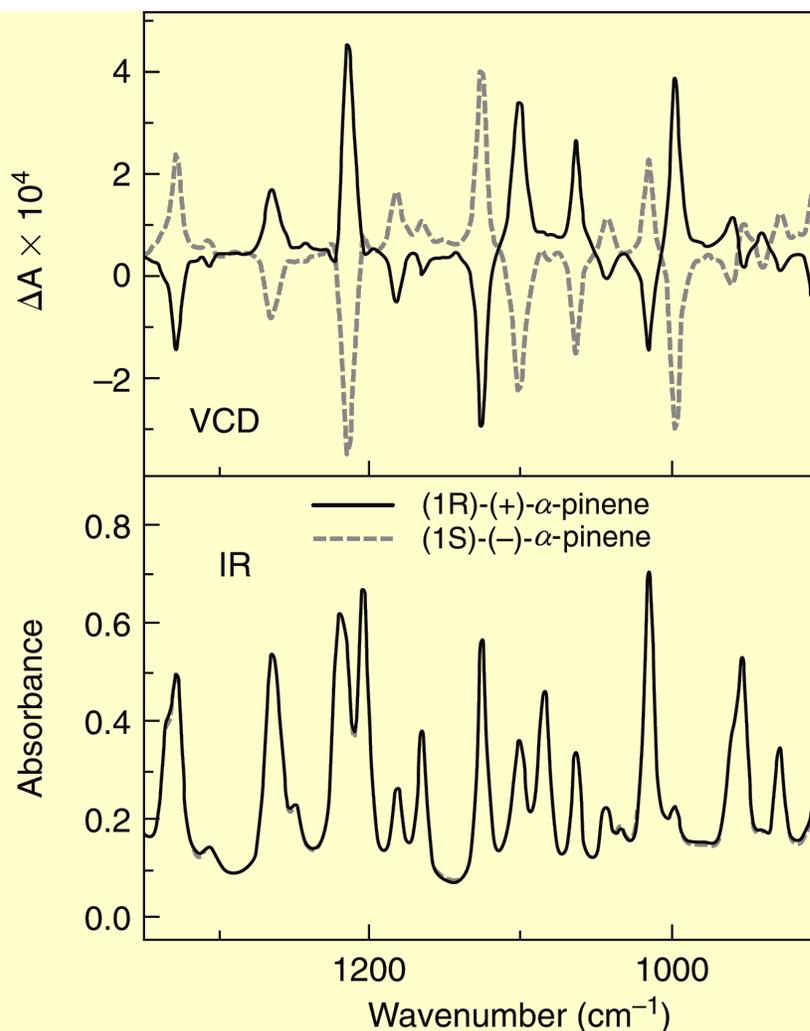
VCD and IR Spectra α -Pinene Enantiomers



(S)-(-)- α pinene



(R)-(+)- α pinene



Enantiomers: IR spectra are identical, VCD spectra are opposite in sign

Figure 2. IR (lower) and VCD (upper) of (1S)-(-)- α -pinene (dash) and (1R)-(+)- α -pinene (solid). To the left are presented the quantum chemistry-optimized stereostructures of (1S)-(-)- α -pinene (upper) and (1R)-(+)- α -pinene (lower). The IR and VCD spectra were measured at 4 cm^{-1} spectral resolution, and the VCD spectra were averaged for 1 h.

Sampling requirements for VCD are similar to those needed to obtain a good IR spectrum. Ideally, as shown in *Figure 2*, analysts choose a combination of path length and sample concentration to obtain an average IR absorbance value A in the range between 0.2 and 0.8 and concentrations between 0.1 and 1.0 M, depending on the molecular weight of the sample. Typical path lengths for organic solvents are in the range of 50–100 μm , and sample quantities needed are typically 5–10 mg, although amounts as small as 2 mg are possible. Typical solvents for VCD measurement of organic molecules are deuterated solvents that have reduced solvent absorption in the mid-IR region. Besides hydrogen-free solvents, such as carbon tetrachloride (CCl_4), other commonly used solvents for VCD are deuterated chloroform (CDCl_3) and deuterated dimethyl sulfoxide (DMSO-d_6).

IR and VCD spectra can be plotted either in absorbance units, as A and ΔA that reflect the sampling conditions, or in molar absorption coefficients ϵ and $\Delta\epsilon$ that remove the concentration and path length dependence of the intensity to give a molecular-level property

that can be compared quantitatively to calculated IR and VCD intensities. The conversion between these two sets of quantities is given by:

$$A = \epsilon C \times l$$

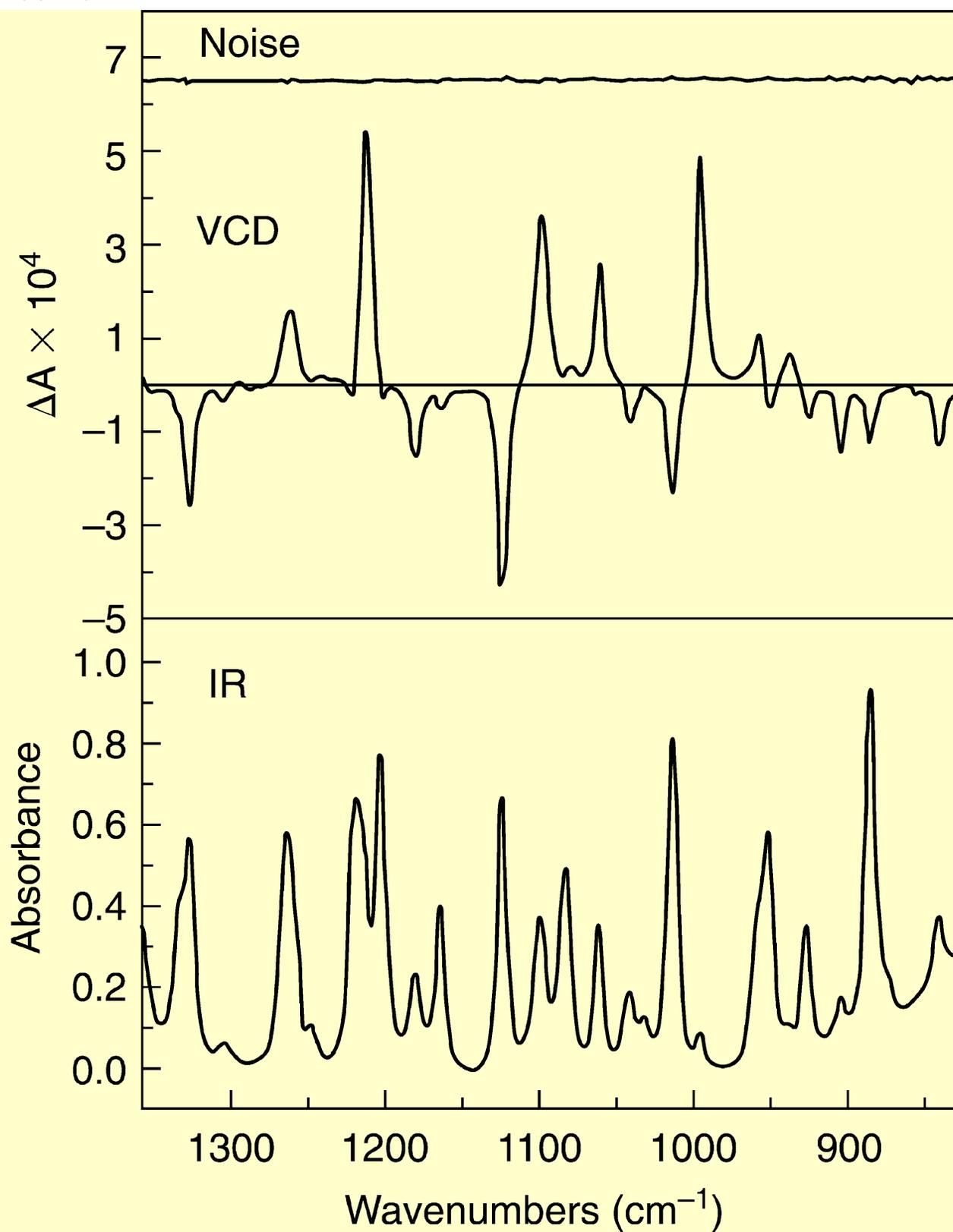
- A = IR absorbance
 ϵ = molar absorption coefficient
 C = concentration (mol/L)
 l = path length (cm)

and

$$\Delta A = \Delta \epsilon C \times l$$

- ΔA = difference in the IR absorbance
 $\Delta \epsilon$ = difference in molar absorption coefficient

A useful dimensionless measure of the intrinsic strength of a VCD band is the anisotropy ratio, defined as $g = \Delta A/A = \Delta \epsilon / \epsilon$. Including the spectral frequency dependence of IR and VCD, the measured spectra are expressed respectively either as $A(\nu)$ and $\Delta A(\nu)$ or as $\epsilon(\nu)$ and $\Delta \epsilon(\nu)$. VCD spectra require baseline corrections for instrumentation and solvent intensity. Deviations from a perfectly flat baseline with zero offset must be removed before a final calibrated VCD spectrum is complete. Solvent baseline correction can be achieved for VCD spectra by subtraction of the VCD spectrum of the solvent in the same sample cell. If the opposite enantiomer or racemic mixture of the sample is available, baseline correction can be achieved as one-half the difference between these two equal and opposite VCD spectra in the case of enantiomers or as simple subtraction in the case of the racemic mixture. Baseline deviations are caused by the unavoidable presence of linear birefringence in the optical elements of the instrument and the sample cell that can become important for all but the largest VCD intensities. An example of a baseline-corrected VCD spectrum is presented in *Figure 3*, along with the corresponding IR and VCD noise spectrum. The spectrum of this sample of neat (–)-(*S*)- α -pinene was collected for a period of 1 h at 4 cm^{-1} spectral resolution in barium fluoride (BaF_2) with a path length of $75 \text{ }\mu\text{m}$. The noise spectrum is less than 1×10^{-5} absorbance (A) units across the spectrum. The IR, VCD, and noise spectra presented in *Figure 3* may be taken as a validation standard for the performance of a mid-IR FT-VCD instrument, as described in *Vibrational Circular Dichroism Spectroscopy* (782).



Simultaneous, two enantiomers, total 3.5 hour

Figure 3. IR absorbance A (bottom), baseline-corrected VCD (middle), and VCD noise spectrum (top) for (-)-(1S)- α -pinene as a neat liquid averaged for 1 h at 4 cm^{-1} spectral resolution and

in a 75- μm path-length barium fluoride cell.

5. QUALITATIVE AND QUANTITATIVE ANALYSIS

5.1 Ensure That Signs and Intensities Are Correct

Qualitative analysis can be carried out with VCD by comparing the VCD spectrum of an unknown to a reference spectrum of that molecule to identify common features and to confirm that the VCD spectrum of the sample is a close match to the reference spectrum.

Quantitative analysis using VCD involves measuring the VCD intensity of the sample molecule and comparing it to a standard reference spectrum for that molecule. In this way, the concentration and the %EE of the sample can be determined as follows. The intensity of the IR spectrum relative to the IR standard of the sample determines the concentration for measurements with the same path length. After normalizing the IR intensity of the unknown sample to the standard, the relative intensities of the VCD determine the %EE of the sample. Such comparisons need to be carried out with all sampling conditions being the same, including the solvent and instrument spectral resolution.

To determine that the VCD intensities are correct, the uncalibrated VCD intensities must be calibrated. This is accomplished by placing a multiple-wave plate in the sample position, followed by a polarizer that is either parallel or perpendicular to the instrument polarizer in *Figure 1*. Measurement of the VCD spectrum of this multiple-wave plate set-up for two settings of the wave plate and polarizer that differ by 90° produces two sets of curves that have crossing points. The curve connecting the crossing points follows the shape of a first-order Bessel function and represents unit VCD intensity. Dividing this curve into the uncalibrated VCD spectrum produces a VCD spectrum with the correct calibrated intensities. The two calibration curves and their crossing-point curve are illustrated in *Figure 4*.

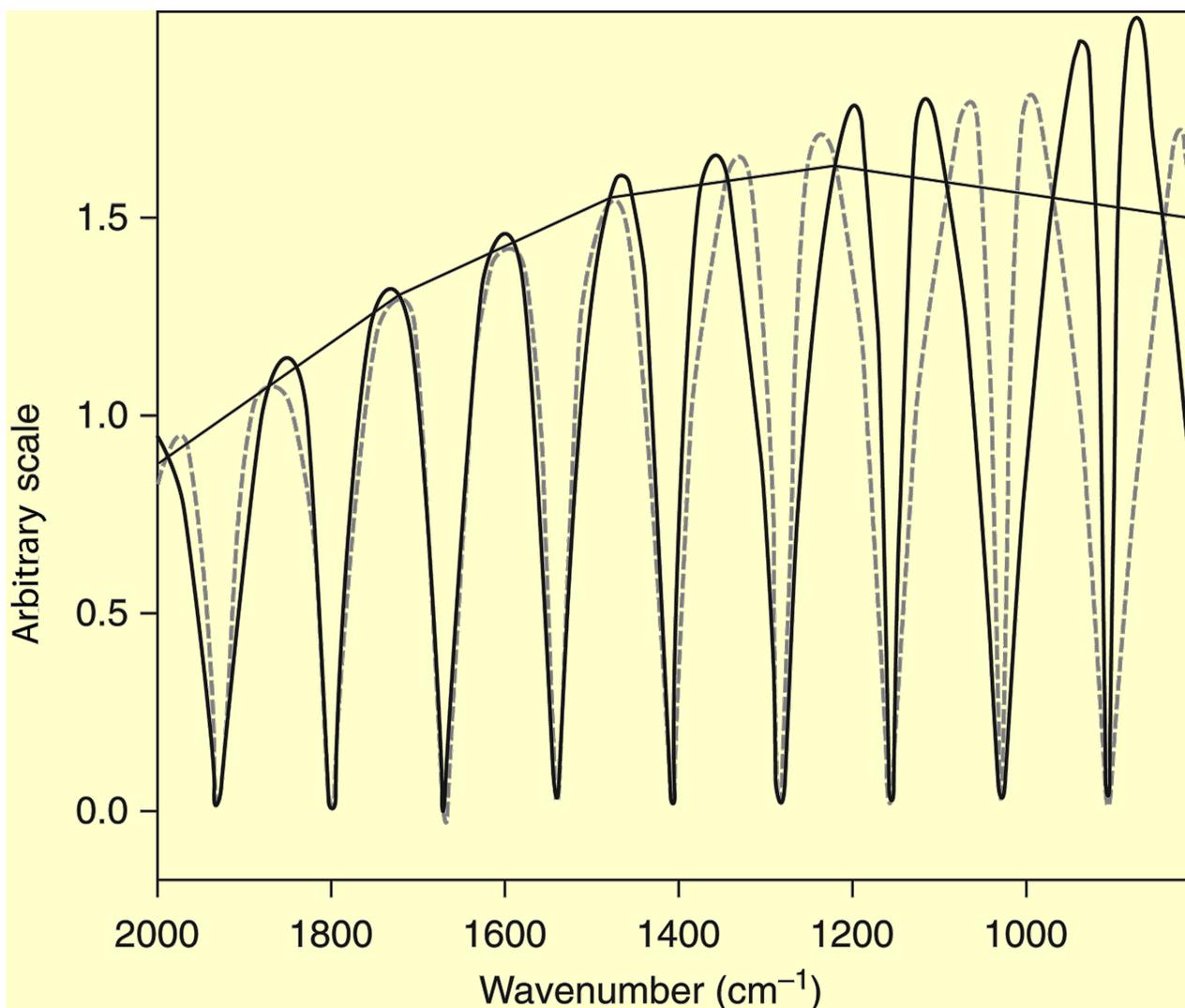


Figure 4. Calibration curves for VCD intensity generated by placing a multiple-wave plate and a polarizer in the IR beam and connecting the crossing points with a curve representing unit VCD intensity for maximum VCD intensity at 1200 cm^{-1} .

5.2 Determination of the Noise Level at Each Point in the Spectrum

A critical factor in quantitative analysis using VCD is the degree of accuracy of the spectrum as given by the VCD noise level and baseline stability of the measurement. The noise level depends on a number of factors such as the D^* value of the detector, the amount of radiation passing through the sample from the source, and the amount of absorption of the sample. The noise level is obtained by dividing the VCD measurement into two halves. Addition of these halves gives the VCD spectrum, including noise, whereas subtraction eliminates the VCD features and leaves the equivalent noise spectrum. An example of a VCD noise spectrum is given in *Figure 3*, which shows, from bottom to top, the IR, VCD, and VCD noise spectra for a measurement of $(-)$ -*S*- α -pinene. The VCD noise level can be reduced by increasing the measurement time through co-addition of the spectra, as desired.

5.3 VCD Baseline Accuracy

A second critical factor in the measurement of a VCD spectrum is the characteristics of the

VCD baseline, namely its location, straightness, and stability. The VCD zero intensity line between positive and negative VCD intensities needs to lie as close as possible to the electronic zero of the measurement (zero of spectral display). Due to the sensitivity of the VCD intensity scale, some offset of the baseline from electronic zero almost always occurs, as discussed above in reference to the need for baseline correction of the VCD spectra of α -pinene in *Figure 2* and as carried out for *Figure 3*. Use of α -pinene as a validation standard is advantageous, because α -pinene has an unusually large intensity and thus, its spectrum is relatively easy to measure on a regular basis. α -Pinene can also be used as a measure of the baseline of a VCD measurement relative to the electronic zero of the measurement. This is illustrated in *Figure 5*, where the VCD spectra of (–)- α -pinene (*dash*) and (+)- α -pinene (*black*) are presented along with the VCD spectrum of a racemic mixture [equal quantities of (–)- α -pinene and (+)- α -pinene, hence cancelling the VCD intensity] of α -pinene (*gray*). The VCD spectrum of the racemic mixture of α -pinene is the VCD baseline for this measurement. A typical standard for baseline quality is deviations from electronic zero of <10% of the maximum separation of positive and negative VCD intensity of the neat α -pinene spectrum, or approximately 1×10^{-4} . The baseline illustrated in *Figure 5* satisfies this standard to within 5%. The baseline at 1350 cm^{-1} is slightly above electronic zero and has maximum excursions away from electronic zero of <0.00005 , or 5×10^{-5} at 1170 cm^{-1} , which can be compared to the maximum positive and negative intensity values of (–)- α -pinene of $+5 \times 10^{-4}$ at 1220 cm^{-1} and a negative peak value of -4×10^{-4} at 1130 cm^{-1} .

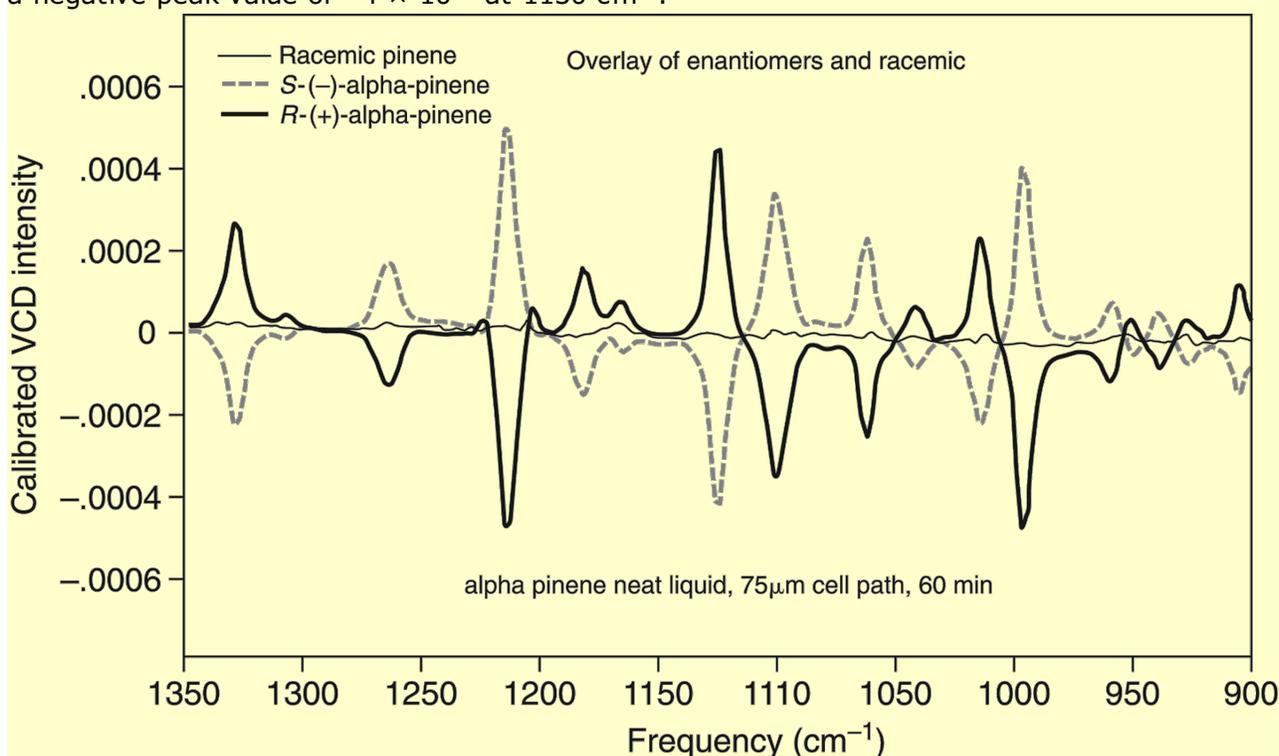


Figure 5. VCD spectrum of *R*-(+)- α -pinene (black), *S*-(-)- α -pinene (dash), and Racemic (gray) α -pinene as a neat liquid for a 60-min measurement in a 75- μm path-length barium fluoride cell.

5.4 VCD Baseline Absorption Artifacts and Single-Enantiomer Measurement Capability

For tests of baseline accuracy and stability, the sample camphor in carbon tetrachloride

solution is used as a qualification standard and provides a more stringent test than α -pinene, because the VCD spectrum of camphor is approximately one-fifth the magnitude, relative to the IR absorbance spectrum, of that of neat α -pinene. The VCD spectra of both enantiomers, as well as two measures of the VCD baseline, are provided in *Figure 6*. It is clear, as in *Figure 2* and *Figure 5*, that the VCD spectra of enantiomers, in this case (+)-camphor and (-)-camphor, are equal in intensity and opposite in sign relative to the VCD baseline to within the noise level of the measurement. The two VCD baselines provided are both zero VCD measurements. The VCD spectrum of racemic camphor [equal mixture of (+)-camphor and (-)-camphor] is the true VCD baseline for the individual (+)-camphor and (-)-camphor spectra, even if there are offsets (artifacts) due to absorption bands in the IR spectrum of camphor. The absence of such artifacts to within the noise level of the measurement is demonstrated by the congruence of the VCD spectrum of the racemic mixture and that of the solvent, carbon tetrachloride. Because carbon tetrachloride has no significant level of absorbance in the region of the spectrum displayed, the agreement of these two baselines within the noise level demonstrates the absence of baseline artifacts in the VCD spectrum of racemic camphor, and therefore, for the VCD measurement of either of the two enantiomers of camphor.

Achieving a VCD baseline that is free of absorption artifacts is usually done by optical alignment of the VCD instrument such that the baseline is as close as possible to the true electronic zero of the instrument of the measurement. In *Figure 6*, the VCD baseline can be seen to wander above and below zero across the spectrum, but in this case, the deviations from the electronic zero are small (approximately 2×10^{-5}) and $<20\%$ of the positive and negative maximum VCD intensities of the spectrum (approximately 1×10^{-4}). This value of deviation is consistent with the maximum baseline magnitudes discussed above for α -pinene and displayed in *Figure 5*, because α -pinene has a much larger (approximately $5\times$) VCD spectrum, relative to its IR spectrum, than camphor. The baselines for camphor in *Figure 6* are noisier than that of α -pinene in *Figure 5*, because the VCD spectrum scale is smaller and the measurement time is 20 min instead of 1 h. As in *Figure 3*, the VCD baseline can be corrected by subtraction of an appropriate baseline spectrum, racemic or solvent. In *Figure 7*, the baseline-corrected VCD spectra for both enantiomers and the racemic mixture have been obtained by subtraction of the VCD spectrum of the solvent carbon tetrachloride. As a result the VCD zero baseline and the instrument electronic zero are the same to within the noise level, and the mirror symmetric properties of these spectra are even more apparent. This VCD test verifies that by subtracting the VCD spectrum of the solvent, an accurate VCD spectrum of camphor can be obtained using only one enantiomer, either the (+)- or (-)-enantiomer, and hence, the instrument possesses a single-enantiomer VCD measurement capability.

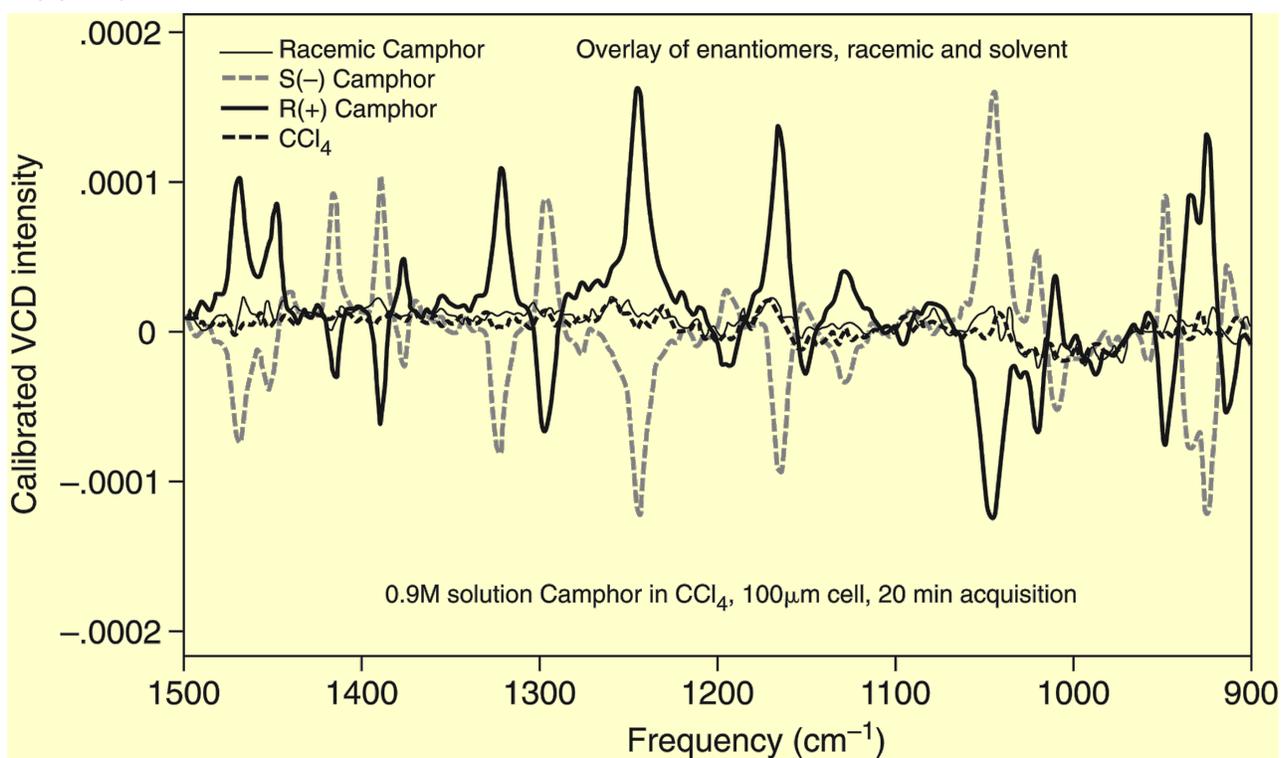


Figure 6. VCD spectrum of *R*(+)-Camphor (black), *S*(-)-Camphor (gray dash), and Racemic Camphor mixture (gray) as a 0.9 M solution in carbon tetrachloride. Also provided with the same cell and path length is the VCD spectrum of pure carbon tetrachloride (black dash). The sampling conditions were a spectral collection time of 20 min with a 100- μm path-length barium fluoride cell.

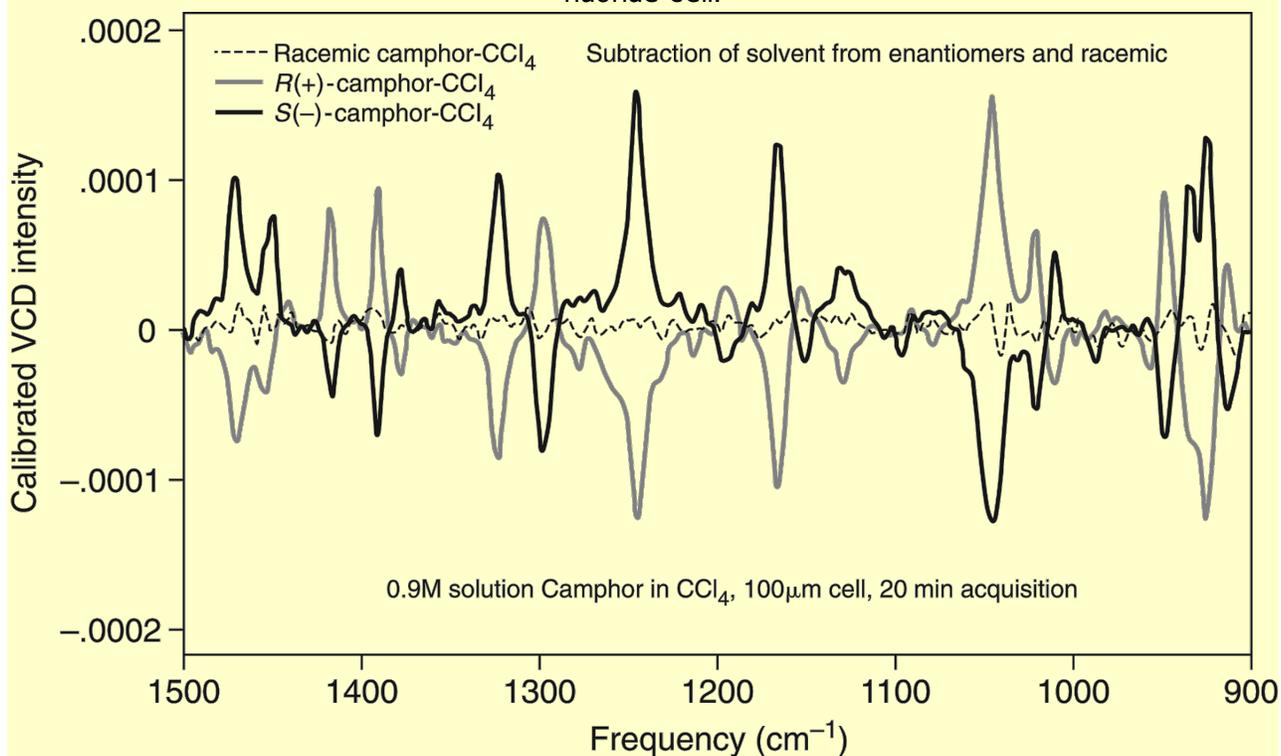


Figure 7. Solvent baseline-corrected VCD spectrum of *R*(+)-camphor- CCl_4 (gray), *S*(-)-camphor- CCl_4 (black), and Racemic camphor- CCl_4 mixture (dash) of the VCD spectra shown in Figure 6.

5.5 VCD Measurement Stability and Noise Level Reduction

Comparison of VCD measurements of camphor for 20 min to that measured over a period of 4 h in blocks of 20 min permits evaluation of instrument measurement stability as well as the reduction of noise level over time. In *Figure 8*, 12 such 20-min VCD spectra measured in 4 h are presented to show the stability of a VCD spectrum over time. It is clear that there are no deviations of VCD spectra outside the noise level of any one 20-min spectrum, and hence, there is no long-term drift in the VCD baseline over this time period.

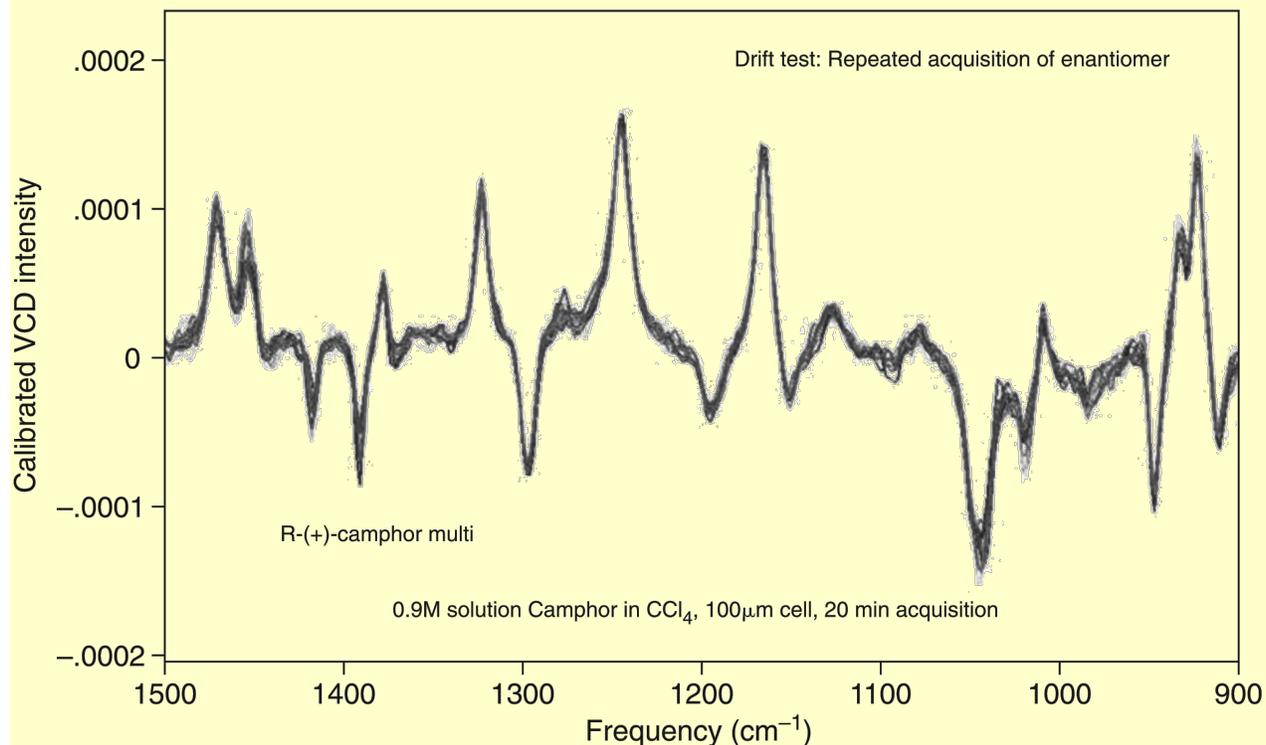


Figure 8. Superposition of twelve 20-min VCD spectra of *R*-(+)-camphor under the conditions of *Figure 6* and *Figure 7*.

If the twelve blocks of 20-min VCD spectra in *Figure 8* are averaged, the resulting 4-h VCD spectrum can be compared to a 20-min VCD spectrum of the same sample to demonstrate the noise reduction that is achieved upon signal averaging, provided there are no other systematic noise sources in the VCD spectrometer. In this case, the noise reduction should be a factor of the square root of 12, or approximately 3.5. Thus, the signal-to-noise ratio should improve by a factor of 3.5. Such a comparison is provided in *Figure 9*.

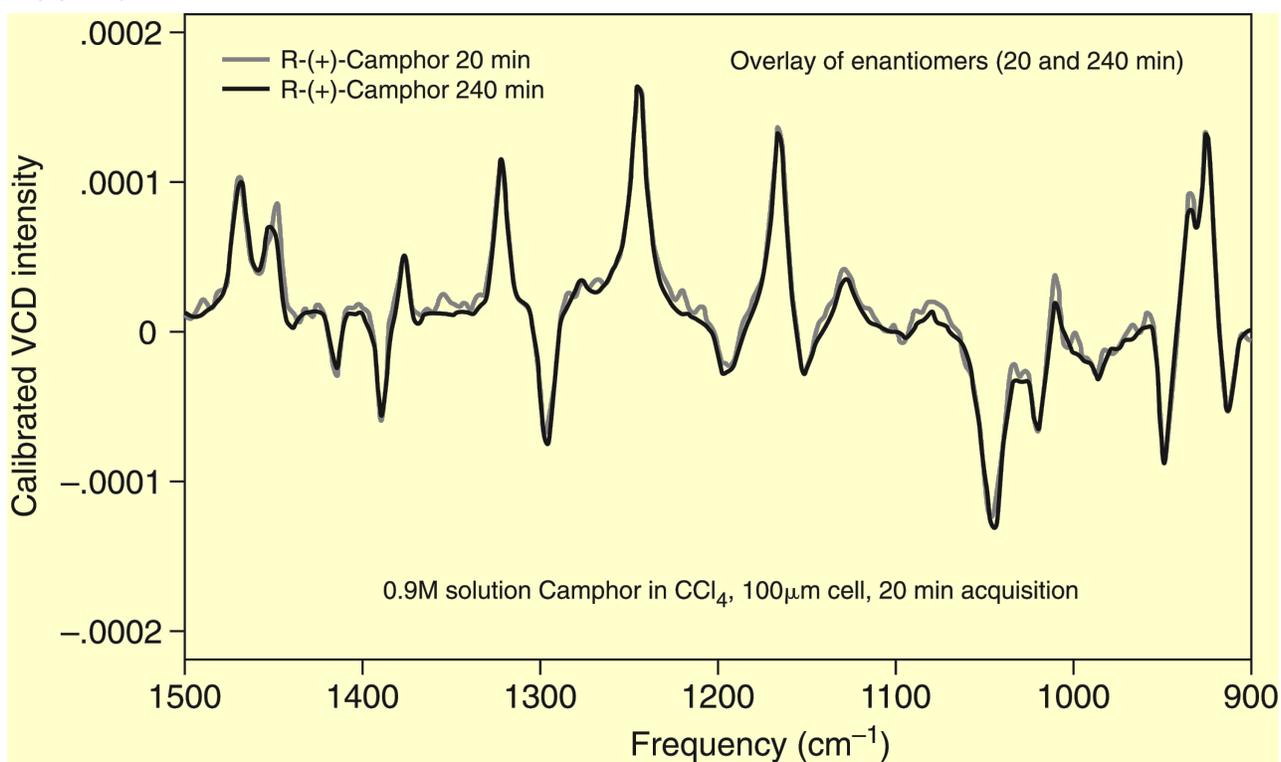


Figure 9. Comparison of the 4-h average of twelve 20-min VCD spectra [*R*-(+)-camphor 240 min; (gray)] to one 20-min measurement of camphor [*R*-(+)-camphor 20 min; black], under the conditions of *Figures 6, 7, and 8*, showing improvement in signal-to-noise ratio with increased collection time.

The noise level can be visualized directly on a 5× more sensitive scale to better evaluate the noise levels by comparing the noise spectrums, as illustrated and described for *Figure 3*, for the 4-h and 20-min VCD measurements. This is presented in *Figure 10*.

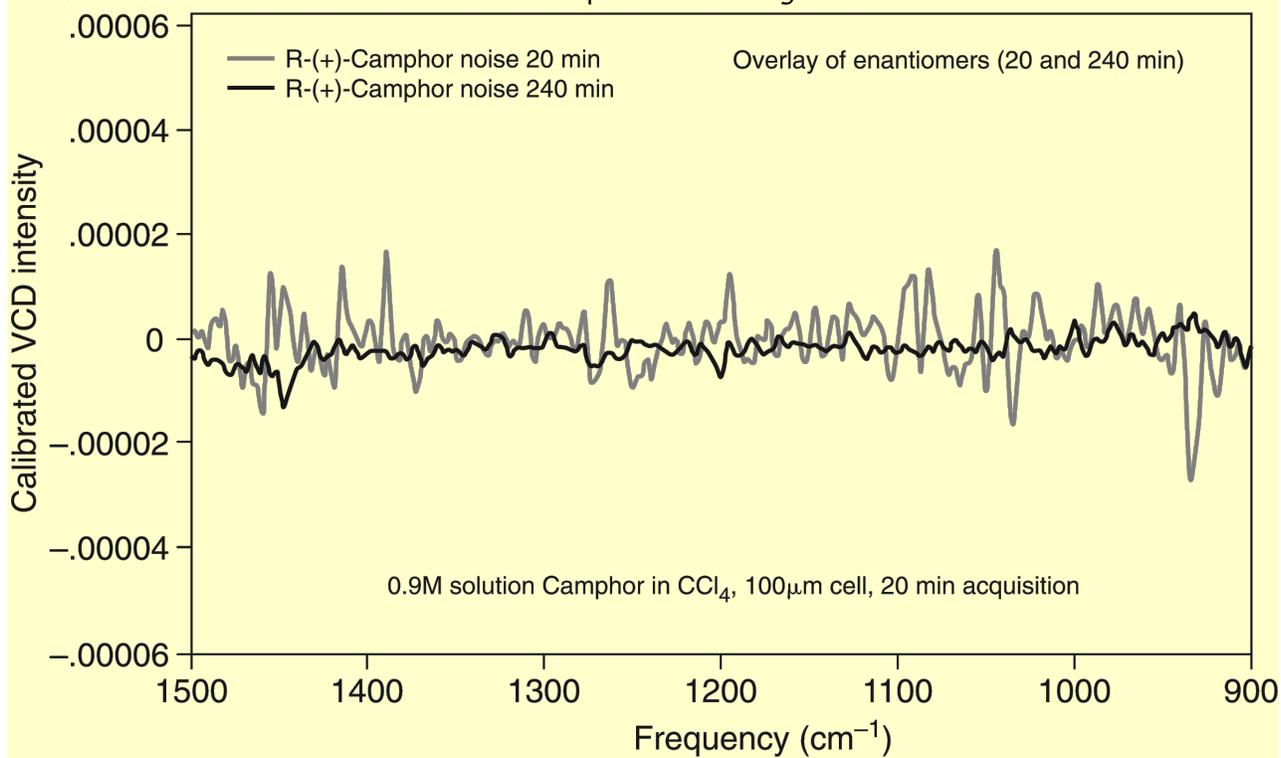
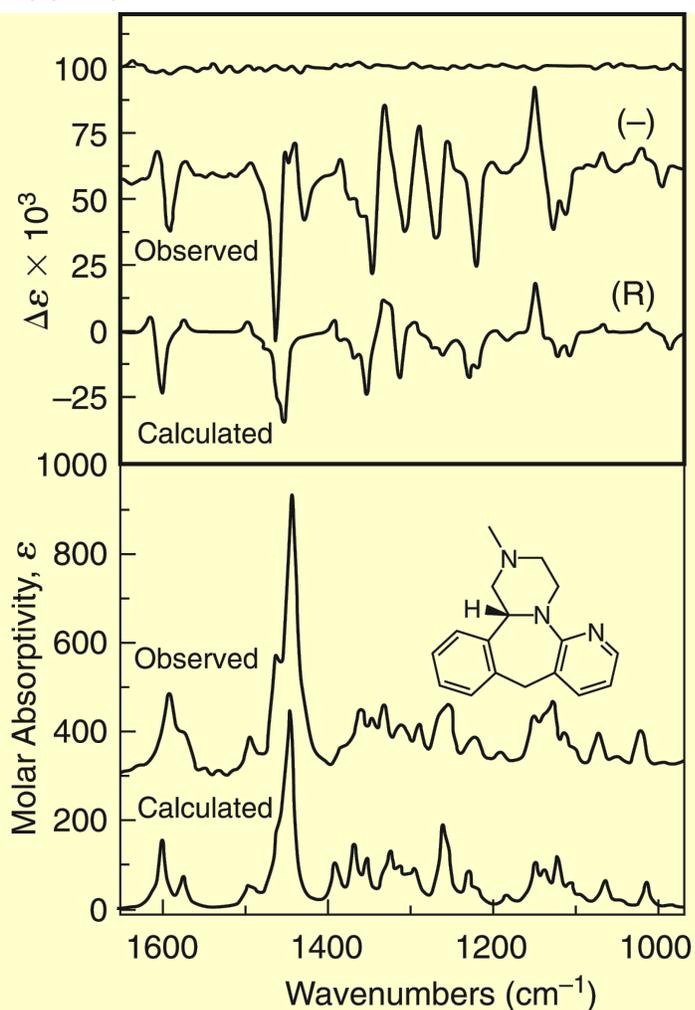


Figure 10. Comparison of the VCD noise spectra of *R*-(+)-camphor for one 20-min measurement [*R*-(+)-camphor noise; (gray)] versus a 4-h measurement [*R*-(+)-camphor noise; (black)] under the conditions of *Figure 5*, *Figure 6*, *Figure 7*, and *Figure 8*, where the intensity scale is approximately 5× more sensitive than those in *Figure 7* and *Figure 8*.

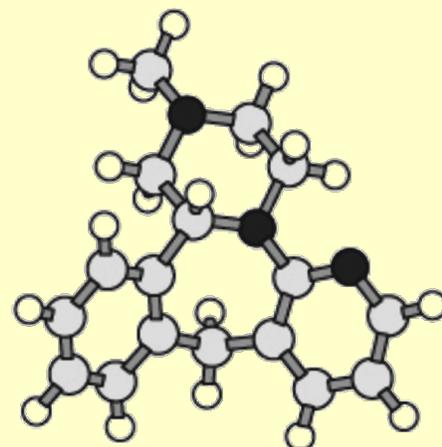
6. DETERMINATION OF ABSOLUTE CONFIGURATION

The IR and VCD spectra of a sample are measured in solution in a matter of min to h without the requirement of prior crystallization or high sample optical purity. The AC of an unknown sample is determined by comparison of its measured VCD spectrum to a VCD quantum chemistry calculation of the same molecule, where the AC used in the calculated VCD spectrum is chosen arbitrarily. If the signs of the bands in the measured VCD spectrum match those in the calculated VCD spectrum, the AC of the sample is identical to that used in the calculation. If the signs are opposite, then the sample has the opposite AC compared with that calculated. An example of the determination of the AC of a pharmaceutical molecule is shown in *Figure 11*. The AC of mirtazapine, which is sold in racemic form, is determined by comparison of the measured IR and VCD spectra of the (–)-enantiomer to the calculated IR and VCD spectra of the *R*-enantiomer. The close match in VCD signs—vibrational frequencies, intensities of the observed and calculated spectra, and IR frequencies and intensities—demonstrates clearly that the AC of mirtazapine is (–)-*R*. Also shown in *Figure 11* is the stereo-specific quantum chemistry calculated structure of *R*-mirtazapine.



Helv. Chim. Acta, 85, 1160 (2002)

Figure 11. Comparison of the measured (Observed) and calculated IR and VCD spectra of mirtazapine, allowing assignment of its AC to (-)-R. Reproduced in part with permission from John Wiley & Sons.



Vibrational circular dichroism (VCD) is used to identify **unambiguously** the absolute configuration of Mirtazapine as (-)-R and solution conformation as shown above

If the AC of a molecule has previously been established, for example, by X-ray crystallography, a VCD calculation of the molecule is not necessary (but could be performed as a check of the X-ray assignment), and the measured VCD spectrum of the molecule may be taken as a Reference Standard for the AC of the molecule, supported, for example, by USP. For a molecule with unknown structural chirality, the determination of its AC by VCD requires a comparison of the measured and calculated IR and VCD spectra, $\epsilon(\nu)$ and $\Delta\epsilon(\nu)$, as shown in *Figure 11*. The key point of the comparison is correlating the major VCD bands to determine whether they have the same or opposite signs. The IR spectrum is a guide in the process. Seeing a close correspondence between the calculated and measured IR spectra provides confidence that a good vibrational force field has been calculated and the most important vibrational bands are correlated. If a corresponding correlation of the signs of the major VCD bands can be made, as in *Figure 11*, then the AC has been determined.

When the measured and calculated VCD spectra are compared, the magnitudes of the corresponding intensities are not critical. What is important is the relative intensities of the bands in the two spectra, and in fact, the comparison of measured and calculated spectra can

be made using different sets of intensities for $\epsilon(\nu)$ and $\Delta\epsilon(\nu)$ for the calculated spectra and $A(\nu)$ and $\Delta A(\nu)$ for the measured spectra. In general, an exact match of the measured and calculated band frequencies is not expected because of several limitations of the calculations. Examples of these limitations include the assumption of the harmonic approximation when some anharmonicity is present in the measured spectra, the need to ignore the effects of solvent interactions with the chiral solute molecule, limitations in the size of the basis set used for the quantum calculation, and the choice of density functional required for the density functional theory (DFT) calculation.

7. CALCULATION OF VCD SPECTRA

To determine the AC of a molecule with unknown chirality using the VCD method as an alternative to, or supplement to, X-ray crystallography, analysts conduct a quantum chemistry calculation of the IR and VCD intensities of the molecule. Since the late 1990s, commercial DFT software has been available and can be used effectively by a trained user because it does not require special expertise or extensive experience in quantum chemistry calculations. For example, VCD calculations are now part of some undergraduate chemistry curricula. The following steps are required to perform a DFT calculation of IR and VCD:

1. The stereo-specific structure of the molecule is entered through a visual graphics interface
2. The quantum chemistry program then automatically performs the following steps:
 - a. The geometry is optimized to a minimum energy conformation.
 - b. A force field is calculated that determines the vibrational modes of the molecule, with $3N-6$ modes for a molecule with N atoms.
 - c. Atomic polar tensors that yield the IR spectrum are calculated.
 - d. Atomic axial tensors that yield the VCD spectrum are calculated.
3. The output of the calculation consists of a table where, for each mode i there is a frequency ν_i , IR intensity (dipole strength) D_i , and VCD intensity (rotational strength) R_i .
4. Commercially available programs calculate the IR and VCD spectra for the molecule using the following steps.
 - a. A lineshape $f(\nu)$ is applied to each dipole and rotational strength value, D_i and R_i , centered at each vibrational frequency ν_i .
 - b. Summation of these normal mode line-shaped intensities automatically produces the final calculated IR and VCD spectra $\epsilon(\nu)$ and $\Delta\epsilon(\nu)$ that can be compared directly to the corresponding measured IR and VCD spectra.

Another important factor that often is encountered in the assignment of the AC of pharmaceutical molecules and natural products (because of the structural complexity of these types of molecules) is the presence of more than one important conformer under the

measurement conditions. The fractional populations of different conformers can be predicted by using their relative calculated energies in the Boltzmann distribution. The lowest-energy conformer has the highest fractional population and so on, to increasingly less-populated conformers with higher relative energies. To calculate the VCD, one must calculate the IR and VCD of each important conformer and then add these spectra together, weighted by the fractional Boltzmann population. Usually, one can ignore the spectral contributions of conformers that account for less than a few percent of the total population.

Programs for calculating VCD and IR spectra to the level of accuracy sufficient for comparison to measured IR and VCD are available commercially from a variety of sources. First, one needs a program for evaluating and finding the geometry of all the lowest-energy conformations of a chiral molecule. A variety of programs using molecular mechanics are available for this purpose. Second, one needs a full quantum chemistry software program for calculating the ab initio force fields, vibrational frequencies, and VCD and IR intensities for each of the lowest-energy conformers. A common chemical model of sufficient accuracy for AC determination is DFT, using a basis set minimum of 6-31G(d), and a choice of hybrid functionals, such as B3LYP or B3PW91. Higher-level basis sets and alternative choices of hybrid functionals may be made. Finally, one needs a software method for comparing measured and calculated VCD and IR spectra to assess the degree of spectral agreement between the measured and calculated spectra, and hence the level of confidence that the correct assignment of AC has been predicted. Additional details are described below.

In *Figure 12*, the AC of the bioactive enantiomer of the analgesic (*S*)-(+)-ibuprofen is determined by comparing the measured and calculated IR and VCD spectra. The calculated IR and VCD are the sums of the IR and VCD conformer spectra, one for each conformer, weighted by the fractional populations 0.37, 0.36, 0.12, and 0.07, plus smaller contributions from another eight conformers that have a total contribution of 0.08. Clearly, there are two important conformers that are nearly equally populated, and the basic features of the final IR and VCD spectra are determined by these spectra. As a result of this analysis, not only has VCD been able to confirm the AC of (+)-ibuprofen as *S*, but two dominant solution-state conformers have been identified, along with two additional less-populated conformers.

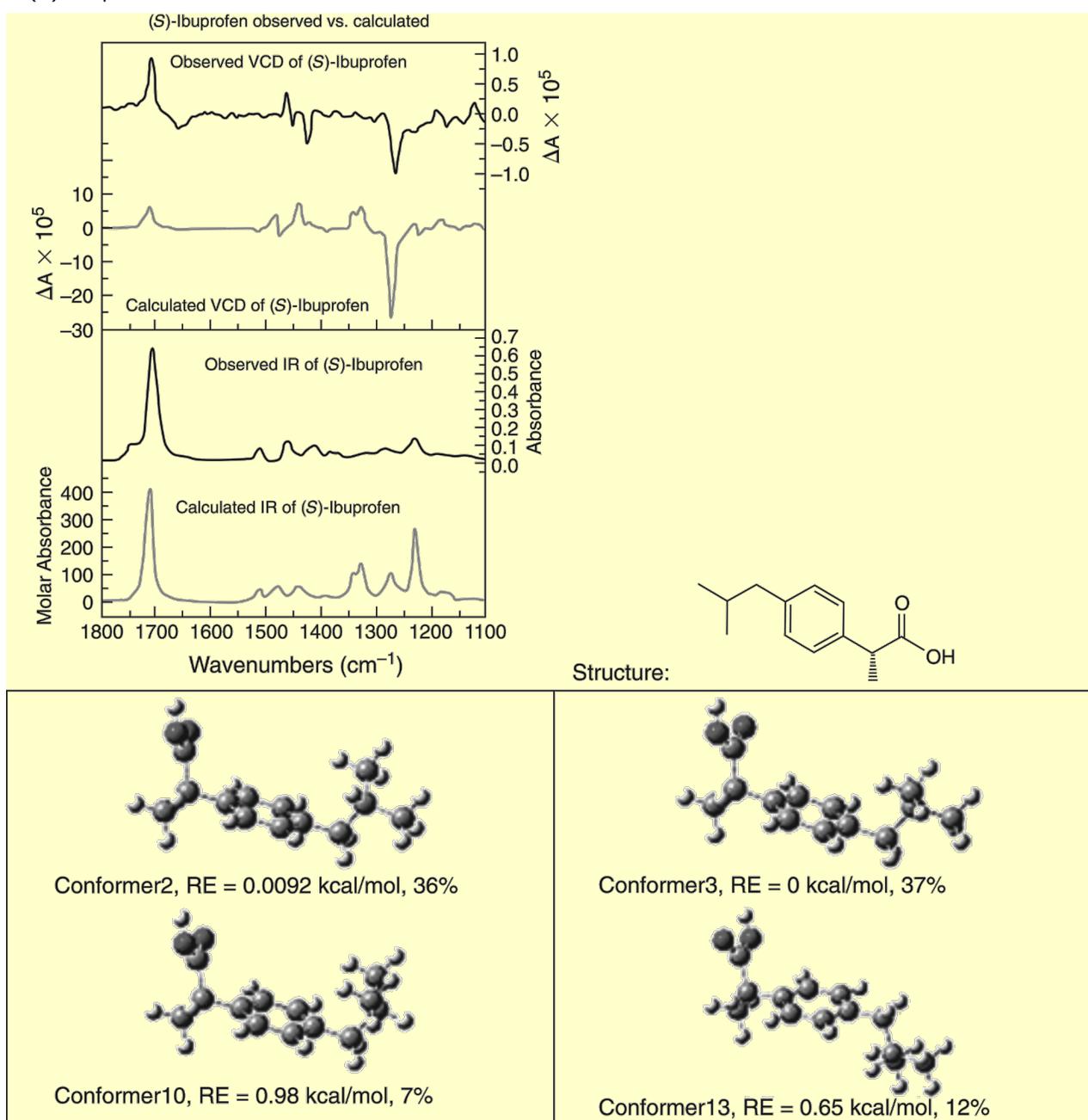


Figure 12. On the right are displayed the measured (Observed; +) and calculated (S) IR and VCD spectra of (+)-ibuprofen (above) and the stereostructure of (S)-ibuprofen (below). To the left are displayed the stereostructures, relative energy (RE), and percentage Boltzmann population (BP) for the four most important solution-state conformers.

8. COMPARISON OF MEASURED AND CALCULATED SPECTRA

To provide an unbiased statistical measure of the degree of similarity between measured and calculated IR and VCD spectra, an analytical method based on a convolution algorithm has been developed. The degree of similarity, or congruence, between a measured and a calculated spectrum can be calculated and used to determine a degree of confidence that the AC determined by visual inspection is correct.

8.1 Degree of Confidence of Correct Assignment

The results of the use of such statistical measures are shown in *Figure 13*. The degree of similarity (the total neighborhood similarity, TNS) of the measured and calculated IR is 92.5, where 100 is a perfect match. The same TNS measure for the VCD is 82.2. Because VCD bands can be either positive or negative, a more refined analysis is carried out for VCD, and only regions of sign agreement are compared for each enantiomer. In this case, the value of the signed neighborhood similarity (SNS) for the *S*-enantiomer is 92.8, and for the *R*-enantiomer it is only 12.1. Clearly, the analysis confirms the visual agreement of the measured VCD spectrum (*upper solid line*) and the calculated VCD spectra (*lower solid line*) for the *S*-enantiomer versus the *R*-enantiomer (*dashed line*). An additional numerical comparison is calculated as the enantiomeric similarity index (ESI), which equals $SNS(S) - SNS(R)$. The result (*black dot*) is then plotted against a database of 89 prior correct VCD assignments, where the axes are SNS (*vertical*) and ESI (*horizontal*). The closer a statistical point is to the *upper right-hand corner* of the plot, the higher the similarity of the comparison and the higher the degree of confidence. The upper right-hand corner of the plot is defined as 100% spectral similarity. The degree of confidence is a statistical measure that is set to be 100% confidence for points clustered in the *upper right-hand region* of the plot. Statistical measures such as these eliminate the reliance on only visual judgment for the comparison of measured and calculated VCD spectra and provide a statistical basis for assigning a degree of similarity between the measured and calculated spectra.

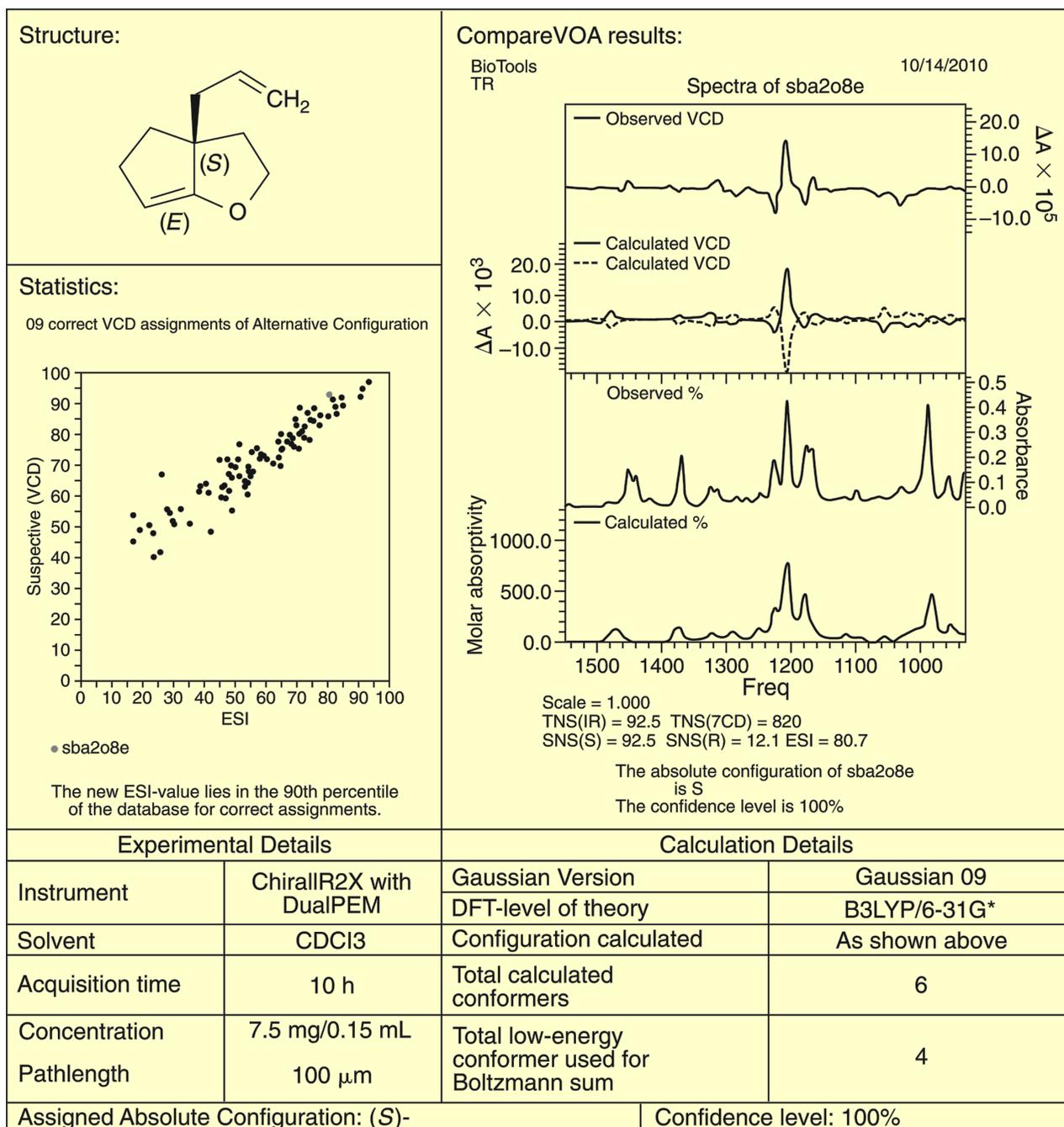


Figure 13. Output of a commercial program for comparison of measured (Observed) VCD and IR spectra with calculated VCD spectra of both enantiomers and the calculated IR spectrum. Statistical data are explained in the text.

9. DETERMINATION OF ENANTIOMERIC EXCESS

After AC, the next most important property of a chiral sample is the EE, as described in the *Introduction*. For enantiomers labeled *R* and *S*, the EE for the *R* enantiomer is defined as $EE = (N_R - N_S) / (N_R + N_S)$ and $\%EE = EE \times 100\%$. Here, N_R represents the number of moles of the *R* enantiomer present in the sample; or for a solution, N_R can represent the concentration of the *R* enantiomer. Thus, $\%EE$ for the *R* enantiomer can vary from +100% to -100%.

VCD spectra also can be used to determine the EE of a sample once the sample has been calibrated by a single IR and VCD measurement of a sample with a known EE. VCD scales linearly with EE with a maximum VCD intensity for a given IR intensity at 100% EE, one-half VCD intensity for the same IR intensity at 50% EE, and zero VCD for the racemic mixture of 0% EE. These points are illustrated in *Figure 14* for 11 measurements of *R*-(+)- α -pinene for which the %EE decreases from 100% to 6.7%. The IR spectra are identical, but the VCD spectra grow smaller linearly as EE is reduced by the addition of measured amounts of the opposite enantiomer to the sample cell. The degree of accuracy of this determination of %EE was slightly >1%, as indicated in the plot of actual prepared EE versus VCD predicted EE, using a partial least-squares chemometric analysis as presented in *Figure 15*.

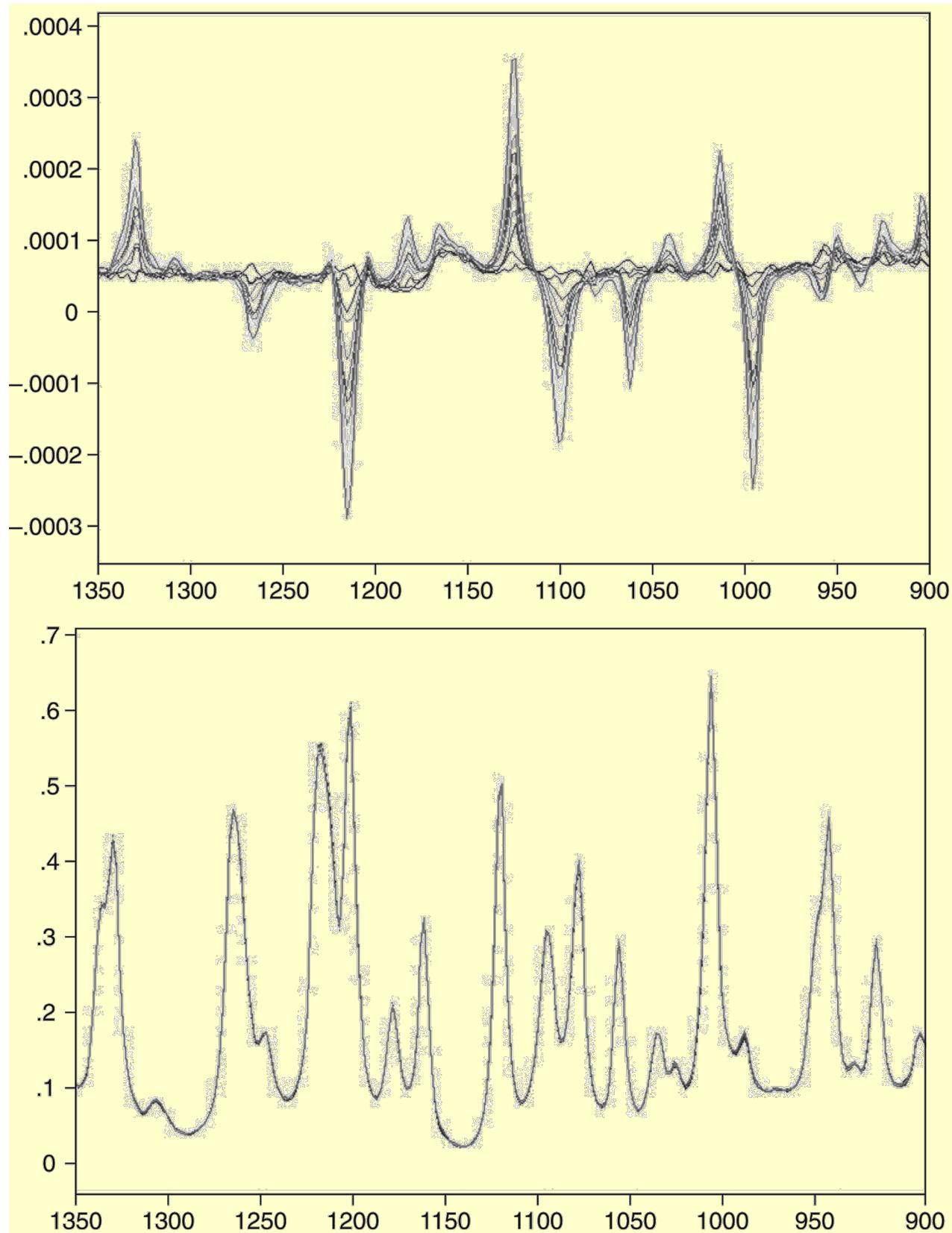
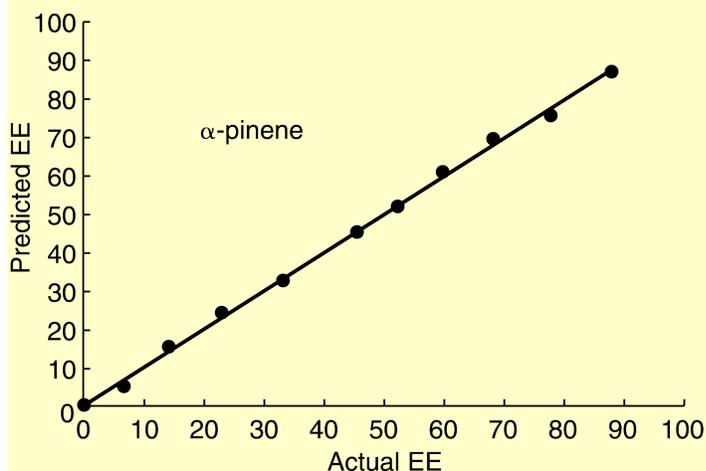


Figure 14. Superposition of 11 VCD (upper) and IR (lower) spectra of a 3.1 M solution of (+)-*(R)*- α -pinene in carbon tetrachloride for samples with %EE values of 100.0%, 88.2%, 77.8%, 68.4%, 60.2%, 52.4%, 45.5%, 33.3%, 23.1%, 14.3%, and 6.7%. Reproduced in part with

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Actual EE%	Predicted EE%	Variance (EE%)
88.2	87.2	1.0
77.8	75.9	1.9
68.4	69.7	-1.3
60	61.3	-1.3
52.4	52.2	0.2
45.5	45.3	0.2
33.3	32.8	0.5
23.1	24.4	-1.3
14.3	15.4	-1.1
6.7	4.9	1.8
0	0.3	-0.3
RMSD	1.15	
STDEV	1.21	

Figure 15. Plot and corresponding table of actual versus VCD predicted values of %EE using partial least-squares (PLS) chemometric analysis that achieves a root-mean-square deviation (RMSD) of 1.15 and a standard deviation error of cross-validation (STDEV) of 1.21.

10. CONCURRENT USE OF VCD FOR ABSOLUTE CONFIGURATION AND ENANTIOMERIC EXCESS

10.1 Chiral Raw Material Identification

Currently, there is no routine, real-time chiral measurement for material identification (ID). Chiral drug substances must comply with the *USP* monograph tests that typically rely upon nonchiral test procedures such as mid-IR spectroscopy (*Mid-Infrared Spectroscopy* (854) and *Mid-Infrared Spectroscopy—Theory and Practice* (1854)). In practice, near-IR [see the proposals in *PF* 41(1) for *Near-Infrared Spectroscopy* (856) and *Near-Infrared Spectroscopy* (1119)] is used in many manufacturing facilities. For near-IR analysis of solids, analysts commonly use fiber-optic probes and then confirm the raw material by comparison of the measured spectrum against that of the *USP* Reference Standard. To determine the AC and EE of a chiral material, analysts must make a separate optical rotation measurement, which requires large amounts of sample in a 10-cm path-length cell, where uncertainty errors are possible for materials with small OR values.

A single measurement of the VCD spectrum and its associated IR spectrum in the mid-IR or near-IR region of a raw material, either as a crystalline solid or in solution, simultaneously contains information for the three critical measures: ID, AC, and EE. By comparison to a *USP* Reference Standard, the simultaneous measurement of IR and VCD spectra of a test material identifies the sample and the presence of impurities and separates impurities into achiral (IR only) or chiral (IR and VCD). The signs of the VCD identify the AC of the dominant enantiomer, and the ratio of the VCD to the IR gives the EE, as described above. Typical VCD accuracy for EE determinations is in the range of 0.1%–1%.

10.2 Chiral Quality Control

VCD can be used as a chiral measure for characterization of raw materials for process analytical technology (PAT) during development, synthesis, formulation, and final production of drug

substances and drug products. VCD also can be used to test the interaction between formulated chiral drug substances and excipients. Currently, there are no protocols for monitoring EE as a quality control measure in the pharmaceutical industry. Because of the importance of chirality as a critical measure of sample integrity, there is a need for the incorporation of a new technology, such as VCD, to ensure the desired level of chiral quality of pharmaceutical products from discovery through to final formulation.

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BRIEFING

⟨1821⟩ **Radioactivity—Theory and Practice.** The current general chapter *Radioactivity* ⟨821⟩ is divided into two major sections, *General Considerations* and *Identification and Assay of Radionuclides*, both of which contain subsections with general information about radioactivity. Because of the nature of the information presented in these subsections, they are being moved to this proposed new general information chapter *Radioactivity—Theory and Practice* ⟨1821⟩.

(CHM4: R. Ravichandran.)

Correspondence Number—C154279

Comment deadline: November 30, 2015

Add the following:

■ ⟨1821⟩ RADIOACTIVITY—THEORY AND PRACTICE

1. INTRODUCTION
2. TYPES OF DECAY
 - 2.1 Alpha Decay
 - 2.2 Beta Decay
 - 2.3 Electron Capture Decay
 - 2.4 Isomeric Transition
3. GENERAL CONSIDERATIONS
 - 3.1 Radioactivity
 - 3.2 Fundamental Decay Law
 - 3.3 Counting Efficiency
 - 3.4 Background
 - 3.5 Statistics of Counting
 - 3.6 Minimum Detectable Activity
 - 3.7 Limit of Quantification
 - 3.8 Counting Losses
 - 3.9 Linearity and Range
 - 3.10 Calibration Standards
 - 3.11 Production of Radionuclides
 - 3.12 Carrier

- 3.13 Radiochemical Purity
- 3.14 Radionuclidic Purity
- 3.15 Chemical Purity
- 3.16 Labeling
- 3.17 Naming Conventions for Isotopes

4. INSTRUMENTATION FOR DETECTION AND MEASUREMENT OF RADIOACTIVE EMISSIONS AND APPLICATIONS

- 4.1 Ionization Chambers
- 4.2 Liquid Scintillation Counters
- 4.3 Nuclear Spectroscopy Systems
- 4.4 Detector Systems for Chromatographic Applications

5. GLOSSARY

6. REFERENCES

1. INTRODUCTION

Radioactive drugs and devices require specialized techniques in their production, testing, handling, dispensing, and administration to ensure optimal effectiveness and maintain safety for workers, patients, and the public. All operations involving these articles should be carried out by or under the supervision of personnel who have been appropriately trained in the handling of radioactive materials.

The facilities for the production, storage, and use of radioactive drugs and devices are generally subject to licensing by the U.S. Nuclear Regulatory Commission, an appropriate State agency, or similar governmental agencies outside of the United States. Most radioactive drugs and devices, although not identified as hazardous drugs, are classified as hazardous materials and are therefore subject to other regulations relating to transportation, environmental release, and workplace safety.

The purpose of this chapter is to provide information regarding radioactivity—including definitions, types of decay, and general considerations relating to radioactive decay, counting, radionuclide production, purity, and labeling—as well as instrumentation for detection and measurement of radioactive emissions.

Specific information on standards for radionuclide identification and assay, including instrument qualification, performance checks, identification of radionuclides and radionuclidic impurities, and assay of radionuclides are provided in *Radioactivity* (821).

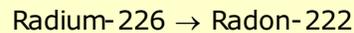
2. TYPES OF DECAY

Radioactive decay is the process by which an unstable nuclide transitions to a lower energy configuration. Depending on the particular starting radionuclide, the result of the transition may be either a stable nuclide or a different radionuclide. Typically, these transitions are accompanied by the emission of radiation from the nucleus, which is broadly classified as either particulate or nonparticulate. Some radionuclides emit multiple types of radiation in this process, whereas others emit only a single type. The main types of particulate radiation commonly seen in nuclear medicine are alpha, beta, and positron. Nonparticulate types of

radiation include gamma rays and X-rays. Strictly speaking, X-rays do not originate in the nucleus but rather in the electron orbitals; however, X-rays are the result of interactions between radiation from the nucleus and the orbital electrons. Nuclear medicine imaging is accomplished through detection and localization of nonparticulate radiation, whereas therapeutic effects arise from the energy deposited in the target organ by particulate radiation.

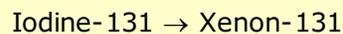
2.1 Alpha Decay

Alpha decay is radioactive decay with the emission of alpha particles, or helium nuclei, and is generally limited to elements with an atomic number >83 . In some cases, beta particles and gamma rays may also be emitted during alpha decay. An example of a radionuclide that decays by alpha decay is:

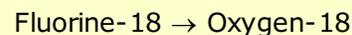


2.2 Beta Decay

Beta decay is radioactive decay with the emission of an electron. This type of decay typically occurs in neutron-excessive radionuclides wherein a neutron is transformed into a proton. In some cases, the emission of a positively charged electron, or positron, may occur. This type of decay typically occurs in neutron-deficient radionuclides with a lower atomic number wherein a proton is transformed into a neutron. In some cases, gamma rays may also be emitted during beta decay. An example of beta decay through emission of an electron is:



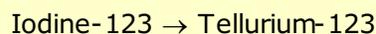
An example of beta decay through emission of a positron (β^+) is:



Because a positron is an anti-electron, when it interacts with an electron, the two particles annihilate, and their combined mass is transformed into energy in the form of two 511 kiloelectronvolt (keV) gamma rays. These gamma rays are produced simultaneously and travel away from the point of interaction in nearly opposite directions. These two characteristics form the basis for positron emission tomography (PET) imaging techniques.

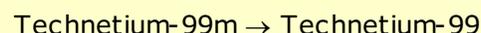
2.3 Electron Capture Decay

Electron capture is radioactive decay that involves nuclear capture of an inner orbital electron, nuclear transformation of a proton into a neutron, and emission of one or more gamma rays. Electron capture generally occurs in higher atomic number radionuclides that are neutron deficient. An example of a radionuclide that decays by electron capture decay is:



2.4 Isomeric Transition

An isomeric transition is radioactive decay that involves a transition between nuclear isomers with the emission of one or more gamma rays. In contrast to other types of decay, the number of protons and neutrons remains the same in an isomeric transition. Isomeric transition generally occurs in radionuclides that are metastable. An example of a radionuclide that decays by isomeric transition is:



3. GENERAL CONSIDERATIONS

3.1 Radioactivity

Radioactive decay is a first-order process (i.e., a fraction of atoms decay per unit time). The rate of decay for each radionuclide is a unique and constant value, which gives rise to the term "decay constant".

Each radionuclide's rate of decay is a unique and constant value (its decay constant) and can be described by the following equation:

$$A = \lambda N$$

A = amount of radioactivity in a source at a given time

λ = rate of decay of the radionuclide

N = number of radioactive atoms

The traditional unit for radioactivity is the Curie (Ci), which is equal to 3.7×10^{10} atoms undergoing radioactive decay, or disintegrations per second (dps). Commonly used prefixes associated with the Ci include the millicurie (mCi) and the microcurie (μ Ci). The SI unit for radioactivity is the Becquerel (Bq), which is equal to 1 dps. Commonly, prefixes associated with the Bq include the megabecquerel (MBq) and the gigabecquerel (GBq). Hence, 1 Ci = 37 GBq.

3.2 Fundamental Decay Law

The decay of a radioactive source is described by the equation:

$$N_t = N_0 e^{-\lambda t}$$

N_t = number of radioactive atoms remaining at elapsed time t

t = time elapsed (time unit, such as s, min, or h)

N_0 = number of radioactive atoms when $t = 0$

λ = decay constant of the specific radionuclide

The above equation can be rewritten in terms of radioactivity:

$$A_t = A_0 e^{-\lambda t}$$

A_t = amount of radioactivity at elapsed time (t)

t = time elapsed (time unit, such as s, min, or h)

A_0 = amount of radioactivity when $t = 0$

λ = decay constant of the specific radionuclide

"Decay tables" that provide radionuclide-specific decay factors (i.e., fraction remaining) calculated from $A_0 e^{-\lambda t}$ at various elapsed times (t) are commonly available.

The half-life is defined as the time interval required for a quantity of radioactivity to decay to one-half of its initial value and is related to the decay constant λ by the equation:

$$T_{1/2} = 0.69315/\lambda$$

$T_{1/2}$ = half-life of the radionuclide

λ = decay constant of the specific radionuclide

The activity of a pure radioactive substance as a function of time can be obtained from the exponential equation, from decay tables, or by graphical means based on the half-life (*Figure 1*).

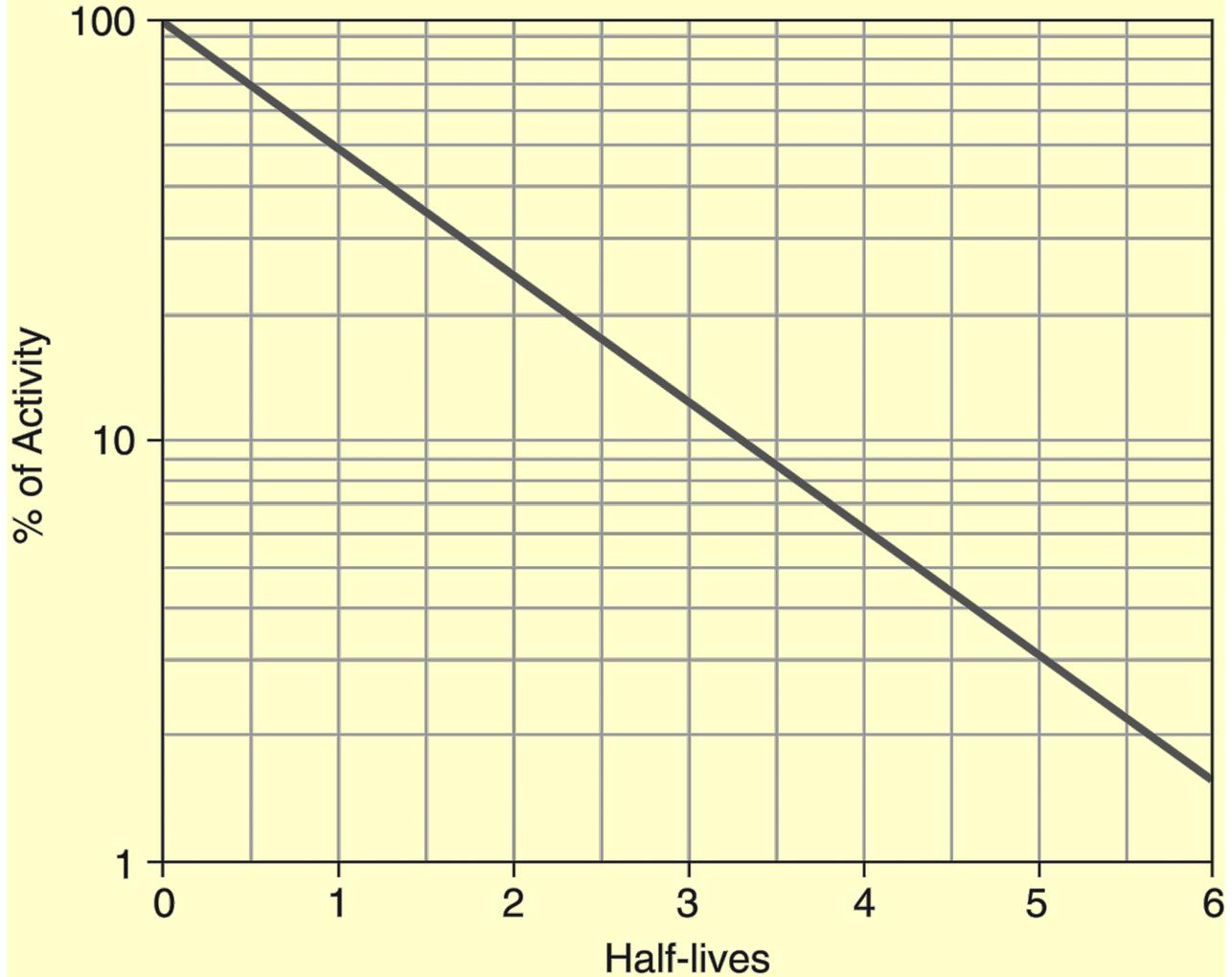


Figure 1. Normalized decay chart.

3.3 Counting Efficiency

The validity of any radionuclide measurement is dependent upon the reproducibility of the relationship between the source, the detector, and its surroundings. Appropriate allowances should be made for source configuration. Measurements of radioactivity require calculation of a calibration factor, or efficiency, and are dependent on the type of detector, the container in which the radioactivity is placed, and the source-detector geometry. The basic efficiency equation for detectors designed to count discrete decay events over a period of time is given as:

$$\text{disintegrations/s} = (\text{counts/s}) / \epsilon$$

ϵ = efficiency or calibration factor

Because 1 dps is defined as 1 Bq, the above equation gives radioactivity in units of Bq. Through the application of the appropriate unit conversion, Bq can be transformed into μCi or any other unit of radioactivity. Detectors should be calibrated with a source of known radioactivity and in a fixed geometry.

3.4 Background

Cosmic rays, radioactivity present in the detector or shielding materials, and radiation from improperly shielded sources contribute to the background radiation. All radioactivity measurements should be corrected by subtracting the background count rate from the gross count rate in the test sample.

3.5 Statistics of Counting

Modern radiation detection systems often incorporate statistical analysis into their software. The user should understand the use and limitations of these programs to ensure accurate results.

Because the process of radioactive decay is a random phenomenon, the events being counted form a random sequence in time. Therefore, counting for any finite time can yield only an estimate of the true counting rate. The precision of this estimate, being subject to statistical fluctuations, is dependent upon the number of counts accumulated in a given measurement and can be expressed as:

$$\Sigma = \sqrt{n}$$

Σ = standard deviation

n = number of counts accumulated in a given measurement

The probability of a single measurement falling within $\pm 100/\sqrt{n}\%$ of the mean of a great many measurements is 0.68, which means that if each count were to lie within $\pm 100/\sqrt{n}\%$ of the mean for approximately two-thirds of the observations, then approximately one-third of the observations would lie outside of this interval.

Because of the statistical nature of radioactive decay, repeated counting of an undisturbed source will yield count-rate values in accordance with the frequency of a normal distribution.

Any deviations in these values from the normal distribution conform to the chi-square (χ^2) statistical test. For this reason, the χ^2 test is frequently applied to determine the performance and correct operation of a counting assembly. The term "figure of merit" of a radioactive counting instrument is expressed as:

$$\text{Figure of merit} = \epsilon^2/B$$

ϵ = counter efficiency

B = background count rate (dps)

In the selection of instruments and conditions for assay of radioactive sources, the figure of merit should be maximized.

3.6 Minimum Detectable Activity

In situations where only very small quantities of radioactivity are to be measured, the lower limit of the ability of the instrument to detect that particular radionuclide should be known. The "minimum sensitivity", also referred to as "limit of detection", is defined as the net count rate above background that must be exceeded before a sample is deemed to contain detectable radioactivity with a specified level of confidence. The minimum sensitivity is generally considered to be 3 standard deviations above the mean background count rate and is calculated as:

$$\text{Minimum sensitivity} = (3 \times \sqrt{B})/t$$

B = background count rate (dps)

t = count time

The minimum detectable activity is defined as the smallest quantity of radioactivity that can be measured under the specific conditions of minimum sensitivity and counting efficiency of the instrument. It is calculated as:

$$\text{Minimum detectable activity} = (\text{Minimum sensitivity})/(\epsilon \times F)$$

ϵ = counting efficiency

F = unit conversion factor

For example, if minimum sensitivity is in units of dpm and minimum detectable activity is desired to be in units of Bq, then $F = 60 \text{ dpm/Bq}$. If minimum sensitivity is in units of cpm and minimum detectable activity is desired to be in units of μCi , then $F = 2.2 \times 10^6 \text{ dpm}/\mu\text{Ci}$.

3.7 Limit of Quantification

The limit of quantification is the smallest quantity of radioactivity that can be quantitatively determined with suitable precision and accuracy. The limit of quantification is used particularly for the determination of impurities and degradation products. In practical terms, the limit of quantification is usually considered to be 10 standard deviations above the mean background count rate.

3.8 Counting Losses

The minimum time interval that is required for the counter to resolve two consecutive signal pulses is known as the dead time. The dead time is typically on the order of microseconds for proportional and scintillation counters and to hundreds of microseconds for Geiger counters. Nuclear events occurring within the dead time of the counter will not be registered. The corrected count rate, R , can be calculated using the formula:

$$R = r/(1 - r \times \tau)$$

r = observed count rate

τ = dead time

The correction formula assumes a nonextendable dead time. The observed count rate, r , refers to the gross sample count rate and is not to be corrected for background before use in this

equation. For general validity, the value of $r \times T$ should not exceed 0.1.

3.9 Linearity and Range

When a radiation detection instrument is used in a quantitative measurement, the instrument should be suitable for the type(s) of radiation to be measured, and the instrument response should be linear over the range of measurements or a correction factor should be applied. Normally, a minimum of five different quantities of radioactivity are used to establish linearity. These quantities should bracket the range of radioactivity levels that are routinely measured in a particular application.

3.10 Calibration Standards

All radioactivity assays should be performed using measurement systems that have been calibrated with appropriately certified radioactivity standards. Such calibration standards may be purchased either directly from an appropriate National Metrology Institute (NMI) or from other sources that have established traceability to the NMI, through participation in a program of inter-comparative measurements. Where such calibration standards are unavailable, nuclear decay data required for calibration can be obtained from the Evaluated Nuclear Structure Data File maintained at the Brookhaven National Laboratory.¹

3.11 Production of Radionuclides

The various radionuclides found in nature generally have undesirable properties for nuclear medicine applications, including very long half-lives (e.g., thousands or millions of years), decay with emissions of alpha or beta particles, and low isotopic purity because of the presence of other isotopes of that element. Because of these properties, naturally occurring radionuclides are rarely used for radiopharmaceuticals, except for some alpha-emitting members of the actinide decay series, which are used for certain therapeutic radiopharmaceuticals, e.g., radium-223.

There are four common production routes for artificially produced radionuclides: fission, neutron activation, charged-particle-induced reactions (cyclotron), and radionuclide generators. Fission by-products refer to radionuclides that are obtained as by-products of the fission of uranium (uranium-235). These radionuclides, whether directly produced fission fragments or subsequent members in a decay chain originating from a fission fragment, can be chemically separated into individual forms from the mixture of fission products. Desirable properties of fission by-products include high isotopic purity and moderate cost. Undesirable properties may include beta-particle emissions, low specific activity of certain radionuclides, and the limited selection of radionuclides produced. Examples of fission by-products used in nuclear medicine applications include iodine-131 and xenon-133. Currently, the most widely used fission by-product is molybdenum-99, which is used in technetium-99m generators.

Neutron activation refers to the production of radionuclides in a nuclear reactor by bombarding target atoms with thermal neutrons. Nuclear transformations, induced by neutron capture, result in isotopes possessing one additional neutron, and thus an atomic mass increased by one. The excess energy of the newly formed isotope is emitted as a gamma ray. These reactions are often termed (n,γ) reactions. Desirable properties of neutron-activated

radionuclides include a wide variety of isotopes that can be produced and the moderate cost to produce them. Undesirable properties may include decay with beta-particle emissions and relatively low isotopic purity (i.e., unreacted stable target atoms are mixed with their radioisotope products). Because of their beta emissions, however, several of these radionuclides have been used in therapeutic radiopharmaceuticals. Examples of neutron-activated radionuclides used in therapeutic radiopharmaceuticals include strontium-89, yttrium-90, iodine-131, samarium-153, and lutetium-177.

Cyclotron production of radionuclides occurs by bombarding stable atoms with charged particles (e.g., protons or deuterons) that have been accelerated in the cyclotron's oscillating electromagnetic field. Nuclear transformations induced by particle capture usually result in a radioisotope of a different element with the emission of one or more neutrons or protons. For example, if a proton is captured and a neutron is emitted, the reaction is often termed a (p,n) reaction. Desirable properties of cyclotron-produced radionuclides include: the wide variety of isotopes that can be produced, the availability of alternate production schemes, radionuclide decay by electron capture or positron decay rather than by beta decay, and high isotopic purity. Undesirable properties may include: contaminating radioisotopes from side reactions and the relatively high cost of radionuclide product. Examples of cyclotron-produced radionuclides used in nuclear medicine applications include carbon-11, fluorine-18, gallium-67, indium-111, iodine-123, and thallium-201.

Generators refer to a special method of radionuclide production whereby a short-lived radionuclide results or is generated from the decay of a longer-lived radionuclide. The longer-lived parent radionuclide is generally bound to a column, and the short-lived radionuclide daughter product is then extracted (eluted) from the column. After elution, subsequent decay of the long-lived parent radionuclide generates more of the short-lived radionuclide daughter product, which can then be extracted. A generator provides a specific radionuclide in sequential elutions over a prolonged period of time. Desirable properties of generator-produced radionuclides include: ready availability, portability, low-to-moderate cost, variety of radionuclides and type of decay, and relatively high isotopic purity. Undesirable properties may include the limited number of parent-daughter pairs and the potential for generator breakthrough of the parent radionuclide in the eluate. Examples of generator-produced radionuclides used in nuclear medicine applications include technetium-99m (daughter of molybdenum-99), rubidium-82 (daughter of strontium-82), and gallium-68 (daughter of germanium-68). The characteristics of all four production methods are summarized in *Table 1*.

Table 1. Production Methods of Radionuclides

Production Method	Nuclear Reactor (fission by-product)	Nuclear Reactor (neutron activation)	Cyclotron	Radionuclide Generator
Bombarding particle	Neutron	Neutron	Proton, deuteron, triton, alpha	Production by decay of parent
Product	Neutron excess	Neutron excess	Neutron poor	Neutron poor or excess
Typical decay pathway	β^-	β^-	Positron emission, electron capture	Several modes
Typically carrier free	Yes	No	Yes	Yes
High specific activity	Yes	No	Yes	Yes
Relative cost	Moderate	Moderate	High	Low to moderate
Radionuclides for nuclear medicine applications	Molybdenum-99, iodine-131, xenon-133	Iodine-131, strontium-89, yttrium-90, samarium-153, lutetium-177	Thallium-201, iodine-123, gallium-67, indium-111, fluorine-18, carbon-11	Technetium-99m, krypton-81m, gallium-68, rubidium-82

Other methods of radionuclide production have been developed but currently are not used to produce radionuclides used in radiopharmaceuticals.

3.12 Carrier

The total mass of radioactive atoms or molecules in a radiopharmaceutical is directly proportional to the amount of radioactivity and is usually too small to be measured by ordinary chemical or physical methods. For example, 37 MBq (1 mCi) of iodine-131 has a mass of 8×10^{-9} g. Because such small quantities behave in an anomalous manner, such as nonspecific adsorption to container walls, a carrier may be added during processing to permit ready handling. Amounts of the carrier, however, should be sufficiently small so that undesirable physiological, pharmacological, or toxicological effects are not produced. Also, because the carrier is chemically identical to the radionuclide, the amount of carrier should be limited to avoid competitive interference with the desired chemical reactions and overall radiochemical yield.

The term "carrier free" refers only to radioactive preparations in which other isotopes of the radionuclide are absent (i.e., free from the presence of carrier). In practice, a true carrier-free state may be difficult or impossible to achieve because of the ubiquity of certain elements or molecules. Hence, the term "no carrier added" may more appropriately describe a preparation that may contain a trivial amount of carrier but for which additional carrier has not been purposefully added. Radionuclides produced by neutron activation reactions generally contain substantial amounts of nonradioactive isotope remaining from unreacted target material and thus cannot be considered carrier free. However, there are select cases in which this is not the case, such as (*n,p*) reactions.

The radioactivity per unit volume of a medium or vehicle containing a radionuclide is referred to as the "radioactivity concentration", "specific concentration" or "strength" and is expressed in units such as Bq/mL or Ci/mL. The radioactivity of a radionuclide per unit mass is referred to as "specific activity" and is expressed in units such as Bq/g, Ci/g, or Bq/mol. The maximum specific activity of a radioactive preparation exists when it is in a carrier-free state; the addition of a carrier results in lowered specific activity.

3.13 Radiochemical Purity

The radiochemical purity of a radiopharmaceutical preparation refers to the fraction of the stated radionuclide present in the stated chemical form. Radiochemical purity is important for radiopharmaceuticals because radiochemical impurities may affect the biodistribution and interfere with image interpretation (diagnostic accuracy). In addition, radiochemical impurities may alter radiation absorbed doses to various organs. When using therapeutic radiopharmaceuticals, radiochemical purity is very important because altered biodistribution associated with radiochemical impurities may result in insufficient irradiation of the target tissue (suboptimal treatment response) or excessive irradiation of other tissues (undesired radiation damage).

Radiochemical impurities in radiopharmaceuticals may result from by-products of the preparative method or from decomposition. Radiation causes decomposition of water, a main component of aqueous radiopharmaceutical preparations, leading to the production of reactive hydrogen atoms and hydroxyl radicals, hydrated electrons, hydrogen, hydrogen ions, and hydrogen peroxide. Hydrogen peroxide is formed in the presence of oxygen radicals, originating from the radiolytic decomposition of dissolved oxygen. Many radiopharmaceuticals show improved stability if oxygen is excluded or restricted. Radiation may also affect the radiopharmaceutical itself, giving rise to ions, radicals, and excited states. These species may combine with one another and/or with the active species formed from water. Radiation decomposition may be minimized by the use of chemical agents that act as electron or radical scavengers.

Radiochemical purity must meet compendial standards throughout the time of use until the stated expiration of the radiopharmaceutical.

Determination of radiochemical purity is typically a two-stage process: 1) the different chemical species are separated by paper, thin-layer, or column chromatography or other suitable analytical separation technique; and 2) the radioactivity content in each of the separated chemical species is measured with a suitable radiation detector and counting device. The ultimate confirmation of acceptable radiochemical purity of a radiopharmaceutical is its intended biodistribution after administration.

3.14 Radionuclidic Purity

The radionuclidic purity of a radiopharmaceutical preparation refers to the fraction of radioactivity attributable to the desired radionuclide in the total radioactivity measured. Hence, a radionuclidic impurity is the presence of an unwanted radionuclide. Radionuclidic purity is important for radiopharmaceuticals, because unwanted radionuclides may cause several undesired consequences.

- Radionuclidic impurities may cause the radioactive assay of the radiopharmaceutical to deviate from the prescribed amount.
- Radionuclidic impurities may deliver higher-than-desired, radiation-absorbed doses to various organs and tissues.

- In some situations, radionuclidic impurities may interfere with image interpretation (diagnostic accuracy). It should be remembered that radionuclidic purity will change with time and is generally specified as a percentage of the desired radionuclide's activity at the time of calibration or at the time of administration (e.g., molybdenum-99 in technetium-99m).

The radionuclidic purity must meet compendial standards throughout the useful life of the radiopharmaceutical. In addition, the impurity(ies) themselves will decay. Consideration must be given to the acceptable minimum and maximum allowable times between expiration of the product and analysis for impurities.

Radionuclidic impurities commonly arise during radionuclide production relating to impurities in target materials, differences in the values of various competing production cross-sections, and different excitation functions of competing reactions at the energy of the bombarding particles. In the case of generator-produced radionuclides, some generator breakthrough of parent radionuclide typically occurs and represents a radionuclide impurity in the eluate of the daughter radionuclide.

Determination of radionuclidic purity is typically based on evaluation of radioactive emissions, the principal analysis of the gamma spectrum obtained from a sample of the product. For short-lived isotopes, the half-life measurement could be an appropriate approach to assess radionuclidic purity. In cases involving radionuclidic impurities that have long half-lives relative to the desired radionuclide, measurement of the radionuclidic impurities can be performed after a sufficient time delay to allow the desired radionuclide to fully decay. In cases involving radionuclidic impurities that have substantially higher energy gamma emissions relative to the desired radionuclide, measurement of the radionuclidic impurities can be performed after placing the product inside a properly calibrated radiation shield that affords differential attenuation of the gamma rays emitted from the desired radionuclide versus those emitted from the radionuclidic impurities. Positron-emitting radionuclidic impurities typically cannot be differentiated, because their emitted energy (511 keV) is the same for each radionuclide. In any case, the appropriate instrument should be chosen to detect potential impurities and should be properly calibrated to accurately quantify any identified impurities.

3.15 Chemical Purity

The chemical purity refers to the fraction of the total chemical species present in the product as the specified chemical component(s). Hence, a chemical impurity is the presence of an unwanted nonradioactive chemical. Chemical purity is important for radiopharmaceuticals, because chemical impurities may cause undesirable consequences such as chemical interactions (e.g., precipitation) and toxic biologic effects.

Chemical impurities are typically associated with production procedures and may include contaminants from raw materials, synthetic by-products, solvents, excipients, equipment, preparative or purification columns, and containers. For certain radiopharmaceuticals, chemical impurities may also be associated with generator breakthrough of resin material from the generator column (for example, alumina) in the eluate solution.

Determination of chemical purity is generally not performed and reported as a single attribute. Rather, determinations of individual chemical impurities are performed and compared to specifications (limits) for the respective individual chemical impurities. Such determinations of chemical impurities use analytical techniques as appropriate and described in the individual radiopharmaceutical monograph.

3.16 Labeling

Individual radiopharmaceutical monographs indicate that the labeling is to include the date and time of calibration, the amount of radioactivity associated with the radiopharmaceutical expressed as total MBq (μCi or mCi) and concentration as MBq (μCi or mCi)/mL at the time of calibration, the expiration date (and time, if appropriate), and the statement, "Caution—Radioactive Material". The labeling indicates that in making dosage calculations, a correction is to be made for radioactive decay and also indicates the radioactive half-life of the radionuclide. Other labeling requirements may apply to biologics or articles intended for injection. Beyond-use dates of compounded preparations should be included as appropriate.

3.17 Naming Conventions for Isotopes

Various naming conventions exist for isotopes that are associated with radiopharmaceuticals and radioactive devices. For example, the name of an isotope may or may not use a superscripted value for the mass number. Superscripted values should precede the elemental symbol for the isotope, and non-superscripted values should follow the elemental symbol, preferably with a hyphen between the symbol and the mass number. Examples include ^{68}Ga and O-18, respectively. Square brackets should be used to denote a specific isotope when necessary within a chemical name, for example, 2- ^{18}F fluoro-2-deoxyglucose. Individual radiopharmaceutical monographs use non-proprietary names assigned by the USAN Council, which use the elemental symbol followed by the mass number separated by a space, for example, Thallous Chloride Tl 201 Injection. Although exceptions to these conventions undoubtedly exist, efforts should be made to adopt standardized conventions for radiopharmaceuticals and radioactive devices that fall within the scope of this general chapter.

4. INSTRUMENTATION FOR DETECTION AND MEASUREMENT OF RADIOACTIVE EMISSIONS AND APPLICATIONS

4.1 Ionization Chambers

Radioactive materials are not readily detected by ordinary chemical or physical methods. Instead, detection methods for radioactive materials rely on the ionization of matter that results from the emitted radiation. The charge separation created during this process forms the basis of radiation detection systems, which may be based on the ionization properties of gaseous, liquid, and solid materials.

An ionization chamber is an instrument that directly measures ions produced in a gas as the result of the interaction of radiation with the gas. The most common usage in nuclear medicine applications is as the detector used in a dose calibrator. The dose calibrator is an instrument used to measure the quantity of radioactivity in a radiopharmaceutical. The key component of a dose calibrator is an argon-filled chamber with an applied electrical potential that allows the detection of ions produced by the passage of gamma rays through the chamber. Calibration of the system may involve one or more radionuclides with gamma energies and quantities that span the range of typical analyses. The calibration of the ionization chamber should be performed, when possible, with suitable NMI-traceable radionuclide standards. Routine system suitability testing should include checks for these parameters. Frequency of testing should

occur as appropriate. Please refer to (821) for additional details on typical instrument requirements.

The position of the radioactive sample in the dose calibrator is ideal when it simulates 4 π geometry. The geometric goal is placement of the sample at a point in the center of the cylindrical detector. Reproducibility of placement within the chamber is critical, because the response typically drops off at the top and bottom of the cylinder because of a combination of geometry and electronic effects. The value of the ionization current per unit of radioactivity, known as the "calibration factor", is characteristic of each gamma-ray-emitting radionuclide. The current produced in a dose calibrator is related to the mean energy of the emitted radiation and is proportional to the intensity of the radiation. The calibration of the dose calibrator for a specific radionuclide is ideally performed with a radioactive calibration source of the same radionuclide. Alternatively, it may be performed by measuring radioactive calibration sources with gamma energies above and below the gamma energy for the radionuclide to be measured and interpolating these values, also correcting for differences in gamma abundance, to establish the calibration factor for that radionuclide.

The upper limit of the dose calibrator is normally specified by the manufacturer. If not, testing is required to ascertain this upper limit. With a deep re-entrant well-type chamber, reproducibility within approximately 5% or less can be readily obtained in a few seconds for quantities of radioactivity in the MBq (mCi) range and within about 30 s for quantities in the kBq (μ Ci) range.

The calibration of a dose calibrator should be maintained by relating the measured response of a standard to that of a long-lived reference standard, such as radium-226 in equilibrium with its daughters, cesium-137 in equilibrium with its daughters, barium-133, cobalt-60, or cobalt-57. The instrument should be checked on each day of use with the reference standard source to ascertain the stability over a long period of time. This check should include reference standard readings at all radionuclide settings used. Any necessary corrections for radioactive decay of the reference standard source should first be applied. It is also recommended that the reproducibility and/or stability of multi-range instruments be checked with the use of standards with appropriate activities for all ranges.

The size, shape, and location of a radioactive sample within the well will affect the response of a dose calibrator. This is usually referred to as "geometry". The shape, composition, and dimensions of the container holding the radioactive material can affect the result. Effects relating to container properties are generally more pronounced with radionuclides that emit beta particles (because of differences in Bremsstrahlung production) or emit low energy gamma or X-rays (because of differences in photon attenuation). It is important that geometric correction factors, if needed, be determined for each combination of radionuclide and configuration (i.e., size, shape, location with the well chamber, volume within the container, and container properties). The manufacturer's calibration factor for each radionuclide is determined using a specific geometry and container, which may not match the geometry or container used operationally.

4.2 Liquid Scintillation Counters

The liquid scintillation counter (LSC) detection method uses liquid scintillation cocktails to transform emitted radiation into detectable light photons. Alpha- and beta-emitting radionuclides may be assayed with the use of a liquid-scintillation detector system. In the liquid

scintillator, the emitted radiation is converted into light quanta that are usually detected by two multiplier phototubes arranged to detect only coincidence radiation. The liquid scintillator is a solution consisting of a solvent, primary and secondary solutes, and additives. As the emitted particle dissipates energy in the solvent, a fraction of this energy is converted into fluorescent light by the primary solute. The function of the secondary solute is to absorb the primary fluorescence and re-emit the light at a longer wavelength that is more efficiently detected by the multiplier phototubes. Traditionally used solvents (cocktails) are toluene and *p*-xylene; primary solutes are 2,5-diphenyloxazole (PPO) and 2-(4'-*tert*-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole (butyl-PBD); and secondary solutes are 2,2'-*p*-phenylenebis[4-methyl-5-phenyloxazole] (dimethyl-POPOP) and *p*-bis(*o*-methylstyryl)benzene (bis-MSB). Many aqueous scintillating solution cocktails that are less hazardous are available. Aqueous solutions tend to have a shorter shelf-life; therefore, it is important to ensure that they have not expired before use. As a means of attaining compatibility and miscibility with aqueous samples to be assayed, additives, such as surfactants and solubilizing agents, are also incorporated into the scintillator. For an accurate determination of radioactivity of the sample, care should be exercised to prepare a sample that is truly homogeneous. Quenching is a major concern for liquid scintillation and refers to any mechanism that causes a reduction of emitted light by the source. Quenching can result from multiple factors, including oxygen and dilution effects; therefore, it is critical to perform quenching corrections by counting comparative measurements of standard samples using the same conditions of volume, additives, and solvent to accurately account for these effects.

Alternatively, an external source, typically barium-133 or europium-152, is placed in close proximity to the sample vial to release Compton electrons. The shape of the resulting spectrum is analyzed to compute a quench-indication parameter. This parameter can then be related to the counting efficiency by measuring sources of known radioactivity at a determined level of quenching agent. The resulting quench curve allows the determination of the radioactivity of an unknown sample knowing the count rate and value of the quenching parameter. The scintillation fluid may require special handling for disposal, in addition to any residual radioactivity. Static electricity on the vials may also cause spurious counts in the system, especially in the case where low-energy beta emitters are being assayed. This problem is often greater in a low-humidity environment.

The disintegration rate of a beta-emitting source may be determined by a procedure in which the integral count rate of the sample is measured as a function of the pulse-height discriminator bias, and the emission rate is then obtained by extrapolation to zero bias. Energetic alpha-emitters may be similarly measured by this method.

4.3 Nuclear Spectroscopy Systems

GAMMA RAY SPECTROMETRY

Each gamma-emitting radionuclide has a unique spectrum of mono-energetic photons emitted that allows the identification and quantification of radioactive materials in a sample by comparing the energy(ies) of the photon(s) detected and the intensity at each energy. This gamma spectrum allows for both the quantitative determination of purity as well as identity of the radionuclide. Gamma-spectrum analysis can be performed by using either a scintillation crystal, typically sodium iodide activated with thallium [NaI(Tl)], or by using a semiconductor detector consisting of a germanium-lithium crystal, often referred to as a high-purity germanium detector (HPGe). Semiconductor detectors have a much higher energy resolution than NaI(Tl) detectors, with the ability to resolve gamma rays differing in energy by only a few

keV, as opposed to the 20–80 keV required for a NaI(Tl) detector. Because of their increased resolution, semiconductor detectors are the preferred analytical method for gamma spectral analysis. A lanthanum–bromide detector is also available that has a significantly better resolution (10–12 keV) than the NaI(Tl) detector, without the necessary requirement of liquid nitrogen cooling for a HPGe detector. The use of spectroscopy software to automate the analysis is acceptable; however, the operator should have an understanding of the parameters chosen to ensure that the performance of the system is adequate to meet test requirements. When all or part of the energy of beta or gamma radiation is dissipated within scintillators, photons of an intensity proportional to the amount of dissipated energy are produced. These pulses are detected by an electron multiplier phototube and converted to electrical pulses, which are subsequently analyzed with a pulse-height analyzer to yield a pulse-height spectrum related to the energy spectrum of the incident radiation. In general, a beta-particle, scintillation pulse-height spectrum approximates the true beta-energy spectrum, provided that the beta-particle source is prepared in such a manner that self-absorption is minimized. Beta-particle energy spectra may be obtained by using calcium fluoride or anthracene as the scintillator, whereas gamma-ray spectra are usually obtained with NaI(Tl) crystals, a HPGe semiconductor detector, or a lanthanum–bromide detector. The spectra of charged particles may also be obtained using silicon semiconductor detectors and/or gas proportional counters. Semiconductor detectors are, in essence, solid-state ionization chambers, but the energy required to create an electron-hole pair or to promote an electron from the valence band to the conduction band in the semiconductor is about one-tenth the energy required for creation of an ion-pair in a gas-filled ionization chamber or proportional counter. This energy threshold is also far less than the energy needed to produce a photon in a NaI(Tl) scintillation crystal. The energy resolution is a measure of the ability to distinguish the presence of two gamma rays closely spaced in energy and is defined by convention as the full width of the photopeak at its half maximum (FWHM), expressed as a percentage of the photopeak energy. For example, with 1.33 MeV gamma rays from cobalt-60, a HPGe detector has an energy resolution of about 0.3% FWHM, whereas a 3- × 3-in NaI(Tl) crystal has a value of about 6% .

Gamma-ray spectra exhibit one or more sharp, characteristic photopeaks, or full-energy peaks, as a result of total absorption in the detector of the full energy of incident gamma radiations. These photopeaks are useful for identification purposes. Other secondary peaks are observed as a consequence of backscatter, annihilation radiation, coincidence summing, fluorescent X-rays, and other factors, accompanied by a broad band known as the Compton continuum, which arises from the scattering of photons in the detector and surrounding materials. Because the photopeak response varies with gamma-ray energy, calibration of the gamma-ray spectrometer should be achieved with radionuclide standards having well-known gamma-ray energies and emission rates from an NMI. The shape of the gamma-ray spectrum is dependent upon the shape and size of the detector, the types of shielding materials used, and the electronic processing characteristics of the instrument.

One of the most useful applications of gamma-ray spectrometry is the identification of radionuclides and the determination of radionuclidic impurities. When confirming the identity of a radionuclide by gamma-ray spectrometry and/or quantifying the radioactivity, it is necessary to ensure that the detector has been accurately calibrated using a known source, as described above, and in the same geometry as the unknown sample. Where the radionuclides emit coincident gamma or X-radiations, the character of the pulse-height distribution often changes quite dramatically because of the summing effect of these coincident radiations in the detector as the efficiency of detection is increased, e.g., by bringing the source closer to the detector; this is referred to as "cascade summing". Such an effect is particularly evident in the case of

iodine-125. Most commercially available software packages include an ability to correct this source of error.

When identification of a radionuclide by means of a calibrated spectrometry system is not possible, the identity of the radionuclide may instead be established by measuring two or more of the following nuclear decay scheme parameters: 1) half-life; 2) energy of each gamma- or X-ray emitted; 3) the abundance of each emission; and (4) E_{max} , the maximum energy of emitted beta particles, for those radionuclides that decay with beta-particle emissions. Such measurements should be performed as directed in (821). Agreement of two or more of the measured parameters within 10% of the corresponding published nuclear decay scheme data confirms the identity of the radionuclide.

As with other types of detectors, the background should be determined and subtracted from the measurement. In addition, the background should be stable, especially in situations where long counting times are required. This can be achieved by running a background spectrum before analysis and comparing it to a previously obtained background spectrum. Generation of a background spectrum will also allow the calculation of a minimum detectable activity for each possible impurity.

BETA PARTICLE COUNTING SYSTEMS

Beta (β) particles are emitted with a distribution of energies ranging from zero to a definite maximum value. The maximum energy of the electrons is characteristic of a particular radionuclide and is normally the E_{max} shown in nuclear data tables. The determination of the maximum beta energy may aid in the identification of the beta-emitting radioisotope, and careful measurements can routinely quantify activities. Emitted beta particles rarely possess the maximum energy. On average, emitted beta particles possess one-third of the maximum energy. Beta particles can be difficult to detect because they only penetrate small thicknesses of solid materials. [Note—Radioisotopes that emit gamma radiation in addition to beta radiation are more easily quantified and identified by using gamma ray spectroscopy. In some cases, gamma ray detection is the preferred method for measuring these radioisotopes and is usually the best means of identification of a beta/gamma-emitting radionuclide.]

Several detectors can be used for the detection and measurement of beta particles. These can be ionization chambers, proportional counters, and scintillation counters with their associated electronics. Self-absorption and back scattering can be an issue in beta-particle analysis and can result in a lower or higher number. Ionization chambers and proportional counters can be used for the quantitation of beta particles but are less suited for identification, because they cannot measure the maximum beta energy. Scintillation counters can be used for both the quantitation and identification of beta particles.

The penetration power of beta particles is significantly larger than for alpha particles; a few mm of aluminum will stop beta particles. Care should be taken for the "Bremsstrahlung" X-rays that are created during the deceleration of the beta particles, because they may influence the measurement and can be a radiation safety concern.

Beta emitters can be quantitatively measured with ionization chambers and proportional counters that measure electrical current generated by the ionization of a select gas in a high-voltage field. Depending on the energy of the beta particle, the composition of the sample and container, and the design of the detector, the measurement may be based on Bremsstrahlung. For a radionuclide in solution, most of the beta energy is absorbed by the solvent, leaving only Bremsstrahlung exiting the sample. In the case of ionization chambers (e.g., dose calibrators), the walls housing the ionizable gas effectively convert all beta energy to secondary photons.

As is the case for all radiation measurements, quantitative determinations with an ion chamber or a proportional counter depend on rigorous calibration and standardization of the sample type and geometry.

Depending on the detection setup, alpha particles can interfere with measurement of the beta particles. This can be easily prevented by positioning an absorber for alpha particles between the source and the measuring equipment. However, because low-energy beta particles (<200 keV) may also be absorbed, the count rate should be corrected for the absorption of these low-energy beta particles.

An LSC can also be used for the quantification of beta particles and aid in radionuclide detection. Because of the high efficiency of the method, LSC is particularly useful in measuring very low levels of radioactivity and is commonly used for complex biological samples. The sample is normally dissolved in a solution containing a phosphor that converts the beta emission to light pulses, which are detected by a very sensitive system of photomultiplier tubes. At energies greater than ~100 keV, the energy conversion and therefore the counting efficiency, is essentially 100%. The method is somewhat compromised by quenching effects, but these can be easily overcome with careful calibration.

When high energy (>800 keV) beta particles are measured, it is possible to count without scintillation cocktails, because the beta particles create Cherenkov radiation, which can be directly detected with a photomultiplier. [Note—Although alpha particles can interfere with beta counting, this problem can be corrected for by use of thin absorbers or by instrumental energy discrimination.]

The identification of pure beta-emitting radioisotopes without accompanying gammas is best done by a combined measurement of the half-life of the radioisotope and the maximum energy of the beta radiation emitted by the radioisotope.

The approximate maximum energy of a beta particle can be determined by two procedures: 1) by measuring the radioactivity as a function of absorber thickness; and 2) by plotting the logarithm of the count rate versus the thickness of the absorber in mg/m^2 ; then, an absorption curve can be made. The absorption curve can be compared with standardized absorption curves, which aids in the identification of the radioisotope.

Using LSC, by measuring the pulse height over a calibrated energy range, a beta-energy spectrum can be generated from which the maximum energy can be estimated. LSC instruments typically automate the calibration factor, but care should be taken in correcting for quenching effects.

ALPHA PARTICLE COUNTING SYSTEMS

Several detectors can be used for the detection and measurement of alpha particles. These can be ionization chambers, proportional counters, silicon semiconductor detectors, and scintillation counters with their associated electronics. For the identification and quantification of alpha particles, ionization chamber and proportional counters are not suitable.

Special precautions should be taken in the measurements of alpha particles because of their high energy but limited penetration power (about 40 μm in human skin).

For the identification and assay of alpha-particle emitters, spectrometry using liquid scintillation is mostly used. For the identification and determination of radionuclidic purity of alpha-particle emitters, spectrometry using a silicon-diode semiconductor detector can be used.

In a scintillation counter, the energy of the alpha particle is transformed to a light pulse, which can be detected by a photomultiplier tube. The intensity of the pulse is a measure of the energy of the detected alpha particle. Solid scintillation counters using ZnS or phosphor

detectors can be used as well as liquid scintillation.

When solid-state detectors are used, the sample is usually electroplated on a planchet disc, and the detector is brought close to or immediately on the sample. Depending on the type of detector used, the maximum counting efficiency is about 50%. In liquid scintillation counting, when the sample is dissolved in the appropriate medium, the efficiency can be almost 100%. Each method of counting has its own advantages and disadvantages. For solid detectors, advantages are lower background noise and a better differentiation between alpha and beta particles. Disadvantages are that no volatile samples can be measured, and self-absorption by the sample material (related to thickness of the sample layer) can lower the detected count rate, giving a falsely low outcome of the assay.

For liquid scintillation, advantages are the ease of sample preparation and no self-absorption, because the sample is mixed with the scintillation fluid. Disadvantages are higher background noise levels and poor separation between alpha and beta particles.

4.4 Detector Systems for Chromatographic Applications

Chromatographic applications, wherein the radioactive components of a mixture are separated based on their distribution between a stationary phase and a mobile phase, represent a unique usage of radiation detection systems. The most common chromatography applications include thin-layer chromatography (TLC), gas chromatography (GC), and liquid chromatography (high-pressure liquid chromatography and high-performance liquid chromatography, typically denoted as HPLC). Depending on the chromatographic application and the type of emitted radiation, various detector systems may be used in the analysis of separated radioactive components. Gamma-ray detector systems can be used to dynamically measure separated radioactive components in GC and HPLC applications. In these applications, the eluate from the chromatography column is directed over or through the scintillating detector. Typically, these detector systems are not used for radionuclide identification; therefore, NaI(Tl) crystals tend to be the detector of choice in these applications. Depending on the required sensitivity of the technique, the geometry of detector may consist of a well that surrounds the eluate tubing, or a flow cell that passes the eluate across the surface of the detector. In either case, the design of the system should provide a reproducible geometric relationship between the tubing and the detector. In addition, the detector should be shielded sufficiently to prevent spurious peaks or baseline drift due to background radiation. For this application, the output pulses of the photomultiplier tube may be converted into an analog signal whose voltage is proportional to the number of pulses (i.e., the amount of radioactivity). In this manner, the resulting electric signal is used by a data acquisition and chromatography system to provide a chromatogram in a similar fashion as more traditional chromatography detection systems. The flow rate of the eluate and the amount of radioactivity in the eluate should be controlled to provide a count rate that is within the linear range of the detector system.

LSC methods can also be used in HPLC separations of beta-emitting components. Several techniques may be used in this application. In the first, the eluate from the chromatographic column is collected in discrete fractions. Each fraction is then mixed with the liquid scintillation cocktail before analysis in the LSC. Additional processing steps may be necessary before the addition of the cocktail to minimize quenching. In the second technique, the scintillation cocktail may be mixed with the eluate from the column before passage of the resulting mixture through an online flow cell surrounded by the photomultiplier tubes. Finally, inline solid scintillators may be used with in situ mixing of the eluate with a liquid scintillation cocktail. Beta-particle detector systems can be used to measure separated radioactive components in

TLC applications. In this application, windowless gas ionization detectors are most commonly used, and the detector is automatically scanned over the TLC plate, yielding a two-dimensional plot of the radioactivity. Because of the nature of the sample, self-adsorption is negligible, and the beta radiation is efficiently counted. If all of the components on the TLC plate contain the same beta emitter, the output signal is proportional to the fraction of radioactivity in the separated components. If the separated species contain different radionuclides, the detector should be calibrated with each radionuclide to correct for different signal responses.

Alternatively, if such a detector is not available, the TLC plate can be cut into multiple strips and counted by an appropriate detector. The individual strips should be counted using the same geometry. For the highest sensitivity, the strips may be extracted with solvent and then counted by LSC.

5. GLOSSARY

Alpha particles (α): Positively charged particles emitted from nuclei during radioactive decay. Alpha particles are essentially helium-4 nuclei, consisting of two protons and two neutrons but no electrons.

Beta particle (β^-): Negatively charged particles that are emitted from nuclei during radioactive decay. Beta particles are essentially electrons.

Beyond-use date: Date (and time, if appropriate) that establishes a limit for the use of a compounded preparation. The acceptable use period (i.e., the period of time between the date and time of compounding and the beyond-use date and time) is based on a knowledge of the radioactive properties of the preparation, the results of stability studies on the preparation, and, as appropriate, the assurance of sterility of the compounded sterile preparation.

Bremsstrahlung: Electromagnetic radiation produced by the deceleration of a charged particle through interaction with another charged particle, typically an electron and an atomic nucleus, respectively. The moving particle (e.g., beta particle) loses kinetic energy, which is converted into photons (X-rays). This electromagnetic radiation exhibits a continuous spectrum, with peak intensity as a function of the energy of the incident particle.

Calibration factor: The coefficient used to convert the measured ionization chamber current to a nominal radioactivity. This term is often referred to as the "calibration coefficient".

Calibration time: An arbitrary time at which the specified amount of radioactivity is present on a specific date.

Carrier free: A preparation free from stable isotopes of the same element as the radionuclide.

Counting assembly: An instrument that consists of a sensing unit and an electronic scaling device. The sensing unit may be a Geiger-Müller tube, a proportional counter, a scintillation detector in which a photomultiplier tube is used in conjunction with a scintillator, or a solid-state semi-conductor.

Dose calibrator: A well-type ionization chamber commonly used to assay radiopharmaceuticals. Display units are typically in Curies (μCi or mCi or Ci) or Becquerels (kBq or MBq or GBq).

Expiration date: The date (and time, if appropriate) that establishes a limit for the use of the manufactured product that is based on the knowledge of the radioactive properties of the product and the results of stability studies on the finished dosage form.

Gamma rays (γ rays): Electromagnetic radiation emitted from nuclei during radioactive decay. Gamma rays have a wide range of energies. The gamma rays emitted from a given radionuclide are always at the same energy(ies), providing a unique signature that enables the identification

of a gamma-emitting radionuclide.

Geiger-Müller counter: Often referred to as a G-M counter or Geiger counter. An instrument in which a high-voltage potential is applied across a volume of inert gas for the purpose of collecting ions produced by a radiation field. The negative electrons are internally multiplied to produce a readily detectable current pulse. Display units are typically counts per min (cpm) or milliroentgen per h (mR/h).

Geometry: The characteristics of a radioactive source (i.e., container type, container wall thickness, volume and position of the container in the well chamber) that along with the physical characteristics of the ionization chamber affect the magnitude of the calibration coefficient for a specific radionuclide. The principal geometry considerations that may affect the accuracy of a source measurement in a dose calibrator are container configuration, source volume, position of the source in the chamber well and the radionuclide itself. [Note—It is customary to compare a standardized preparation and radiopharmaceutical drug or preparation using identical geometry conditions for assay, identification and other parameters. The validity of the result is critically dependent upon the reproducibility of the spatial relationships of the source to the detector and its surroundings and upon the accuracy of the standardized preparation.]

Ionization chamber: An instrument in which an electric field is applied across a volume of inert gas for the purpose of collecting ions produced by a radiation field. The positive ions and negative electrons drift along the lines of force of the electric field and are collected on electrodes, producing an ionization current. The most commonly used form of ionization chambers for measurement of the activities of radiopharmaceuticals is a well-type ionization chamber known as a dose calibrator.

Isobars: Nuclides with the same mass number (protons + neutrons).

Isomers: Atoms with the same number of protons and neutrons, but a different nuclear energy configuration. Short-lived radioactive isomers are generally referred to as metastable. Different isomers are different nuclides based on their nuclear energy configurations.

Isotones: Nuclides with the same number of neutrons and a different number of protons. Isotones are different elements with different atomic masses.

Isotopes: Nuclides with the same number of protons and a different number of neutrons. Isotopes are the same element with a different atomic mass.

Isotopic carrier: A stable isotope of the element concerned either present in or added to the radioactive preparation in the same chemical form of the radionuclide.

Liquid scintillation counter (LSC): An instrument that detects scintillation light from the absorption of radiation energy in a scintillation liquid. This instrument is used primarily for beta-emitting radionuclides that do not also emit gamma photons. For best results, the radioactive sample must be able to be dissolved in the scintillation liquid.

Minimum detectable activity: The smallest quantity of radioactivity that can be detected above the background with a specified level of confidence.

National Metrology Institute (also known as NMI): A measurement standards body that is a laboratory of metrology that establishes standards for a country or organization. e.g., National Institute of Standards and Technology (NIST) is the NMI for the United States.

No carrier added: A preparation where no stable isotopes of the same element as the radionuclide being tested are intentionally added in the stated chemical form or at the position of the radionuclide in the molecule being tested.

Nuclide: An atom with a specific number of protons and neutrons in a given nuclear energy state.

Positrons (β^+): Positively charged particles emitted from a nucleus during radioactive decay.

Positrons are anti-electrons.

Radioactivity: 1) The spontaneous transformation of nuclei by radioactive decay. Radioactivity is typically described as atoms undergoing radioactive decay per unit time (or disintegrations per unit time). 2) The quantity of radioactive material, as measured in units of Curies (US units) or Becquerels (SI units). The quantity of radioactive material may also be referred to as activity.

Radiochemical identity: The molecular structure of the intended active radioactive drug ingredient that is present in the radiopharmaceutical preparation.

Radiochemical purity: The ratio, expressed as a percentage, of the radioactivity of the intended active radiopharmaceutical ingredient to the total radioactivity of all radioactive ingredients present in the radiopharmaceutical preparation.

Radioisotope: A radioactive atom, generally used in the context of an isotope of an element.

Radionuclide: An unstable nuclide that undergoes radioactive decay; a radioactive nucleus. The terms radionuclide and radioisotope are commonly used interchangeably.

Radionuclidic identity: The intended radionuclide in the radiopharmaceutical preparation.

Radionuclidic purity: The ratio, expressed as a percentage, of the radioactivity of the intended radionuclide to the total radioactivity of all radionuclides in the radiopharmaceutical preparation.

Radiopharmaceutical (radiopharmaceutical preparation/radioactive drug): A finished dosage form that contains a radioactive substance in association with one or more other ingredients and that is intended to diagnose, stage a disease, monitor treatment, or provide therapy. A radiopharmaceutical includes any nonradioactive reagent kit or radionuclide generator that is intended to be used in the preparation of any such substance. The terms radiopharmaceutical and radioactive drug are commonly used interchangeably.

Scintillation crystal counter: An instrument consisting of a crystal scintillator, such as NaI(Tl), with an attached photomultiplier tube and associated electronics. Scintillation light produced from the absorption of gamma and X-rays in the crystal is converted to electrons and amplified in the photomultiplier tube. The resultant current pulse may be further analyzed with regard to photon energy. A commonly used form of this instrument that has a hole in the crystal of sufficient size to allow placement of a test tube or similar container is known as a well counter.

Semiconductor detector: An instrument consisting of a semiconductor material, such as silicon or germanium crystals, that detects ionizing radiation through generation of charge carriers (passage of electrons through holes). The current pulse produced by migration of these charge carriers, under the influence of a voltage potential across the material, can be further amplified and analyzed to determine the quantity and energy of the incident radiation.

Solid-state detector: A crystal-based detector, in contrast to a gas-based detector; often is used as a synonym for a semiconductor detector.

Specific activity: The radioactivity of a radionuclide per unit mass of the element or compound. The unit of specific activity is radioactivity per mass expressed on a gram or mole basis [e.g., mCi/ μ g (MBq/ μ g); Ci/mmol (GBq/mmol)].

Strength: The radioactivity concentration of the radiopharmaceutical at the calibration time. The unit of strength is the amount of radioactivity on a volume basis (e.g., mCi/mL or MBq/mL).

Total radioactivity: The radioactivity of the radionuclide, expressed per unit (e.g., vial, capsule, ampule, generator, and others) at the calibration time.

Validation: Establishment of documented evidence that a method, process, or system meets its intended requirements.

Verification: Confirmation that an established method, process, or system meets

predetermined acceptance criteria.

X-rays: A type of electromagnetic radiation emitted from the electron orbitals. Although they do not arise from the nucleus, they are often present immediately after a decay event if there are interactions between the emitted radiation and the orbital electrons.

6. REFERENCES

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■ 2S (USP39)

¹ <http://www.nndc.bnl.gov/ensdf>

BRIEFING

⟨1823⟩ **Positron Emission Tomography Drugs—Information.** Until 2011, USP had two general chapters related to positron emission tomography (PET) drugs. The first was *Automated Radiochemical Synthesis Apparatus* ⟨1015⟩. This chapter described a quality assurance (QA) program for equipment, reagents, documentation, and software used in the production of PET drugs. The second was the *Positron Emission Tomography for Compounding, Investigational, and Research Uses* ⟨823⟩. This chapter provided an extensive QA program for compounding PET drugs. Since the introduction of standards for PET drugs in 1989, technological, marketplace, and regulatory changes necessitated revision of the standards. In addition, in 2009 the Food and Drug Administration (FDA) issued a guidance document on PET drug manufacturing in which the standards in ⟨823⟩ in USP 32 were recognized as an alternative for current good manufacturing practice (cGMP) for investigational and research PET drugs. In 2011, ⟨823⟩ was revised in its entirety to align with these changes and to represent current compendial thinking about the preparation of PET drugs. During the revision of ⟨823⟩, the need for a new chapter to provide additional information on concepts, technologies, and procedures related to PET drug manufacturing and controls to supplement ⟨823⟩ was recognized by the USP General Chapters—Physical Analysis Expert Committee (GCPA-EC). The proposed *Positron Emission Tomography Drugs—Information* ⟨1823⟩ includes relevant information regarding PET radionuclides, definitions of common terminology, along with information on production and quality assurance of PET drugs. The GCPA-EC hopes that this proposed chapter will serve the needs of patients, research subjects, medical institutions, clinical researchers, and all members of the PET community. This proposed ⟨1823⟩, when adopted into USP, eliminates the need for ⟨1015⟩, which is proposed for omission in this *PF* issue.

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Comment deadline: November 30, 2015

Add the following:

■ (1823) POSITRON EMISSION TOMOGRAPHY DRUGS—INFORMATION

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1. INTRODUCTION

Positron emission tomography (PET) drugs contain radionuclides that undergo nuclear transformation, or radioactive decay, predominantly by the emission of a positron. Positrons undergo annihilation upon interaction with electrons to produce two photons that are emitted in nearly opposite directions to each other. Each photon possesses an energy of 511 keV, which lies in the gamma ray portion of the electromagnetic spectrum. These photons are used in a wide range of PET imaging studies, including research, investigational, and clinical applications. The radionuclides used in PET imaging studies typically possess short physical half-lives (denoted as $T_{1/2}$). Some common examples of PET radionuclides and their associated half-lives are included in *Table 1*. Note that *Table 1* includes radionuclides currently in predominant use and is not intended to illustrate all positron-emitting radionuclides used in PET.

Table 1

PET Radionuclide	Half-Life, $T_{1/2}$
Fluorine-18	109.8 min
Carbon-11	20.4 min
Nitrogen-13	10.0 min
Oxygen-15	2.0 min
Copper-64	12.7 h
Gallium-68	68 min
Rubidium-82	75 s

Because these radionuclides have the same chemical and physical properties as their stable counterparts found in biological systems, PET offers a unique platform for in vivo imaging studies of complex biochemical pathways. As a result, PET radionuclides have found widespread use in cardiology, oncology, and neurology applications. PET drugs have also attracted interest as potential tools to accelerate and reduce the cost of therapeutic drug discovery efforts. Most PET radionuclides are produced at the point of use by a particle accelerator (e.g., a cyclotron) or a radionuclide generator. A cyclotron accelerates charged particles such as protons or deuterons to velocities sufficient to induce a nuclear transformation of the target nucleus into a different element. High-energy particles and/or radiation are emitted from the target nucleus during the transformation process. An example of a transformation is the

bombardment of stable Oxygen-18 nuclei with accelerated protons to produce Fluorine-18 nuclei along with the concomitant emission of a neutron. This process may be summarized according to the following shorthand notation:

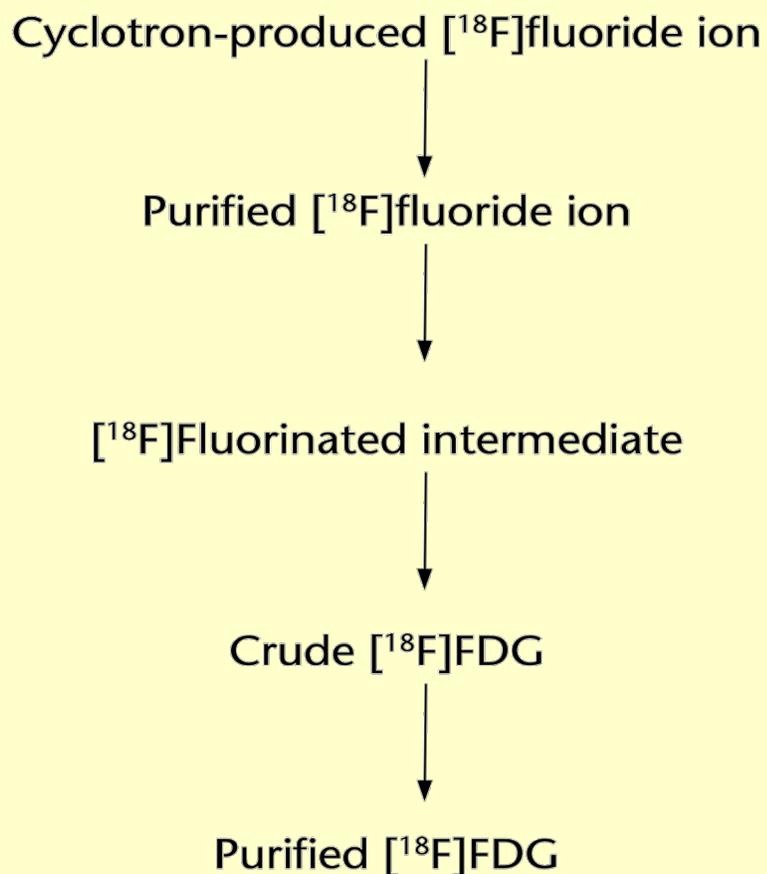


The chemical form and physical state of the bombarded nuclei vary, depending on the target and the subsequent usage of the PET radionuclide. This allows the production of PET radionuclides in gaseous, solid, and solution phases.

Radionuclide generators offer an alternative method for point-of-use access to some PET radionuclides. A radionuclide generator contains an immobilized parent radionuclide that undergoes radioactive decay to a daughter radionuclide, which may be eluted from the generator. An example of a PET radionuclide generator is the Germanium-68/Gallium-68 generator. The parent Germanium-68 ($T_{1/2} = 271$ days) is adsorbed onto the column packing material. The Germanium-68 undergoes radioactive decay by electron capture into Gallium-68, which is not adsorbed onto the column packing material and may be eluted from the generator. Regardless of the production method, other radionuclides may be present in addition to the desired PET radionuclide. In the case of cyclotron-produced PET radionuclides, other radionuclides may result from the bombardment of the target holder and/or other nuclei present in the target material. For generator-produced PET radionuclides, break-through of the parent radionuclide may occur. Safeguards are readily available to avoid unnecessary contamination with radionuclidic impurities of the final PET drug.

Once the PET radionuclide has been produced, it should be processed into a suitable form of a PET drug. The simplest form of processing involves the purification and/or formulation of the PET radionuclide. An example is the purification of the fluoride ^{18}F ion. After production of the fluoride ^{18}F ion by cyclotron bombardment, ion exchange procedures may be used to remove impurities and yield a formulation suitable for PET imaging studies. Another example is the purification of gaseous Oxygen-15 through various solid supports before administration of the finished PET drug by inhalation.

Simple forms of processing are rare, because PET drug products are typically synthesized from the appropriate radionuclide through multiple synthetic processing steps. The exact process depends on the desired PET drug. The synthesis of 2-[F-18]Fluoro-2-deoxyglucose (FDG F18) provides an illustration. The first step in this synthesis is the preparation of the anhydrous Fluoride-F18 ion from cyclotron-produced Fluorine-18. The Fluoride-F18 ion is activated with a phase transfer catalyst such as tetraethylammonium or [2.2.2]-cryptand to enhance its reactivity toward nucleophilic substitution. The resulting complex reacts with mannose triflate to yield a fluorinated ^{18}F intermediate, which is deprotected and purified to yield FDG-F18. This process is summarized in the following set of equations:



In addition to the chemical processing and/or purification steps used to generate the desired radiochemical of interest, other steps are typically required to formulate the PET drug product. For injectable PET drug products, formulation steps may include dilution, addition of a stabilizer, pH adjustment, and other steps. The overall process should take place in a manner that minimizes the presence of bacterial endotoxins and bioburden. Finally, the product should be sterilized, e.g., by passage through a membrane sterilizing filter, to provide a solution suitable for intravenous administration. In the above example, Fludeoxyglucose F-18 becomes the drug product Fludeoxyglucose F 18 Injection.

Before use, the finished PET drug product should be tested to ensure that the product meets suitable standards of identity, strength, and purity. Because of the short half-life of PET radionuclides, testing should be completed in a timely fashion; however, it is not possible to complete certain quality control (QC) tests within a suitable timeframe. In some cases, it may be necessary to adopt a "sub-batch" approach for QC testing, wherein a sub-batch of the PET drug product is prepared solely for purposes of QC testing. Examples of PET drug products with such short half-lives include water-¹⁵O and ammonia-¹³N.

Ultimately, the short half-lives of PET radionuclides create unique constraints for the production and testing of PET drug products and define how these products are used in research, investigational, and clinical settings. PET drugs are a unique class of products defined by the following characteristics:

- The mass of the radioactive ingredient in a PET drug product usually ranges from nanogram to microgram quantities. This affects pharmacological and toxicological considerations for PET drugs, usually by creating large safety margins due to low mass of the active ingredient in the administered dose.
- An entire batch of a PET drug product may be contained in a single vial. Samples withdrawn for QC testing are representative of the entire batch. PET drug products produced in this fashion undergo 100% QC testing.
- PET drug products are produced and handled in environments with overlapping areas of regulatory authority. For example, radiation protection regulations require a negative pressure environment to minimize personnel radiation exposure, but drug regulations for sterile products require a positive pressure environment.
- PET drug products are generally produced at or near their point of use in small-scale facilities with limited personnel and resources. This requires:
 - Allowance for multiple operations in one area with adequate controls
 - Allowance for making and testing multiple PET drug products using shared equipment with appropriate cleaning between batches
 - Appropriate requirements for aseptic operations
 - Appropriate requirements for system suitability and other day-of-use activities
 - Appropriate QC requirements for components, materials, and supplies
 - Self-verification of significant steps in radionuclide production, PET drug production, compounding, and testing
 - Single-person oversight of production or compounding, testing, review of batch records, and release authorization
- PET drug products do not enter a traditional distribution chain. Instead, PET drug products require just-in-time deliveries typically performed by dedicated carriers with experience in handling radioactive materials.
- It is not possible to complete sterility testing for PET drug products before their use. Therefore, considerations are made to provide for the assurance of sterility for injectable PET drugs intended for human use.
- Procedures should be in place to notify the responsible individuals in a timely manner if a PET drug product is found to be in noncompliance after release for human use.

2. TECHNIQUES FOR PRODUCTION AND QUALITY CONTROL

The unique characteristics of PET drug products play a large role in the choice of instrumentation and techniques used in production and QC testing. Production techniques and analytical methods should be efficient and rapid. The selection of techniques and methods is also strongly influenced by the development stage of the PET drug. For example, early development efforts focus on radiolabeling, purification, QC methods development, and others. These efforts are designed to support the usage of the PET drug for *in vitro* studies, animal studies, and may even include first-in-human studies. At this stage, relatively small quantities of the PET drug are required at a limited number of institutions or geographic areas. Manual production techniques or semi-automated equipment may provide sufficient quantities to meet this demand. Analytical methods should also be suitable for the usage of the PET drug at the early stages of development. Typically, method development efforts focus on accuracy, precision, and linearity. Before the use of injectable PET drugs in human studies, provisions are made to ensure that bacterial endotoxins are controlled at suitable levels and that the product

is sterile.

In later development stages, larger quantities of the PET drug may be required in more geographic areas. At this point, the synthesis, purification, and testing of the PET drug product should be well defined to support clinical trials. Development efforts at this stage typically focus on optimization and reliability of the production process. Manual production techniques are rarely used. Instead, semi-automated or fully-automated techniques are typically required to provide sufficient quantities of the PET drug at multiple geographic locations. This approach simultaneously assures consistent quality attributes of the PET drug at multiple production facilities and minimizes radiation exposure of operators. Method development activities at this stage focus on specificity, ruggedness, robustness, and other characteristics required to support QC testing of the PET drug at multiple geographic locations.

Finally, at the commercial stage of production, semi-automated or fully-automated techniques and equipment may be required to provide sufficient quantities of the PET drug in numerous geographic areas. Production techniques and analytical methods for commercial PET drug products should be well defined and adequately described in the appropriate marketing authorization.

Not all PET drugs are intended to advance through the various development stages from *in vitro* studies, to animal studies, to clinical trials, to commercial production. For example, a PET drug product may be produced at several institutions for research purposes without the intention of commercialization. In other instances, a PET drug product may be FDA approved but is only available in a single geographic area. These factors often result in different strategies for production techniques, analytical methodologies, and the underlying studies and documentation to support these strategies.

3. QUALITY ASSURANCE

The goal of quality assurance (QA) is to ensure that the techniques and equipment used in production and testing result in a product that meets established standards for that specific PET drug product. The QA program should be sufficient to establish the reliability and suitability of the techniques and equipment as appropriate to the development stage of the PET drug product. The following topics should be considered in a QA program.

3.1 Reagents and Materials

Reagents and materials used in the synthesis and testing of a PET drug product should conform to established acceptance criteria. Procedures for procuring, receiving, testing, storage, and use of reagents and materials should also be considered. An audit trail may be established for the traceability of specific lots of reagents and materials to specific batches of product. These acceptance criteria, procedures, and the audit trail should be appropriate for the development stage of the PET drug product.

3.2 Change Control

Changes in the synthesis and test methods should be evaluated for their potential for altering the product quality and be approved in advance through established procedures. If the resultant PET drug product does not meet the criteria appropriate to the development stage, the process change is unacceptable. Acceptable changes should be appropriately documented.

3.3 Validation

Production and analytical test methods should be validated at a level that is appropriate for the development stage of the PET drug product. Validation of the production process should result in a process that is reliable and consistent. Analytical method validation demonstrates that a method can quantitatively measure a PET drug product reliably and reproducibly. It is not necessary to validate analytical methods that are described in USP compendia. The emphasis on validation typically increases as the PET drug product moves through the development stages toward commercialization. Validation of the computer software used to control automated production equipment should also be considered as appropriate.

QUALIFICATION

Equipment used in the synthesis and testing of PET drug products should be qualified as appropriate for the development stage of the PET drug product. This may include qualification procedures (installation, operational, and performance), as well as procedures for periodic maintenance and equipment calibration. For commercially available equipment, the equipment vendor may be able to support qualification requirements.

STABILITY STUDIES

Stability studies should be performed on PET drug products to establish suitable storage conditions and the expiration time and date. Quality attributes such as radiochemical purity, appearance, pH, stabilizer, preservative effectiveness, and chemical purity should be evaluated. The frequency and extent of these studies should be appropriate for the development stage of the PET drug product. Significant process changes require stability studies to be repeated.

4. PRODUCTION

4.1 Equipment for Manual Synthesis

Equipment intended for the manual synthesis of PET drug products may be based on apparatus found in a typical chemistry laboratory such as glass reaction vessels, heating blocks, tubing, and other items. These items should be suitable for their intended use and selected according to the needs of the process. Items that are reused and come into contact with the reactants and/or final drug product of the synthesis should be cleaned and depyrogenated as appropriate for the use of the final product.

Written procedures for the manual syntheses of PET drug products should contain sufficiently detailed steps to ensure that a reproducible process is followed for each batch by the operator. Observations of critical process parameters should be documented as appropriate in the batch record.

4.2 Equipment for Automated Synthesis

Preparation of Fludeoxyglucose F 18 Injection and other common PET drug products can be adapted readily to automated synthesis. The use of programmable controllers and/or computers with switches, solenoids, and sensors allows the operator to control the sequence of steps in

an automated fashion. Thus, an automated synthesis requires minimal operator intervention, and batch-to-batch consistency is maintained with each step executed in a reproducible manner. Software may provide feedback to the operator such as alarm conditions, status reports, or an end-of-synthesis report. Modules for automated synthesis may be commercially available. These modules may use individual reagents or cassettes required for the appropriate PET drug product synthesis. The cassettes provide pre-assembled collections of reagents, reaction vessels, tubing, filters, and other items necessary to produce a batch of a PET drug product.

Laboratory robots may be appropriate in situations where complex manipulations are required for the procedure, such as the physical movement of a reaction vessel from one station to another, or the dispensing of doses from a vial into individual syringes. Customized equipment may also be fabricated to perform a specific function in the PET drug product synthesis.

5. QUALITY CONTROL

Quality control (QC) tests should be designed and executed in a manner that is appropriate for PET drugs. The short half-life of PET radionuclides significantly limits the timeframe for the performance of QC tests, and it may not be possible to complete all QC tests before the use of the PET drug product.

5.1 Quality Control Sampling

In most cases, the entire batch of a PET drug is contained within a single vial. The relatively small batch volume of PET drugs limits the amount of material available for QC testing. This is different from traditional manufacturing practices, where a batch may consist of numerous vials and not all vials are sampled for purposes of QC testing. QC sampling of PET drug products use a larger proportion of the drug volume, and thereby offers a greater degree of assurance that quality-related problems will be identified in the QC testing process.

5.2 Reference Standards

Reference standards are necessary to identify the API and impurities in the PET drug product. Reference standards may be commercially available or prepared in-house. Commercial reference standards should include documentation from the supplier to ensure they are properly characterized. The characterization and qualification of reference standards should be appropriate for the development stage of the PET drug product.

5.3 Conditional Release

When a required QC test for a PET drug product cannot be completed because of a malfunction of test equipment, it may be appropriate to conditionally release the batch. It is not appropriate in the absence of critical QC tests to conditionally release batches (e.g., radiochemical identity and purity). Procedures should be developed to describe the conditional release process, including the role of historical data, other QC test results, sample retention, and other procedures.

5.4 Out-of-Specification Results

When the results of a QC test do not meet product specifications, an investigation should be conducted to determine if the result is due to an analytical error (false positive) or if the result is due to a product failure. Because of the short half-life of PET radionuclides, it may not be possible to conduct investigations in the same manner as in traditional drug testing laboratories.

6. ANALYTICAL METHODOLOGIES

Various analytical methodologies and chromatographic techniques may be used for the testing of PET drug products. The most commonly used methodologies are discussed in this section for purposes of illustration. Similar principles should apply to other methodologies not included in this section.

6.1 Thin-Layer Chromatography

Thin-layer chromatography (TLC) involves separation, identification, and quantification of analytes using a stationary phase on a support or plate and a developing solvent that may also be known as mobile phase. The stationary phase may be silica or alumina spread in a uniform layer onto a plate of glass, metal, or plastic. A small sample of the PET drug product is applied (spotted) onto the stationary phase, and the plate is developed in a chromatographic tank. The mobile phase moves through the stationary phase by capillary action, and the analytes are separated based on partition, ion exchange, and/or adsorption. The retardation factor (R_f) and radiochemical purity of the PET drug on the developed plate can be determined by either counting the developed plate on a radio-TLC scanner, or cutting the plate into pieces and counting each piece in a radiation detector. The R_f of an analyte, under specific TLC conditions, is considered an identifying characteristic of the analyte.

6.2 Gas Chromatography

Gas chromatography (GC) involves separation, identification, and quantification of volatile analytes using a stationary phase and a gaseous mobile phase. A small sample of the solution is introduced into the GC instrument, which vaporizes the sample for passage over the stationary phase that is immobilized within a GC column. As the gaseous mobile phase passes over the stationary phase, the analytes are separated based on their partition between the gas and stationary phases. A detector located at the exit of the column provides an electronic signal to produce the gas chromatogram.

A GC instrument consists of a gas source, injection port, column, detector, and a data collection device. The injection port, column, and detector are temperature controlled and may be varied as part of the analysis. The gas source depends on the column and detector in use. The type of detector depends on the nature of the compounds analyzed. Typical detectors include flame ionization and thermal conductivity. In addition, radiation detectors may be used in GC analysis. Detector output is recorded over time, and the instrument response, measured as peak area or peak height, is a function of the amount present. The retention time of an analyte under specific GC conditions is considered an identifying characteristic. GC analysis is most typically used to quantify residual solvents in PET drug products but may also be used to determine radiochemical and chemical purity of the PET drug product formulation.

6.3 High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is an analytical technique used to separate, identify, and quantify the components of a solution. HPLC separations are based on the interaction of the analytes between the stationary and mobile phases, which in turn leads to the retention of the analytes. The mechanism of interaction between the analytes and stationary phase may be partition, size exclusion, adsorption, and/or ion exchange chromatography. The retention time of an analyte under specific HPLC conditions is considered an identifying characteristic.

HPLC instruments consist of a reservoir containing the mobile phase, a pump to force the mobile phase through the system at high pressure, an injector to introduce the sample into the mobile phase, a chromatographic column, a detector, and a data collection device. The type of detector used depends on the nature of the compounds analyzed. In addition to mass detectors such as refractive index, ultraviolet, and conductivity, radiation detectors are also used in HPLC analysis. An HPLC system with a radiation and mass detector allows the simultaneous determination of radiochemical and chemical purity, retention time, and the subsequent identity of the PET drug product. This configuration also allows the determination of specific activity. Elution methods include gradient or isocratic methods with aqueous and/or organic buffers.

The calibration of a GC or HPLC instrument may be achieved by different means. One approach involves the creation of a calibration curve from a range of standards with known concentrations. The calibration curve may be used over a specified period of time for product testing. A second approach for calibration involves the creation of a single-point calibration at the beginning of each testing cycle. The results may be averaged and used to provide a calibration factor for product testing. Regardless of the approach for calibration, the tailing factor and chromatographic resolution (or column efficiency as appropriate) should be determined routinely as a part of system suitability. Routine system suitability testing should include checks for these parameters.

6.4 Radiation Detectors used in Chromatographic Techniques

Radiation detectors used in TLC, GC, and HPLC should be calibrated when possible with suitable NMI-traceable radionuclide standards at appropriate frequency. Routine system suitability testing should be performed on the radiation detection system to ensure proper operation. Because of the nature of TLC, system suitability for a radio-TLC scanner should address uniformity, positional accuracy, detector linearity, and resolution. The calibration of radiation detectors should be repeated as appropriate.

6.5 Multichannel Analyzer

A multichannel analyzer (MCA) is an instrument used to obtain a spectrum of gamma rays emitted by a PET radionuclide. The key component of a MCA is an energy-sensitive detector. On the basis of the gamma spectrum of a sample, radionuclides present in the sample may be identified and quantified. Calibration of the system is typically performed with certified standards and may involve one or more radionuclides with gamma energies and quantities that span the range of typical analyses at appropriate frequency.

6.6 Ionization Chamber

An ionization chamber, often referred to as a dose calibrator, is an instrument used to measure the quantity of radioactivity in a PET drug product. The key component of an ionization chamber is an argon-filled chamber with an applied electrical potential that allows the detection of ions produced by the passage of gamma rays through the chamber. Calibration of the system is typically performed with certified standards at appropriate frequencies and may involve one or more radionuclides with gamma energies and quantities that span the range of typical analyses.

7. QUALITY ATTRIBUTES

Quality attributes should be defined and assessed for each PET drug. These attributes should be suitable for the intended use of the PET drug and should also reflect the dosage form. Because most PET drug products are administered by intravenous injection, this section focuses on quality attributes associated with injectable PET drug products. Different attributes may be appropriate for other dosage forms. In addition, the definition and assessment of quality attributes may vary with the development phase of the PET drug product in comparison to the final drug phase.

7.1 Appearance

Injectable PET drug products should be free of particulate matter. Because of the radioactive nature of PET drug products, the test for appearance should be a visual inspection that meets radiation safety requirements. The use of a visual standard should be considered to ensure the accuracy of appearance determinations.

7.2 pH

Injectable PET drug products should be in the pH range suitable for intravenous administration. Because of the limited volume of a PET drug product, pH measurement is typically performed using narrow-range paper strips. The use of pH standards should be considered to ensure the accuracy of pH determinations.

7.3 Total Radioactivity and Strength

The total radioactivity of a PET drug product may be determined by an ionization chamber and should be stated at a given date and time. From the total radioactivity and the volume of the PET drug product, the strength may be determined. The strength of the PET drug product should be stated, along with the date and time of the determination of total radioactivity.

7.4 Radionuclidic Identity

Half-life (also referred to as approximate half-life) is a characteristic of the radionuclide that may be used for its identification. To adequately confirm the identity of a PET radionuclide, the half-life should be measured in a suitable counting device over a period of time appropriate to the half-life of the radionuclide.

7.5 Radionuclidic Purity

By definition, all positron-emitting radionuclides emit 511 keV gamma rays but may also emit gamma rays with different energies. Therefore, it is generally not possible to determine the radionuclidic purity of PET drug products with a MCA. A suitable solution to this problem is the use of validation studies to ensure the removal and/or decay of radionuclidic impurities during the production process. In addition, periodic analysis of decayed samples in routine production with a MCA may be used to quantify radionuclidic impurities developed.

7.6 Radiochemical Identity and Purity

Depending on the physical and chemical properties of the PET drug product, the radiochemical identity and purity may be determined by TLC, HPLC, or GC. The identity of the active pharmaceutical ingredient (API), and possibly other analytes, should be based on the known retention time of the analyte. The simultaneous use of reference standards during sample analysis should be considered for purposes of radiochemical identification. The radiochemical purity of the PET drug product should be determined based on the sum of all chemical forms of the radionuclide of interest. As appropriate, it should be established during validation that all radiochemical analytes are eluting from the chromatographic system and that the radioactive detector is operating within its linear range.

7.7 Chemical Purity

The chemical purity of the PET drug product should be assessed for the presence of volatile impurities, residual reagents and/or precursors, stabilizers, excipients, and by-products. For example, residual solvents, such as acetonitrile and ethyl alcohol, may be assessed by GC. [2.2.2]-Cryptand, a common reagent used in the preparation of PET drug products, may be assessed by colorimetric techniques.

7.8 Total Mass of the Active Pharmaceutical Ingredient and Specific Activity

Potential toxicity issues and pharmacological effects may be associated with the API in a PET drug product (e.g., the mass-dependent localization of neurotransmitters or other more generalized forms of toxicity). In these instances, the total mass of the API should be determined before use of the PET drug product. The total mass of the API contained in a patient dose should be defined for each PET drug product, which may be determined by HPLC or GC. On the basis of the mass of the API, the specific activity may be calculated. To determine the appropriate quality of the injected drug product in the patient dose, any two of three measured parameters (total mass, total activity, or specific activity) are sufficient. Specific activity should be stated along with the date and time of determination.

7.9 Bacterial Endotoxin

The quantity of bacterial endotoxin in an injectable PET drug product should comply with USP standards (<175 USP Endotoxin Units/patient dose). The test for bacterial endotoxin uses a solution of limulus amoebocyte lysate (LAL). Various methods exist for this test, including gel-clot, chromogenic, and others. Test samples should be obtained and handled in a manner that minimizes contamination before testing. In addition, control measures should be used to ensure accurate test results without interferences from the test solution, such as certain formulations

that enhance or inhibit the interaction of LAL with bacterial endotoxin. This effect may be corrected by dilution of the PET drug product before testing. The necessity of dilution in this test is determined in the validation of the specific formulation of the PET drug product.

7.10 Sterility

Injectable PET drug products should be sterile. For reasons described in the *Introduction*, it is not possible to complete the sterility test before the use of PET drug products. The test for sterility should consist of inoculating a test sample into media capable of supporting the growth of aerobic and anaerobic microbes, which may be established with an appropriate certificate of analysis. The test samples should be obtained and handled in a manner that minimizes contamination before testing. To reduce radiation exposure to operators, the test samples may be allowed to decay before inoculation. The inoculation should use techniques that minimize the potential for false positives during the sterility test. In addition, control measures should be used to ensure accurate test results without interferences from the test solution. After inoculation, the sterility test samples should be incubated for the appropriate period of time at the prescribed temperature. If visible growth is detected during the incubation period, the investigator or physician should be informed in a timely manner, and an investigation should be initiated to determine whether the growth was due to accidental contamination during the sample handling process or to a true product failure. The investigation should include identification of the microbe(s) in the sterility test sample to determine the source of the contamination. This aspect of the investigation may be limited if production and sterility test sampling occur in the same laboratory.

8. STERILITY ASSURANCE

A suitable sterility assurance program should be established for PET drug products that are intended for intravenous injection. This section describes appropriate measures that assure the routine production of a sterile product suitable for injection.

8.1 Sterile Membrane Filtration

Injectable PET drug products are typically sterilized by passage of the solution through a sterile membrane filter into a pre-sterilized vial. To provide the greatest assurance of sterility, the PET drug product should pass through an appropriate sterilizing filter into a pre-sterilized vial that has been assembled using aseptic techniques.

8.2 Aseptic Techniques

Critical steps that affect the sterility of the PET drug product should be identified and, where appropriate, aseptic techniques should be used to complete these steps. Aseptic techniques should include the use of a suitable gowning, proper handling of components, environmental controls, and others. Aseptic techniques should be described in written procedures.

8.3 Pre-Sterilized Components

PET drug products are typically produced in a container-closure that is commercially available

as a pre-sterilized vial. Before use, the vial and other pre-sterilized components are assembled using aseptic techniques in a suitably controlled aseptic environment.

8.4 Environmental Controls

The environment where aseptic techniques are executed should be controlled to ensure appropriate aseptic conditions. These controls include temperature and humidity, ventilation and air filtration, cleaning and disinfection, equipment maintenance, proper garb, and microbiological monitoring. Air filtration standards should conform to local and/or national standards relevant to environmental standards, e.g., ISO standards.

8.5 Media Fills

A media fill, also known as a "process simulation", is the performance of an aseptic procedure using a sterile microbiological growth medium in place of the PET drug product. The goal of a media fill is to test whether the aseptic procedure is adequate to prevent microbiological contamination during the actual process. Media fills may be used to evaluate aseptic techniques used in the assembly of pre-sterilized components and to qualify operators for aseptic techniques. A media fill should be designed to ensure that the simulation is representative of the aseptic manipulations performed during the actual process, including personnel, components, gowning, locations, batch size, number of replicates, and other factors. After completing the media fill, components filled with media should be incubated appropriately to permit the growth of microbes. The use of negative controls should be considered during the media fill procedure.

8.6 Suitability of Media

Sterility test media should be tested before use to ensure that the media adequately support the growth of microbes. These tests are typically referred to as growth-promotion tests and may be conducted in-house, by the supplier, or by a contract laboratory. Media should be used within the manufacturer's expiration date and within 3 months of completing the growth-promotion test.

8.7 Suitability of the Sterility Test Method

The sterility test should be tested to ensure that the PET drug product is not bacteriostatic or fungistatic. The suitability of the sterility test may be conducted in-house or by a contract laboratory.

8.8 Membrane Filter Integrity Test

To ensure the proper sterilization of the PET drug product by passage through a membrane sterilization filter, the bubble point of the filter should be determined after completing the filtration process. The bubble point of the filter, measured in pounds per square inch (psi), is the pressure required to force air through the pores of the wetted membrane in the device. Other suitably validated procedures may be used.

8.9 Operator Training and Qualification

Operators involved in aseptic techniques should be trained in proper gowning, environmental controls, handling sterile components, and other techniques. Operators should be qualified through successful completion of media fills. Media fills should be periodically repeated to ensure ongoing competency of the technique.

9. LABELING

The labeling associated with PET drug products may evolve as the drug progresses through the various development stages. For example, the labeling for PET drug products used for *in vitro* and animal studies may be very simple and designed to avoid mix-ups in the routine use of the PET drug product. In later development stages, the labeling may include information required for the investigational use of the PET drug product in humans. Finally, at the commercial stage of production, the labeling should include ingredients, warnings, approved indications, and other elements required by the appropriate regulatory agencies (e.g., FDA, Nuclear Regulatory Commission, and others).

10. GLOSSARY

The following definitions apply to words and phrases as they are used in this chapter:

Accuracy: The closeness of test results obtained by that method to the true value established across the range of the method.

Active pharmaceutical ingredient (API): A radioactive substance that exhibits spontaneous disintegration of unstable nuclei by the emission of positrons and is incorporated into a PET drug product to furnish a direct effect in the diagnosis or monitoring of a disease or a manifestation of a disease in humans, or monitoring treatment of disease or therapeutic procedures (e.g., tumor therapy). Both radioactive and nonradioactive forms of the PET drug are included in the API.

Batch: A quantity of PET drug product that is intended to have uniform character and quality, within specified limits, and that is made in a single, defined operational cycle.

Chemical purity: The purity of a PET drug product based on the nonradioactive components of the formulation, e.g., residual solvents and/or volatile impurities, reagents, and or precursors used in the synthesis and purification, stabilizers, excipients, or by-products produced in the synthesis.

Compounding: The process of synthesis or formulation of a PET drug for use under the practice of pharmacy and medicine.

Conditional final release: A final release for patient administration before completion of a required test because of a breakdown of analytical equipment.

Limit of detection: The lowest amount of analyte in a sample that can be detected but not necessarily quantified under the stated method conditions.

Linearity: The ability to elicit test results that are, directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Lot: A quantity of materials (e.g., reagents, solvents, gases, purification columns, and other auxiliary materials) that have uniform character and quality within specified limits and are used to make a PET drug product.

Lower limit of quantification: The lowest amount of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated method conditions.

National Metrology Institute (NMI): A measurement standards body that is a laboratory of metrology that establishes standards for a country or organization. The National Institute of Standards and Technology (NIST) is the NMI for the United States.

PET drug: A radioactive substance (active pharmaceutical ingredient) that exhibits spontaneous disintegration of unstable nuclei by the emission of positrons and is incorporated into a PET drug product to furnish direct effect in the diagnosis or monitoring of a disease or a manifestation of a disease in humans, or monitoring treatment of disease or therapeutic procedures (e.g., tumor therapy).

PET drug product: A finished dosage form that contains a PET drug, whether or not in association with one or more other ingredients.

Precision (as repeatability): The degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogenous sample within a lab over a short period of time using the same analyst with the same equipment.

Quality assurance (QA): A planned and systematic program to ensure that a PET drug product possesses the quality required for its intended purpose.

Quality control (QC): A system for testing the quality of components, materials, supplies, and PET drug products by procedures, tests, analytical methods, and acceptance criteria.

Radiochemical identity: The molecular structure of the intended active radiopharmaceutical ingredient that is present in the radiopharmaceutical preparation.

Radiochemical purity: The ratio, expressed as a percentage, of the radioactivity of the intended active radiopharmaceutical ingredient to the total radioactivity of all radioactive ingredients present in the radiopharmaceutical preparation.

Radionuclidic identity: The intended radionuclide in the radiopharmaceutical preparation.

Radionuclidic purity: The ratio, expressed as a percentage, of the radioactivity of the intended radionuclide to the total radioactivity of all radionuclides in the radiopharmaceutical preparation.

Range: The interval between the upper and lower levels of a quality attribute that can be determined with a suitable level of precision and accuracy.

Reagents and materials: A reagent is a chemical used in the synthesis and/or testing of a PET drug, whereas a material is an ancillary object, such as tubing, glassware, vials, and others.

Retardation factor (TLC): The ratio of the distance the analyte moved from the origin line divided by the distance the solvent moved from the origin line denoted by the variable R_f .

Retention time (HPLC or GC): The time required, after the injection, for the analyte to move through the column and reach the detector.

Robustness: The measure of the capacity of an analytical method to remain unaffected by small variations in method parameters.

Ruggedness (as reproducibility): The degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different labs, different analysts, different instruments, different lots of reagents, different assays, and different days.

Specific activity: The radioactivity of a radionuclide per unit mass of the element or compound. The unit of specific activity is the amount of radioactivity on a mass basis [e.g., mCi/ μ g (MBq/ μ g) or Ci/mmol (GBq/mmol)].

Specificity: The ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as impurities, degradation products, and matrix components.

Strength: The radioactivity concentration of the active pharmaceutical ingredient in a PET drug product at a given date and time. The unit of strength is the amount of radioactivity on a

volume basis [e.g., mCi/mL (MBq/mL)].

Sub-batch: A quantity of PET drug product having uniform character and quality, within specified limits, that is produced during one succession of multiple irradiations using a given synthesis or purification operation. A group of sub-batches collectively forms a batch that is intended to have uniform character and quality, within specified limits. Sub-batches may be required for PET drug products with very short-lived radionuclides (e.g., ^{13}N and ^{15}O), because QC tests cannot be completed before use.

System suitability: Requirements used to verify that the system performs according to established criteria.

Tailing factor (symmetry factor): A measure indicating the non-ideality of a chromatographic peak resulting from the distribution and the migration of the analyte through the chromatographic column.

Validation: Establishment of documented evidence that a method, process, or system meets its intended requirements.

Verification: Confirmation that an established method, process, or system meets predetermined acceptance criteria. ■2S (USP39)

BRIEFING

(2040) **Disintegration and Dissolution of Dietary Supplements**, *USP 38* page 1774 and *PF 40(6)* [Nov.–Dec. 2014]. It is proposed to make the following changes in this chapter:

1. Clarify the end point in the *Procedure* in the *Rupture Test for Soft Shell Capsules*.
2. Delete the information about flow-through cell (*USP Apparatus 4*), since there is no official procedure using this apparatus in *USP* for dietary supplements. *USP* has received no proposal to use this apparatus.
3. Revise the current *Test 2* dissolution procedure using *USP Apparatus 3* under the sections *Dissolution Conditions for Folic Acid* and *Dissolution Conditions for Index Water-Soluble Vitamins and Index Minerals*.
4. Introduce *Test 3*, a new dissolution procedure for folic acid and index water-soluble vitamins and index minerals, suitable for soft shell capsules using *USP Apparatus 2* with stationary basket.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCDF: N. Davydova.)

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Comment deadline: November 30, 2015

(2040) DISINTEGRATION AND DISSOLUTION OF DIETARY SUPPLEMENTS

INTRODUCTION

This general chapter is provided to determine compliance with the disintegration and dissolution standards for dietary supplements where stated in the individual monographs.

For the purposes of this chapter, dietary supplement dosage forms have been divided into three categories: *Vitamin–Mineral Dosage Forms*, *Botanical Dosage Forms*, and *Dietary Supplements Other Than Vitamin–Mineral and Botanical Dosage Forms*. *Vitamin–Mineral Dosage Forms* include articles prepared with vitamins, minerals, or combinations of these dietary ingredients, as described in *Table 1*. *Botanical Dosage Forms* comprise formulations containing ingredients of botanical origin, including plant materials and extracts. *Dietary Supplements Other Than Vitamin–Mineral and Botanical Dosage Forms* encompass dietary supplements formulated with lawfully recognized dietary ingredients that are different from those pertaining to the two foregoing categories (e.g., amino acids, chondroitin, and glucosamine).

Where a dietary supplement represents a combination of the categories mentioned above, and there is a difference between the requirements for the individual categories, the more stringent requirement applies. [Note—“More stringent requirement” means stricter acceptance criteria and/or milder operational conditions.]

Disintegration and dissolution tests as described in this chapter are quality-control tools to assess performance characteristics of dietary supplement finished dosage forms. These performance standards are intended to detect problems that may arise due to use or misuse, or changes in coatings, lubricants, disintegrants, and other components. These performance tests are also intended to detect manufacturing process issues, such as overcompression and overdrying, that would affect the release characteristics of the final dosage forms. These tests are not intended to be used as a demonstration or as a surrogate for in vivo absorption, bioavailability, or effectiveness, unless an in vitro–in vivo correlation (IVIVC) has been established.

DISINTEGRATION

This test is provided to determine whether dietary supplement capsules or tablets disintegrate within the prescribed time when placed in a liquid medium at the experimental conditions presented below. Compliance with the limits on *Disintegration* stated in the individual monographs for dietary supplements is required, except where the label states that the products are intended for use as troches, are to be chewed, or are designed as extended-release dosage forms. Dietary supplements claiming to be extended-release dosage forms must comply with standards other than disintegration to verify that the release of the dietary ingredients from the dosage form is for a defined period of time. Dietary supplements claiming to be extended-release dosage forms must not be labeled as in compliance with USP unless a *USP* monograph exists for such product. Determine the type of dosage form under test from the labeling and from observation, and apply the appropriate procedure to 6 or more units. For purposes of this test, disintegration does not imply complete solution of the unit or even of its active constituent. Complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the disk (if used) is a soft mass having no palpably firm core.

Apparatus

Apparatus A: Use the *Apparatus* described in *Disintegration* (701) for capsules or tablets that are NMT 18 mm long. For larger capsules or tablets, use *Apparatus B*.

Apparatus B: The apparatus consists of a basket-rack assembly, a 1000-mL low-form beaker for the immersion fluid, a thermostatic arrangement for heating the fluid between 35° and 39°,

and a device for raising and lowering the basket in the immersion fluid at a constant frequency rate between 29 and 32 cycles/min through a distance of 53–57 mm. The volume of the fluid in the vessel is such that at the highest point of the upward stroke, the wire mesh remains at least 15 mm below the surface of the fluid and descends to NLT 25 mm from the bottom of the vessel on the downward stroke. At no time should the top of the basket-rack assembly become submerged. The time required for the upward stroke is equal to the time required for the downward stroke, and the change in stroke direction is a smooth transition rather than an abrupt reversal of motion. The basket-rack assembly moves vertically along its axis. There is no appreciable horizontal motion or movement of the axis from the vertical.

Basket-rack assembly: The basket-rack assembly (see *Figure 1*) consists of three open-ended transparent tubes, each 77.5 ± 2.5 mm long and having an inside diameter of 32.0–34.6 mm and a wall 2.0–3.0 mm in thickness; the tubes are held in a vertical position by two plastic plates, each 97 ± 2 mm in diameter and 7.5–10.5 mm in thickness, with three holes, 36.0–40.6 mm in diameter, equidistant from the center of the plate and equally spaced from one another. Attached to the undersurface of the lower plate is 10-mesh No. 23 (0.025-inch) W- and M-gauge woven stainless-steel wire cloth having a plain square weave. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plastic plates. A suitable means is provided to suspend the basket-rack assembly from the raising and lowering device, using a point on its axis. The design of the basket-rack assembly may be varied somewhat, provided that the specifications for the glass tubes and the screen mesh size are maintained.

Beaker: Low form, 1000 mL; the difference between the diameter of the plastic plates, which hold the tubes in a vertical position, and the inside diameter of the beaker should be NMT 6 mm.¹

Disks: Each tube is provided with a perforated cylindrical disk 15.3 ± 0.15 mm in thickness and 31.4 ± 0.13 mm in diameter. The disk is made of a suitable, transparent plastic material having a specific gravity of between 1.18 and 1.20. Seven holes 3.15 ± 0.1 mm in diameter extend between the ends of the cylinder, one of the holes being in the center and the other six parallel to it and spaced equally tangent to a circle with a radius of 4.2 mm from the center of the disk. All surfaces of the disk are smooth.²

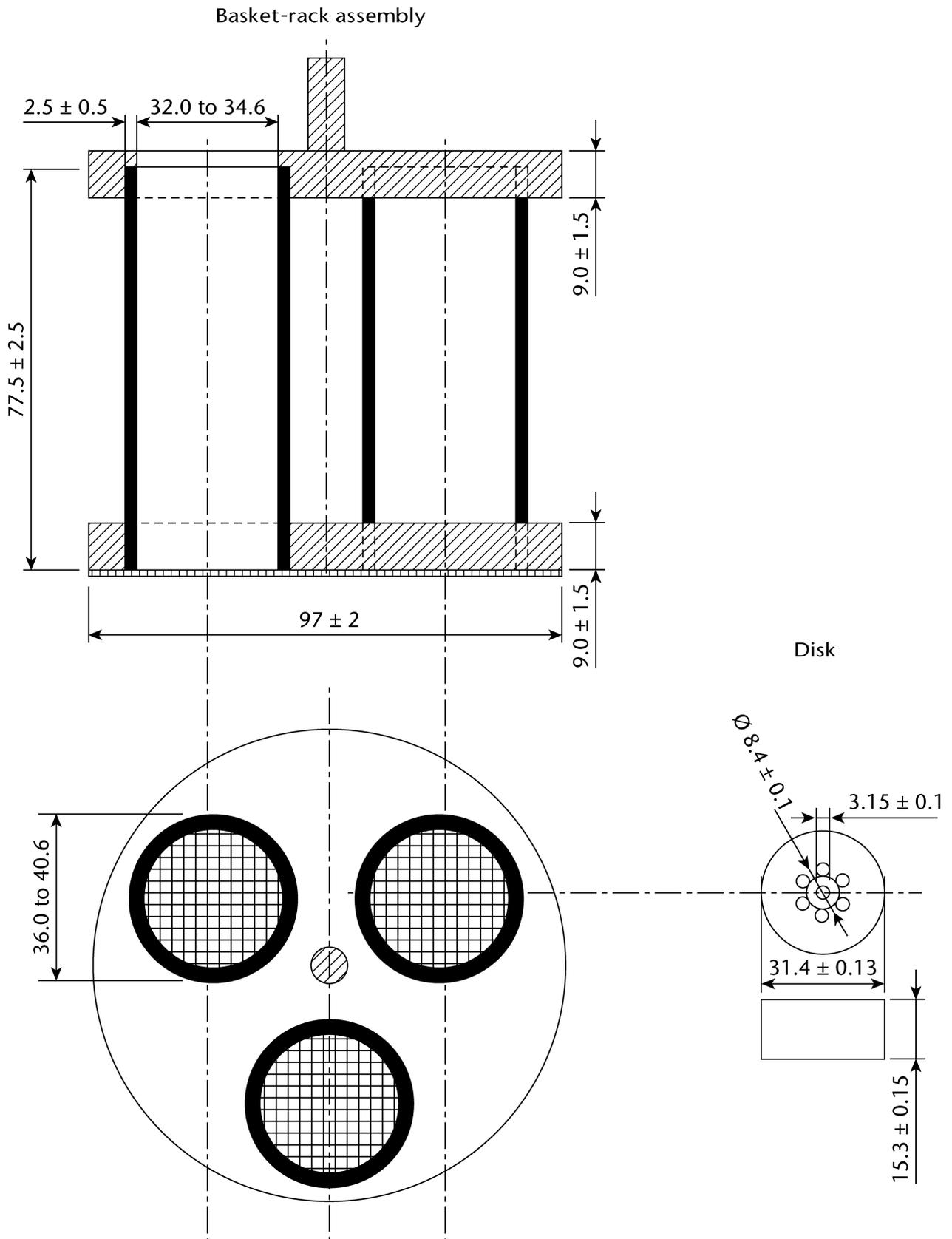


Figure 1. Basket-rack assembly, *Disintegration, Apparatus B* (dimensions in mm).

Procedure

Test 6 dosage units as described below for each type of dosage form. [Note—Two basket arrangements for a total of six tubes are necessary for *Apparatus B*.] If 1 or 2 dosage units fail to disintegrate completely, repeat the test on 12 additional dosage units.

Uncoated tablets: Place 1 tablet in each of the tubes of the basket and, if prescribed, add a disk to each tube. Operate the apparatus, using water or the specified medium as the immersion fluid, maintained at $37 \pm 2^\circ$. At the end of 30 min, lift the basket from the fluid and observe the tablets.

Plain coated tablets: Place 1 tablet in each of the tubes of the basket and, if the tablet has a soluble external sugar coating, immerse the basket in water at room temperature for 5 min. Then, if prescribed, add a disk to each tube and operate the apparatus, using water or the specified medium as the immersion fluid, maintained at $37 \pm 2^\circ$. At the end of 30 min, lift the basket from the fluid and observe the tablets.

Delayed-release (enteric-coated) tablets: Omit the use of a disk. Place 1 tablet in each of the six tubes of the basket, and if the tablet has a soluble external sugar coating, immerse the basket in water at room temperature for 5 min. Then operate the apparatus using simulated gastric fluid TS, maintained at $37 \pm 2^\circ$, as the immersion fluid. After 1 h of operation in simulated gastric fluid TS, lift the basket from the fluid and observe the tablets: the tablets show no evidence of disintegration, cracking, or softening. Operate the apparatus using simulated intestinal fluid TS, maintained at $37 \pm 2^\circ$, as the immersion fluid for the time specified in the monograph. Lift the basket from the fluid and observe the tablets.

Delayed-release (enteric-coated) soft shell capsules: Place 1 softgel capsule in each of the six tubes of the basket. Omit the use of a disk. Operate the apparatus using simulated gastric fluid TS, maintained at $37 \pm 2^\circ$, as the immersion fluid. After 1 h of operation in simulated gastric fluid TS, lift the basket from the fluid and observe the softgels: the softgels show no evidence of disintegration or rupture that would permit the escape of the contents. Operate the apparatus with disks using simulated intestinal fluid TS, maintained at $37 \pm 2^\circ$, as the immersion fluid for NMT 60 min. Lift the basket from the fluid and observe the capsules.

Hard shell capsules: Apply the test for *Uncoated tablets* using as the immersion fluid, maintained at $37 \pm 2^\circ$, a 0.05 M acetate buffer prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain a 1000-mL solution with a pH of 4.50 ± 0.05 . Attach a removable wire cloth, as described in *Basket-rack assembly*, to the surface of the upper plate of the basket-rack assembly. At the end of 30 min, lift the basket from the fluid and observe the capsules.

Soft shell capsules: Proceed as directed in the *Rupture Test for Soft Shell Capsules*.

Use of Disks

Vitamin–mineral dosage forms: Add a disk to each tube unless otherwise specified in the *Procedure* above or in the individual monograph.

Botanical dosage forms: Omit the use of disks unless otherwise specified in the *Procedure* above or in the individual monograph.

Dietary supplements other than vitamin–mineral and botanical dosage forms: Omit the use of disks unless otherwise specified above or in the individual monograph.

Tolerances

All of the 6 dosage units initially tested or NLT 16 of a total of 18 dosage units tested disintegrate completely.

Change to read:

RUPTURE TEST FOR SOFT SHELL CAPSULES

Medium: Water; 500 mL

Apparatus: Use *Apparatus 2* as described in *Dissolution* (711), operating at 50 rpm.

Time: 15 min

Procedure: Place 1 capsule in each vessel, and allow the capsule to sink to the bottom of the vessel before starting rotation of the blade. Use sinkers if the capsules float. ~~Observe the capsules, and record the time taken for each capsule shell to rupture.~~

■ Observe the capsules throughout the test and at the end of the test. The capsule shell is considered ruptured if breached, exposing or allowing to escape the fill contents. ■2S (USP39)

Tolerances: The requirements are met if all of the capsules tested rupture in NMT 15 min. If 1 or 2 of the capsules rupture in >15 min but NMT 30 min, repeat the test on 12 additional capsules: NMT 2 of the total of 18 capsules tested rupture in >15 min but NMT 30 min. ▲ For soft gelatin capsules that do not conform to the above rupture test acceptance criteria, repeat the test with the addition of papain to the *Medium* in the amount that results in an activity of NMT 550,000 Units/L of *Medium* or with the addition of bromelain in the amount that results in an activity of NMT 30 gelatin-digesting units (GDU)/L of *Medium*. [Note—Determine papain activity using the *Assay* test in the monograph for *Papain* and bromelain activity using the procedure in *bromelain*, in the *Reagent Specifications* section.] ▲USP39

Change to read:

DISSOLUTION

This test is provided to determine compliance with the *Dissolution* requirements where stated in the individual monographs for dietary supplements. ▲▲USP39 The operative assumption inherent in this test is that if the index vitamin or mineral or marker compound(s) for a botanical is dissolved within the time frame and under conditions specified, the dosage form does not suffer from formulation- or manufacturing-related problems affecting the adequate release of the active ingredients.

▲

For Dosage Forms Containing or Coated with Gelatin

For hard or soft gelatin capsules and gelatin-coated tablets that do not conform to the dissolution specification because of the presence of cross-linking, the dissolution procedure should be repeated with the addition of enzymes to the medium, as described below.

DISSOLUTION MEDIUM WITH PH \leq 4.0

Enzyme: Pepsin, activity determined by the procedure in *pepsin*, in the *Reagent Specifications* section

Amount: A quantity of pepsin that results in an activity of NMT 750,000 Units/L of dissolution medium

DISSOLUTION MEDIUM WITH PH $>$ 4.0 AND $<$ 6.8

Enzyme: Papain, activity determined by the *Assay* test in the monograph for *Papain*; or bromelain, activity determined by the procedure in *bromelain*, in the *Reagent Specifications* section

Amount: A quantity of papain that results in an activity of NMT 550,000 Units/L of dissolution medium, or a quantity of bromelain that results in an activity of NMT 30 GDU/L of dissolution medium

DISSOLUTION MEDIUM WITH PH \geq 6.8

Enzyme: Pancreatin, protease activity determined by the procedure in *Assay for protease activity (Casein digestive powder)* in the monograph for *Pancreatin*

Amount: A quantity of pancreatin that results in a protease activity of NMT 2,000 Units/L of dissolution medium

DISSOLUTION MEDIUM CONTAINING SURFACTANTS OR OTHER COMPONENTS KNOWN TO DENATURE THE ENZYME

If the dissolution medium contains surfactants or other components known to denature the enzyme to be used, a pretreatment step should be applied. The pretreatment step is performed under the same dissolution conditions (apparatus, rotation, and flow rate), except to use a medium with the corresponding amount of enzyme as directed in the preceding section and without the surfactant or component known to denature the enzyme. To achieve the final specified volume of medium, the pretreatment step may be conducted with a smaller volume of medium without the surfactant or component in such a manner that the final specified volume is achieved after the addition of the surfactant or component at the end of the pretreatment step. Perform the pretreatment step until capsule rupture, but for NMT one-half of the total dissolution time specified in the procedure. The pretreatment time is included in the total dissolution time specified in the procedure.

▲USP39

Apparatus

See <711> for a description of the apparatus used, *Apparatus Suitability Test*, and other related information.

■ Where the procedure specifies the use of a stationary basket, use the quadrangular basket of stainless steel wire gauze as shown in *Figure 2a* and *Figure 2b*.

The capsule is placed in a basket, soldered in one of its upper, narrow sides to the end of a steel rod (see *Figure 2a*). The capsule cover is placed in the horizontal diagonal of the basket. The rod assembly is inserted vertically through the cover of the dissolution vessel, and fixed by

means of two teflon nuts, 3.2 cm from the center of the vessel, or by any other appropriate means. The lower edge of the bottom of the basket is adjusted to about 1 cm above the top of the paddle blade (see *Figure 2b*).

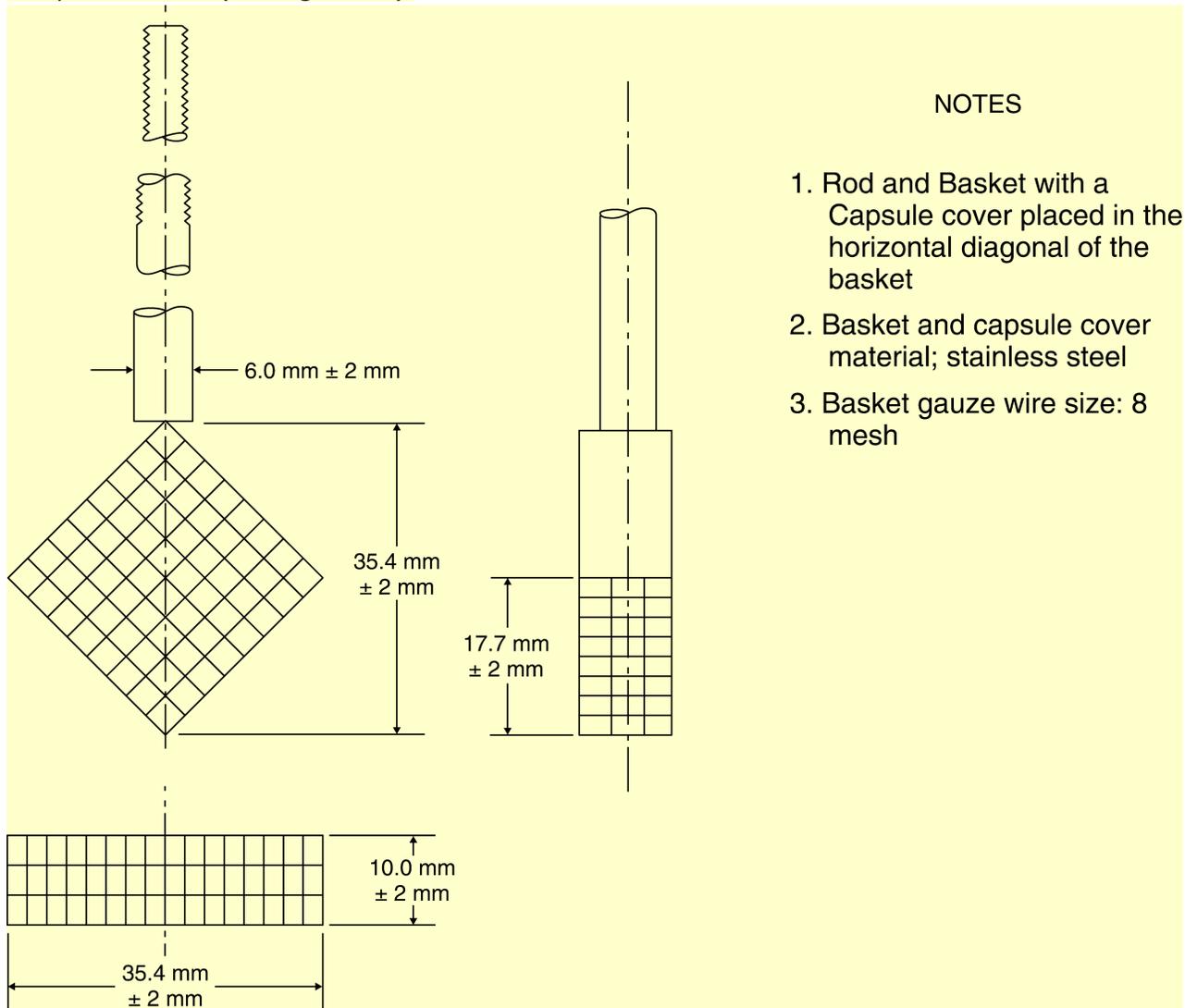


Figure 2a. Stationary Basket

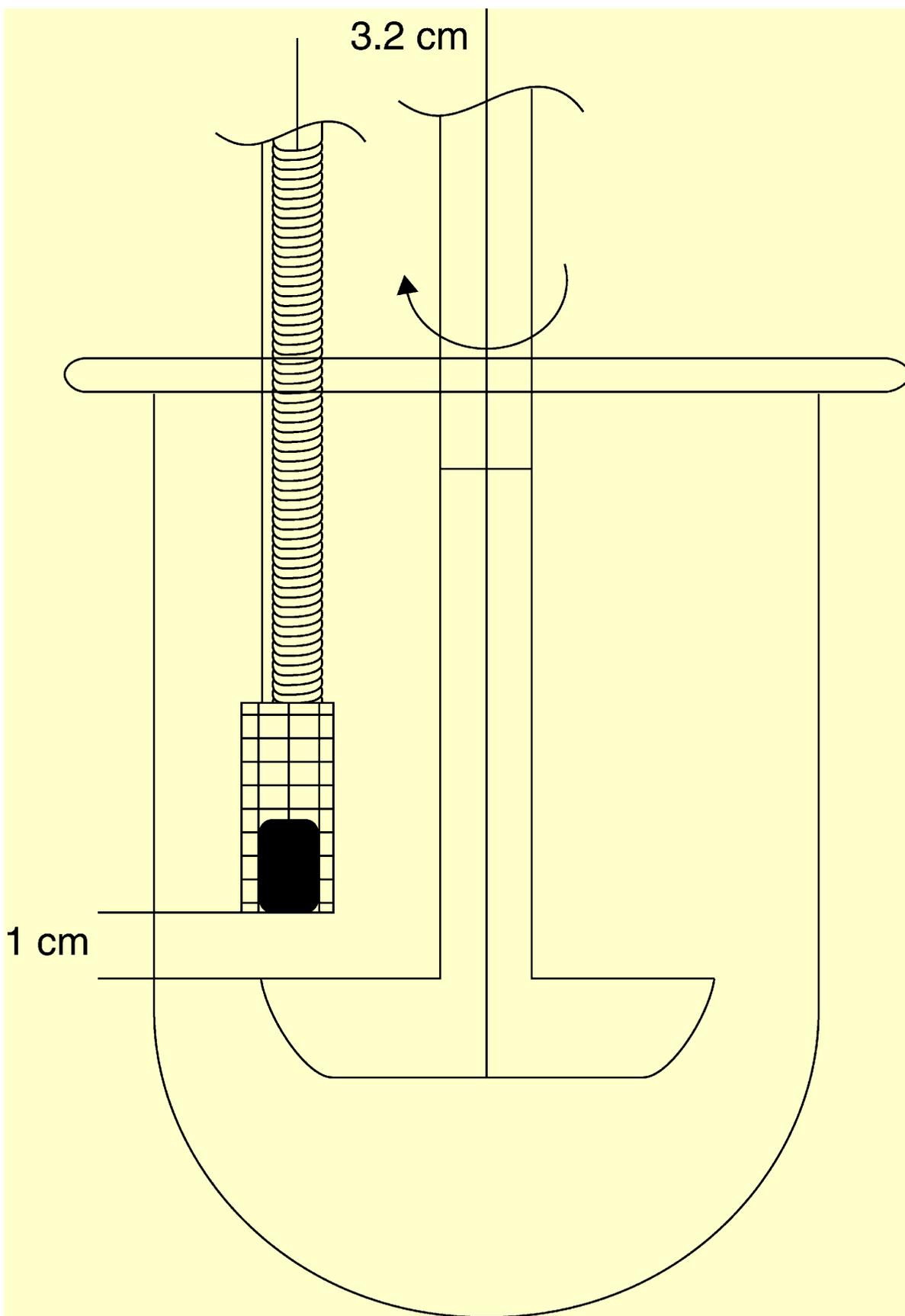


Figure 2b. Stationary Basket Configuration Diagram

~~Figure 2 shows the schematic view of a flow-through cell (USP Apparatus 4) specifically intended for dissolution of lipid-filled soft shell capsules. The lower part [Figure 2 (1)] is made up of two adjacent chambers connected to an overflow device. The dissolution medium passes through chamber A and is subjected to an upward flow. The flow in chamber B is directed downward to a small-size bore exit that leads upward to a filter assembly. The middle part [Figure 2 (2)] of the cell has a cavity designed to collect lipophilic excipients that float on the dissolution medium. A metal grid serves as a rough filter. The upper part [Figure 2 (3)] holds a filter unit for paper, glass fiber, or cellulose filters.~~

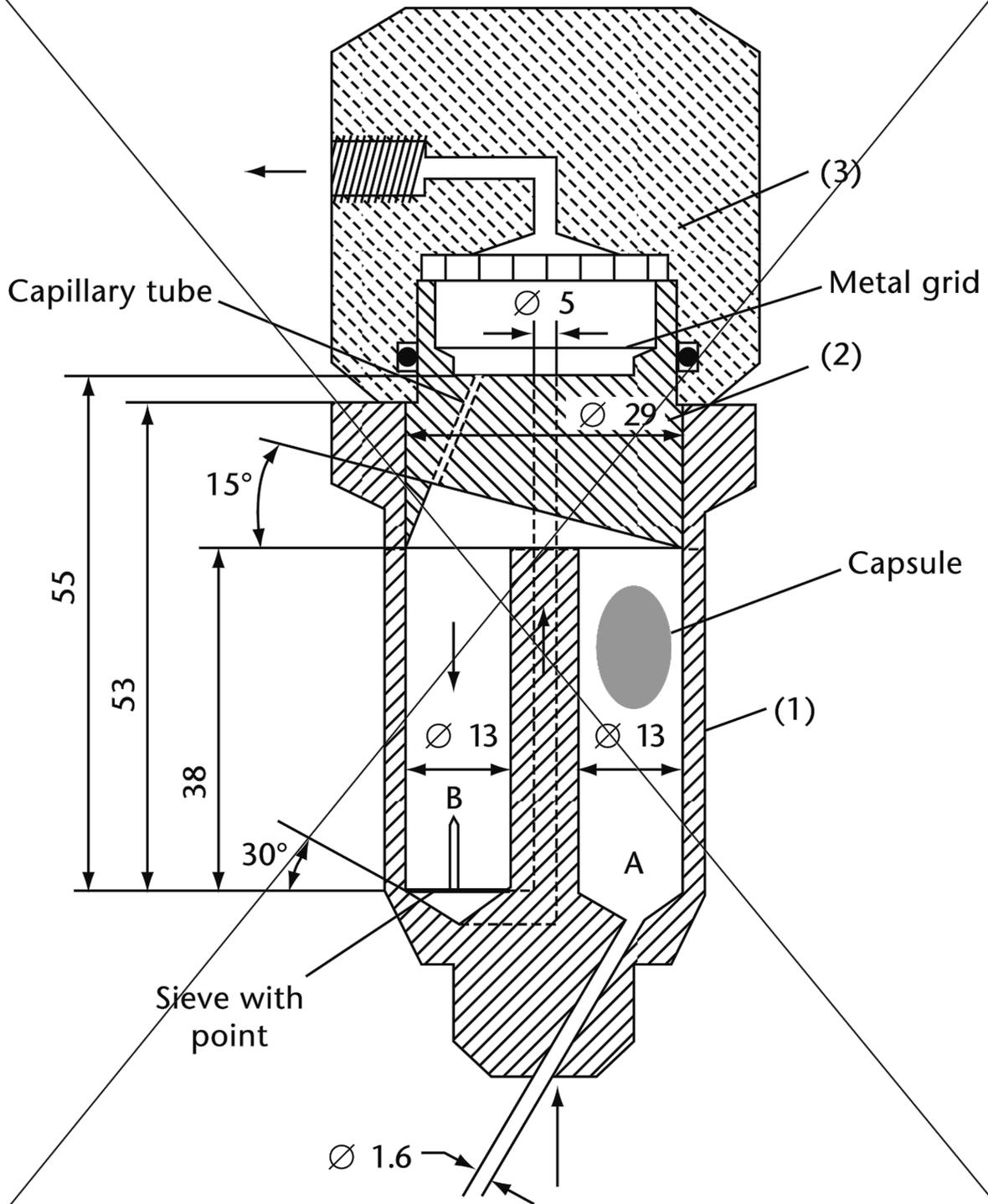


Figure 2. Flow-through cell designed for lipid-filled soft gelatin capsules (dimensions in mm).

■ 2S (USP39)

Of the types of apparatus described in (711), use the one specified in the individual monograph.

▲▲ USP39

Vitamin–Mineral Dosage Forms

All dietary supplement capsules or tablets containing folic acid are subject to the dissolution test and criteria for folic acid described in this chapter. This test is required because of the importance of the relationship between folate deficiency and the risk of neural tube defects. Dietary supplement capsules or tablets containing water-soluble vitamins, minerals, or their combination are subject to the dissolution test and criteria for index vitamins, index minerals, or both, described in this chapter. Dietary supplement tablet and hard shell capsule with solid contents dosage forms containing vitamin A are subject to the dissolution test and criteria for vitamin A described in this chapter. Dissolution standards were not established and therefore are not applicable to vitamin A in dietary supplement soft shell capsules filled with liquids. *Table 1* summarizes the dissolution requirements for the assigned USP classes of dietary supplements. Vitamin–mineral combinations that do not belong to any of the USP classes listed in *Table 1* are subject to the *Dissolution* test and criteria specified in the individual monographs.

Table 1. Dietary Supplements—Vitamin–Mineral Dosage Forms

USP Class	Ingredients	Dissolution Requirements for Tablets and Hard Shell Capsules with Solid Contents	Dissolution Requirements for Soft Shell Capsules Filled with Liquids
I	Oil-soluble vitamins	Vitamin A (if present)	Not applicable
II	Water-soluble vitamins	One index water-soluble vitamin and folic acid (if present)	One index water-soluble vitamin and folic acid (if present)
III	Water-soluble vitamins with minerals	One index water-soluble vitamin, one index element, and folic acid (if present)	One index water-soluble vitamin, one index element, and folic acid (if present)
IV	Oil- and water-soluble vitamins	Vitamin A (if present), one index water-soluble vitamin, and folic acid (if present)	One index water-soluble vitamin and folic acid (if present)
V	Oil- and water-soluble vitamins with minerals	Vitamin A (if present), one index water-soluble vitamin, one index element, and folic acid (if present)	One index water-soluble vitamin, one index element, and folic acid (if present)
VI	Minerals	One index element	One index element
VII	Oil-soluble vitamins with minerals	Vitamin A (if present) and one index element	One index element

SELECTION OF INDEX WATER-SOLUBLE VITAMINS AND INDEX ELEMENTS

Compliance with the dissolution requirements for dietary supplements representing combinations of water-soluble vitamins and combinations of oil- and water-soluble vitamins is determined by measuring the dissolution of a single index vitamin from the water-soluble vitamins present. Riboflavin is the index vitamin when present in the formulation. For formulations that do not contain riboflavin, pyridoxine is the index vitamin. If neither riboflavin nor pyridoxine is present in the formulation, the index vitamin is niacinamide (or niacin), and in the absence of niacinamide (or niacin), the index vitamin is thiamine. If none of these four water-soluble

vitamins are present in the formulation, the index vitamin is ascorbic acid.

Compliance with the dissolution requirements for dietary supplements representing combinations of minerals is determined by measuring the dissolution of only one index element. Iron is the index element when present in the formulation. For formulations that do not contain iron, the index element is calcium. If neither iron nor calcium is present, the index element is zinc. In the absence of all three of these elements, magnesium is the index element.

Compliance with the dissolution requirements for dietary supplements representing combinations of water-soluble vitamins and minerals and combinations of oil- and water-soluble vitamins and minerals is determined by measuring the dissolution of one index water-soluble vitamin and one index element, designated according to the respective hierarchies described above.

DISSOLUTION CONDITIONS FOR VITAMIN A

Note—Perform this test under light conditions that minimize photodegradation.

Medium: 1% (w/v) Sodium ascorbate and 1% (w/v) octoxynol 9 in 0.05 M phosphate buffer, pH 6.8; 900 mL

Apparatus 2: 75 rpm

Time: 45 min

DISSOLUTION CONDITIONS FOR FOLIC ACID

Note—Perform this test under light conditions that minimize photodegradation.

Test 1

Medium: Water; 900 mL. ~~If the units tested do not meet the requirements for dissolution in water, test six additional dosage units for dissolution in a medium of 900 mL of 0.05 M pH 6.0 citrate buffer solution, prepared by mixing 9.5 mL of 0.1 M citric acid monohydrate and 40.5 mL of 0.1 M sodium citrate dihydrate in a 100 mL volumetric flask, diluting with water to volume, mixing, and adjusting to a pH of 6.0 by using either 0.1 M hydrochloric acid or 0.1 M sodium hydroxide solution.~~

■ ■2S (USP39)

Apparatus 1: 100 rpm, for capsules

Apparatus 2: 75 rpm, for tablets

Time: 1 h

■ If the units tested do not meet the requirements for dissolution in water, use the following conditions:

Buffer: Mix 95 mL of 0.1 M citric acid monohydrate and 405 mL of 0.1 M sodium citrate dihydrate, dilute to 1000 mL with water, mix, and adjust to a pH of 6.0 by using either 0.1 M hydrochloric acid or 0.1 M sodium hydroxide solution.

Medium: Buffer; 900 mL

Apparatus 1: 100 rpm, for capsules

Apparatus 2: 75 rpm, for tablets

Time: 1 h ■2S (USP39)

Test 2

■ **(for lipid-filled soft shell capsules):** Proceed as directed for *Test 2* under *Dissolution Conditions for Index Water-Soluble Vitamins and Index Minerals*. ■_{2S} (USP39)

If the article complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

~~Medium:~~ 45 mM citrate buffer, pH 6.0; 250 mL

~~Apparatus 3:~~ 30 rpm

~~Screen (top and bottom):~~ 56 mesh

~~Time:~~ 1 h

■

Test 3 (for lipid-filled soft shell capsules): Proceed as directed for *Test 3* under *Dissolution Conditions for Index Water-Soluble Vitamins and Index Minerals* If the article complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*. ■_{2S} (USP39)

Note—Compliance with the dissolution requirements for folic acid does not exempt the article from compliance with the dissolution requirements of the pertinent index vitamin or the corresponding index mineral.

DISSOLUTION CONDITIONS FOR INDEX WATER-SOLUBLE VITAMINS AND INDEX MINERALS

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm, for capsules

Apparatus 2: 75 rpm, for tablets

Time: 1 h

For formulations containing 25 mg or more of the index vitamin, riboflavin, use the following conditions:

Medium: 0.1 N hydrochloric acid; 1800 mL

Apparatus 1: 100 rpm, for capsules

Apparatus 2: 75 rpm, for tablets

Time: 1 h

Test 2 (not suitable for minerals)

■ **(for lipid filled soft shell capsules):** ■_{2S} (USP39)

If the article complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

~~Medium:~~ 45 mM citrate buffer, pH 6.0; 250 mL

■ 0.25% (w/v) Octoxynol 9, 0.02% (w/v) ascorbic acid, and 0.04% (w/v) simethicone in simulated gastric fluid TS ■_{2S} (USP39)

~~Apparatus 3:~~ 30 rpm

■ 15 rpm ■_{2S} (USP39)

Screen (top and bottom): 56-mesh

■ 20-mesh ■ 2S (USP39)

Time: 1 h

■ **Test 3 (for lipid-filled soft shell capsules):** If the article complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: 0.25% (w/v) Octoxynol 9 and 0.02% (w/v) ascorbic acid in simulated gastric fluid TS; 500 mL

Apparatus 2: 125 rpm; dosage unit placed in stationary basket (*Figure 2a-b*)

Time: 1 h

■ 2S (USP39)

Note—Compliance with dissolution requirements for the pertinent index vitamin or index mineral does not exempt the article from compliance with the dissolution requirements for folic acid, if present.

PROCEDURES

In the following procedures, combine equal volumes of the filtered solutions of the six individual specimens withdrawn, and use the pooled sample as the test specimen. Determine the average amount of vitamin A, folic acid, or the index vitamin or element dissolved in the pooled sample. Make any necessary modifications, including concentration of the analyte in the volume of *Sample solution* taken. Use the *Medium* for preparation of the *Standard solution* and dilution, if necessary, of the *Sample solution*.

Vitamin A: Determine the percentage of retinyl acetate or retinyl palmitate dissolved by using the following procedure.

Standard solution: Dissolve a suitable amount of *USP Retinyl Acetate RS* or *USP Retinyl Palmitate RS* in isopropyl alcohol, and dilute with *Medium* to obtain a concentration similar to that expected in the *Sample solution*. [Note—The amount of isopropyl alcohol should be 5%–10%.]

Sample solution: Withdraw a portion of the solution under test, pass through a suitable filter of 0.45- μ m pore size, and use the pooled sample as the test specimen.

Solution A: Methanol and water (90:10)

Solution B: Methanol and isopropyl alcohol (55:45)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
8	0	100
13	0	100
13.1	100	0
15	100	0

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 325 nm**Column:** 4.6-mm × 10-cm; 3-µm packing L1**Flow rate:** 1.0 mL/min**Injection volume:** 50 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 1.5 for retinyl acetate and NMT 2.0 for retinyl palmitate**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Appropriate Standard solution and Sample solution*

$$\text{Result} = (r_U/r_S) \times (C_S \times V/L) \times 100$$

 r_U = peak area of the all-*trans*-retinyl ester from the *Sample solution* r_S = peak area of the all-*trans*-retinyl ester from the appropriate *Standard solution* C_S = concentration of retinol in the appropriate *Standard solution* (µg/mL) V = volume of *Medium*, 900 mL L = label claim of vitamin A, as retinol (µg/tablet)

Folic acid: Determine the amount of folic acid (C₁₉H₁₉N₇O₆) dissolved by using the procedure set forth in the assay for *Folic Acid* in the individual monograph. Make any necessary modifications.

Niacin or Niacinamide, Pyridoxine, Riboflavin, and Thiamine: Determine the amount of the designated index vitamin dissolved by using the procedure set forth in the *Assay for Niacin or Niacinamide, Pyridoxine Hydrochloride, Riboflavin, and Thiamine* in the individual monographs. Make any necessary modifications.

Ascorbic acid: Determine the amount of ascorbic acid ($C_6H_8O_6$) dissolved by using the procedure set forth in the *Assay for Ascorbic Acid* in the individual monograph. Make any necessary modifications.

Iron, Calcium, Magnesium, and Zinc: Determine the amount of the designated index element dissolved by using the procedure set forth in the appropriate assay in the individual monographs. Make any necessary modifications.

TOLERANCES

The requirements are met if NLT 75% of the labeled content of vitamin A, NLT 75% of the labeled content of folic acid, and NLT 75% of the labeled content of the index vitamin or the index element from the units tested is dissolved.

Botanical Dosage Forms

Compliance with dissolution requirements necessitates the testing of 6 dosage units individually, or testing 2 or more dosage units in each of the six vessels of the dissolution apparatus, and measuring the dissolution of one or more index/marker compound(s) or the extract specified in the individual monograph.

PROCEDURES

Combine equal volumes of the filtered solutions of the six or more individual specimens withdrawn, and use the pooled sample as the *Sample solution*. Determine the average amount of index or marker compound(s) or the extract dissolved in the pooled sample by the procedure specified in the individual monograph. Make any necessary modifications, including concentration of the analyte in the volume of the *Sample solution* taken. Use the *Medium* for preparation of the *Standard solution* and dilution, if necessary, of the *Sample solution*.

TOLERANCES

Unless otherwise specified in the individual monograph, the requirements are met if NLT 75% of the labeled content of the index or marker compound(s) or the extract from the units tested is dissolved in 1 h.

Dietary Supplements Other Than Vitamin–Mineral and Botanical Dosage Forms

Unless otherwise stated in the individual monographs for dietary supplement dosage forms in this category, compliance requires the testing of 6 individual units, measuring the dissolution of the dietary ingredient as the average of the 6 units tested.

PROCEDURES

Combine equal volumes of the filtered solutions of the six specimens withdrawn, and use the pooled sample as the *Sample solution*. Determine the average amount of the dietary ingredient dissolved in the pooled sample by the procedure specified in the individual monograph. Make any necessary modifications, including concentration of the analyte in the volume of the *Sample solution* taken. Use the *Medium* for preparation of the *Standard solution* and for dilution, if necessary, of the *Sample solution*.

TOLERANCES

Because of the diversity of chemical characteristics and solubilities of dietary ingredients pertaining to this category, general tolerances cannot be established. See individual monographs for *Tolerances*.

~~USP Reference Standards (11)~~

~~USP Folic Acid RS~~

~~USP Retinyl Acetate RS~~

~~USP Retinyl Palmitate RS~~

■ ■ 2S (USP39)

¹ 1000-mL low-form beakers, designed in compliance with the current ASTM E 960 Type I or Type II or ISO 3819 specifications, meet the size requirements.

² The use of automatic detection using modified disks is permitted where the use of disks is specified or allowed. Such disks must comply with the requirements for density and dimensions given in this chapter.

BRIEFING

(2750) **Manufacturing Practices for Dietary Supplements**, *USP* 38 page 1789 and page 1319 of *PF* 35(5) [Sept.–Oct. 2009]. On the basis of comments received, the Food Ingredients Expert Committee proposes to revise this general chapter. The revisions are intended to align the text with current regulatory standards. Relevant sections in this chapter, particularly *Record Keeping* and *Testing and Approval or Rejection*, are revised.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(FI: C. Okunji.)

Correspondence Number—C160476

Comment deadline: November 30, 2015

(2750) MANUFACTURING PRACTICES FOR DIETARY SUPPLEMENTS**GENERAL PROVISIONS**

The principles included in this chapter contain recommended minimum current good manufacturing practices for the methods to be used in, and the facilities and controls to be used for, the manufacture, holding, packaging, labeling, and distribution of dietary ingredients and dietary supplements. These principles are set forth to ensure that such products meet the requirements of safety, have the identity and strength, and meet the quality and purity characteristics that they are represented to possess.

Excluded from this chapter are establishments engaged solely in the harvesting, storage, or distribution of one or more “raw agricultural commodities” as defined in Section 201(r) of the Federal Food, Drug, and Cosmetic Act [21 U.S.C. 321(r)], which are ordinarily cleaned, prepared, treated, or otherwise processed before being marketed to the consuming public.

The requirements pertaining to holding dietary ingredients and dietary supplements do not apply

to holding those dietary supplements at a retail establishment for the sole purpose of direct retail sale to individual consumers. A retail establishment does not include a warehouse or other storage facility for a retailer or a warehouse or other storage facility that sells directly to individual consumers.

A *Glossary* of terms used in this chapter is presented at the end.

ORGANIZATION AND PERSONNEL

Responsibilities of a Quality Control Unit

A quality control unit shall be established that has the responsibility and authority to approve or reject all raw materials, product containers, closures, in-process materials, packaging material, labeling, and finished dietary supplements, and the authority to review production records to ensure that no errors have occurred or, if errors have occurred, that they have been fully investigated. The quality control unit should be responsible for approving or rejecting products manufactured, processed, packed, or held under contract by another company. Adequate laboratory facilities for the testing and approval (or rejection) of raw materials, product containers, closures, packaging materials, in-process materials, dietary ingredients, and dietary supplements should be available to the quality control unit.

The quality control unit should have the responsibility for approving or rejecting all procedures or specifications that impact on the identity, strength, quality, and purity of the dietary supplement. All responsibilities and procedures applicable to the quality control unit shall be in writing.

The designated person within the Quality Control Unit who conducts a material review and makes the disposition decision must, at the time of performance, document the material review and disposition decision made.

Personnel Qualifications

Each person engaged in the manufacture of dietary ingredients and dietary supplements should have the proper education, training, and experience (or any combination thereof) needed to perform the assigned functions. Training should be in the particular operation(s) that the employee performs as they relate to the employee's functions. Each person responsible for supervising the manufacture of a dietary ingredient, a dietary supplement, or both should have the proper education, training, and experience (or any combination thereof) to perform assigned functions in such a manner as to provide assurance that the product has the safety, identity, strength, quality, and purity that it is represented to possess. Appropriate documentation of training shall be retained by the company.

An adequate number of qualified personnel to perform and supervise the manufacture of each dietary ingredient, dietary supplement, or both products should be provided.

Personnel Responsibilities

The company management shall take all reasonable measures and precautions to ensure the following:

- **Disease control:** Any person who, by medical examination or supervisory observation, is shown to have, or appears to have: an illness; open lesion, including boils, sores, or infected wounds; or any other abnormal source of microbial contamination by which

there is a reasonable possibility of an in-process or finished dietary ingredient or dietary supplement becoming adulterated; or processing equipment, utensils, or packaging materials becoming contaminated shall be excluded from any operations that may be expected to result in such adulteration or contamination until the condition is corrected. Personnel shall be instructed to report such health conditions to their supervisors.

- **Cleanliness:** All persons working in direct contact with raw materials, in-process or finished dietary ingredients and dietary supplements, processing equipment, utensils, or packaging materials shall conform to hygienic practices while on duty to the extent necessary to protect against adulteration or contamination of such materials. The methods for maintaining cleanliness include, but are not limited to, the following:
 - Wearing outer garments suitable to the operation in a manner that protects against the adulteration of raw materials or of in-process or finished dietary ingredients and dietary supplements, or contamination of processing equipment, utensils, or packaging materials;
 - Maintaining adequate personal cleanliness;
 - Removing cosmetics from parts of the body that may contact raw materials, in-process or finished dietary ingredients and dietary supplements, equipment, utensils, or containers;
 - Washing hands thoroughly (and sanitizing if necessary to protect against contamination with undesirable microorganisms) in an adequate hand-washing facility before starting work, after each absence from the work station, and at any other time when the hands may have become soiled or contaminated;
 - Removing all unsecured jewelry and other objects that might fall into raw materials, in-process or finished dietary ingredients and dietary supplements, equipment, or containers, and removing hand jewelry that cannot be adequately sanitized during periods in which in-process or finished product is manipulated by hand. If such hand jewelry and cosmetics cannot be removed, they may be covered by material that can be maintained in an intact, clean, and sanitary condition and that effectively protects against the adulteration of dietary ingredients and dietary supplements or contamination of processing equipment, utensils, or packaging materials;
 - Maintaining gloves, if they are used in raw materials or in in-process or finished product handling, in an intact, clean, and sanitary condition. The gloves should be of a material that adequately protects the product from contamination;
 - Wearing, where appropriate, in an effective manner, hair nets, caps, beard covers, or other effective hair restraints;
 - Storing clothing or other personal belongings in areas other than where in-process or finished product is exposed or where processing equipment or utensils are washed;
 - Confining the following actions to areas other than where in-process or finished product may be stored or exposed, or where processing equipment or utensils are washed: eating food, chewing gum, drinking beverages, or using tobacco; and
 - Taking any other necessary precautions to protect against adulteration of raw materials or of in-process or finished product; or contamination of processing equipment, utensils, or packaging materials with microorganisms or foreign substances, including but not limited to, perspiration, hair, cosmetics, tobacco, chemicals, and medicines applied to the skin.

GROUND, BUILDING, AND FACILITIES

Grounds

The grounds around a dietary ingredient manufacturing plant and a dietary supplement manufacturing plant under the control of the operator shall be kept in a condition that will protect against the adulteration of dietary ingredients and dietary supplements. The methods for adequate maintenance of grounds include, but are not limited to, the following:

- Properly storing equipment, removing litter and waste, and cutting weeds or grass within the immediate vicinity of the plant building or structures that may constitute an attractant, breeding place, or harborage for pests;
- Maintaining roads, yards, and parking lots so that they do not constitute a source of adulteration in areas where product is exposed;
- Adequately draining areas that may contribute to product adulteration by seepage, foot-borne filth, or providing a breeding place for pests; and
- Operating systems for waste treatment and disposal in an adequate manner so that they do not constitute a source of adulteration in areas where product is exposed. If the plant grounds are bordered by grounds not under the operator's control and not maintained in the manner described above, care shall be exercised in the plant by inspection, extermination, or other means to exclude pests, dirt, and filth that may be a source of product adulteration.

Building Design

Any building or buildings used in the manufacture of a dietary ingredient, a dietary supplement, or both should be of suitable size and shall be constructed in such a manner that floors, walls, and ceilings may be adequately cleaned and kept clean and in good repair; that drips or condensates from fixtures, ducts, and pipes do not adulterate raw materials or in-process or finished dietary ingredients and dietary supplements, or contaminate product containers, utensils, or packaging materials; and that aisles or working spaces are provided between equipment and walls and are adequately unobstructed and of adequate width to permit employees to perform their duties and to protect against adulterating in-process or finished product, or contaminating processing equipment with clothing or personal contact. Adequate screening or other protection against pests and insects should be installed, where necessary. The building should have adequate space for the orderly placement of equipment and materials to prevent mixups between different raw materials, product containers, closures, labeling, in-process materials, or finished products, and to prevent contamination. The flow of raw materials, product containers, closures, labeling, in-process materials, and products through the building or buildings should be designed to prevent contamination.

Operations should be performed within specifically defined areas of adequate size to prevent contamination or mixups or adulteration of in-process or finished dietary ingredients and dietary supplements, or contamination of processing equipment, utensils, or packaging materials with microorganisms, chemicals, filth, or other extraneous materials. The potential for mixups and product adulteration may be reduced by adequate product safety controls and operating practices or effective design, including the separation of operations in which contamination is likely to occur, by one or more of the following means: location, time, partition, airflow, enclosed systems, or other effective means. There should be separate or defined areas as

follows:

- An area for the receipt, identification, storage, and withholding from use of components, product containers, closures, and labeling, pending the appropriate sampling, testing, or examination by the quality control unit before release for manufacturing or packaging;
- An area for the storage of released components, product containers, closures, and labeling;
- An area for storage of in-process materials;
- An area for manufacturing and processing operations;
- An area for packaging and labeling operations; and
- An area for control and laboratory operations.

Any building used in the manufacture of a dietary ingredient or a dietary supplement shall permit the taking of proper precautions to protect dietary ingredients or dietary supplements in outdoor bulk fermentation vessels by any effective means, including the following:

- Using protective coverings,
- Controlling areas over and around the vessels to eliminate harborage for pests,
- Checking on a regular basis for pests and pest infestation, and
- Skimming the fermentation vessels, as necessary.

Lighting

Adequate lighting shall be provided in all areas and should not expose bulk or finished product to adulteration or contamination. Adequate lighting should be provided in hand-washing areas, dressing and locker rooms, and toilet rooms, and in all areas where product is examined, processed, or stored and where equipment or utensils are cleaned; and such lighting should provide safety-type light bulbs, fixtures, skylights, or other glass suspended over exposed product in any step of preparation or otherwise protect against product adulteration in case of glass breakage.

Ventilation, Air Filtration, Air Heating, and Cooling

Adequate ventilation shall be provided, as well as equipment for adequate control over microorganisms, dust, humidity, and temperature when used in the manufacture of a dietary ingredient and a dietary supplement to minimize odors and vapors (including steam and noxious fumes) in areas where they may adulterate dietary ingredients and dietary supplements; and to locate and operate fans and other air-blowing equipment in a manner that minimizes the potential for adulterating raw materials, in-process or finished dietary ingredients and dietary supplements, or contaminating processing equipment, utensils, or packaging materials.

Plumbing

The plumbing in the physical plant must be of an adequate size and design and be adequately installed and maintained to:

- Carry sufficient amounts of water to the required locations throughout the physical plant;
- Properly convey sewage and liquid disposable waste from the physical plant; and
- Avoid being a source of contamination to components, raw materials, dietary

ingredients, dietary supplements, water supplies, or any contact surface, or creating an unsanitary condition.

Potable water at a suitable temperature, and under pressure as needed, should be supplied in a plumbing system free of defects that could contribute contamination to any dietary ingredients and dietary supplements. Potable water should meet the standards prescribed in the Environmental Protection Agency's Primary Drinking Water Regulations (40 CFR Part 141) or any state or local drinking water requirements that are more stringent. Water not meeting such standards should not be permitted in the potable water system for *Purified Water*. If potable water is to be used as a raw material, it should be further purified to satisfy compendial requirements.

Drains should be of adequate size and, where connected directly to a sewer, should have an air break or other mechanical device to prevent back-siphonage.

Sewage and Refuse

Sewage, trash, and other refuse in and from the building and immediate premises shall be disposed of in a safe and sanitary manner.

Washing and Toilet Facilities

Adequate washing facilities shall be provided, including hot and cold water, soap or detergent, air driers or single-service towels, and clean toilet facilities easily accessible to working areas.

General Maintenance and Sanitation

Any building used in the manufacture of a dietary ingredient, a dietary supplement, or both should be maintained in a clean and sanitary condition and shall be kept in repair sufficient to prevent raw materials and in-process or finished dietary ingredients and dietary supplements from becoming adulterated. It shall be free of infestation by rodents, birds, insects, and other vermin. Trash and organic waste matter shall be held and disposed of in a timely and sanitary manner.

Cleaning compounds and sanitizing agents used in cleaning and sanitizing procedures shall be free from undesirable microorganisms and shall be safe and adequate under the conditions of use. Compliance with this requirement may be verified by any effective means, including purchase of these substances under a supplier's guarantee or certification, or examination of these substances for contamination. Only the following toxic materials may be used or stored in a plant where product is processed or exposed:

- Those required to maintain clean and sanitary conditions,
- Those necessary for use in laboratory testing procedures,
- Those necessary for plant and equipment maintenance and operation, and
- Those necessary for use in the plant's operations.

Written procedures assigning responsibility for sanitation and describing in sufficient detail the cleaning schedules, methods, equipment, and materials to be used in cleaning the building and facilities shall be required.

Toxic cleaning compounds, sanitizing agents, and pesticide chemicals shall be identified, used, held, and stored in a manner that protects against adulteration of raw materials or of in-

process or finished product, or contamination of processing equipment or packaging materials. All relevant regulations promulgated by other federal, state, and local government agencies for the application, use, or holding of these products should be followed.

No pests shall be allowed in any area of a dietary ingredient manufacturing plant and a dietary supplement manufacturing plant. Effective measures shall be taken to exclude pests from the processing areas and to protect against the adulteration by pests of product on the premises. The use of insecticides or rodenticides is permitted only under precautions and restrictions that will protect against the adulteration of raw materials, in-process or finished product, or contamination of processing equipment, utensils, or packaging materials.

Written procedures are also required for use of suitable rodenticides, insecticides, fungicides, fumigating agents, and cleaning and sanitizing agents. These procedures should be designed to prevent the contamination of equipment, raw materials, product containers, closures, packaging, labeling materials, or products. Rodenticides, insecticides, and fungicides should be registered and used in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act. Sanitation procedures shall apply to work performed by contractors or temporary employees as well as work performed by full-time employees during the ordinary course of operations.

EQUIPMENT AND UTENSILS

Equipment and utensils used in the manufacture of dietary ingredients and dietary supplements shall be of appropriate design or selection, adequate size, and suitably located to facilitate operations for its intended use and for its cleaning and maintenance and to ensure that the specifications of dietary ingredients and dietary supplements are correct and are met.

Equipment and utensils include, but are not limited to, the following:

- Equipment used to hold or convey;
- Equipment used to measure;
- Equipment using compressed air or gas;
- Equipment used to carry out processes in closed pipes and vessels; and
- Equipment used in automatic, mechanical, or electronic systems.

Construction

All equipment and utensils shall be:

- Constructed so that surfaces that contact raw materials, in-process materials, or finished products are not reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the product beyond the established requirements;
- Made of nontoxic materials;
- Designed and constructed to withstand the environment in which they are used; the action of raw materials, in-process materials, dietary ingredients, or dietary supplements; and, if applicable, cleaning compounds and sanitizing agents; and
- Maintained to protect raw materials, in-process materials, dietary ingredients, and dietary supplements from being contaminated by any source.

Equipment and utensils must have seams that are smoothly bonded or maintained to minimize the accumulation of dirt, filth, organic material, particles of raw materials, in-process materials, dietary ingredients, or dietary supplements, or any other extraneous materials or contaminants,

to minimize the opportunity for growth of microorganisms.

Each freezer, refrigerator, and cold storage compartment used to hold raw materials, in-process materials, dietary ingredients, or dietary supplements:

- Must be fitted with an indicating thermometer, temperature-measuring device, or temperature-recording device that shows, indicates, and records, or allows for recording by hand, the temperature accurately within the compartment; and
- Must have an automated device for regulating temperature or an automated alarm system to indicate a significant temperature change in a manual operation.

The design, construction, and use of equipment and utensils shall preclude the adulteration of raw materials, packaging materials, in-process materials, or finished product with any substances required for operation, such as:

- Lubricants,
- Fuel,
- Coolants,
- Metal or glass fragments,
- Filth or any other extraneous material,
- Contaminated water, or
- Any other contaminants.

Instruments or controls used in the manufacturing, packaging, labeling, or holding of a dietary ingredient, a dietary supplement, or both; and instruments or controls that are used to measure, regulate, or record temperatures, hydrogen-ion concentration (pH), water activity, or other conditions, and to control or prevent the growth of microorganisms or other contamination must be:

- Accurate and precise,
- Adequately maintained, and
- Adequate in number for their designated uses.

For any automated, mechanical, or electronic equipment that is used to manufacture, package, label, or hold a dietary ingredient, a dietary supplement, or both:

- The suitability of the equipment must be determined by ensuring that the equipment is capable of operating satisfactorily within the operating limits required by the process;
- The equipment must be routinely calibrated, inspected, or checked to ensure proper performance. The quality control unit must approve these calibrations, inspections, or checks;
- The appropriate controls for automated, mechanical, and electronic equipment (including software for a computer-controlled process) must be established and used to ensure that any changes to the manufacturing, packaging, labeling, holding, or other operations are approved by the quality control unit and instituted only by authorized personnel; and
- The appropriate controls must be established and used to ensure that the equipment functions in accordance with its intended use. These controls must be approved by the quality control unit.

Compressed air or other gases introduced mechanically into or onto raw materials, in-process materials, dietary ingredients, dietary supplements, or contact surfaces, or that are used to clean any contact surface, must be treated in such a way that the raw material, in-process

material, dietary ingredient, dietary supplement, or contact surface is not contaminated.

Cleaning and Maintenance

Equipment and utensils shall be cleaned, maintained, and sanitized at adequate intervals, between the manufacture of different batches of the same product and between the manufacture of different products, to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the product beyond the established requirements.

In wet processing during manufacturing, all contact surfaces must be cleaned and sanitized, as necessary, to protect against the introduction of microorganisms into components, dietary ingredients, or dietary supplements. When cleaning and sanitizing are necessary, all contact surfaces must be cleaned and sanitized before use and after any interruption during which the contact surface may have become contaminated.

In a continuous production operation or in back-to-back consecutive operations, which involve different batches of the same dietary ingredient or dietary supplement, the contact surfaces must be adequately cleaned and sanitized.

The surfaces that do not come into direct contact with raw materials, in-process materials, dietary ingredients, or dietary supplements must be cleaned as frequently as necessary to protect against contaminating raw materials, in-process materials, dietary ingredients, or dietary supplements.

Single-service articles (such as utensils intended for one-time use, paper cups, and paper towels) must be:

- Stored in appropriate containers; and
- Handled, dispensed, used, and disposed of in a manner that protects against contamination of raw materials, in-process materials, dietary ingredients, dietary supplements, or any contact surface.

Cleaning compounds and sanitizing agents must be adequate for their intended use and safe under their conditions of use.

The portable equipment and utensils that have contact surfaces must be cleaned, sanitized, and then stored in a location and manner that protects them from contamination.

Written procedures for cleaning and maintaining equipment, including utensils, used in the manufacture of a product should be established and followed. These procedures should include, but are not necessarily limited to, the following:

- Assignment of responsibility for cleaning and maintaining equipment;
- Maintenance and cleaning schedules, including, where adequate, sanitizing schedules;
- A description in sufficient detail of the methods, equipment, and materials used in cleaning and maintenance operations, and the methods of disassembling and reassembling equipment, as necessary, to ensure proper cleaning and maintenance;
- Removal or obliteration of previous batch identification;
- Identification and protection of clean equipment from contamination before use;
- Inspection of equipment for cleanliness immediately before use;
- Regular calibration and inspection of equipment, or checking machines, to ensure proper performance and function must be conducted:
 - Before first use, and
 - At a frequency specified in writing by supporting references.
- Instruments or controls that cannot be adjusted to agree with the reference standard

must be repaired or replaced.

A written record of calibration, inspection, maintenance of equipment, and major equipment cleaning and use shall be maintained in individual equipment logs that show the date, product, and lot number of each batch processed. The persons performing the cleaning shall record in the log that the work was performed. Entries in the log should be in chronological order.

The following is specified to keep records related to automated or electric equipment:

- There must be backup file(s) of current software programs (and of outdated software that is necessary to retrieve records that are required to be retained, in accordance with the section *Records and Reports* in this chapter, when current software is not able to retrieve such records) and of data entered into computer systems used to manufacture, package, label, or hold dietary supplements.
 - A backup file (e.g., a hard copy of data entered, diskettes, tapes, microfilm, or compact disks) must be an exact and complete record of the data entered.
 - Backup software programs and data must be kept secure from alterations, inadvertent erasures, or loss.

Change to read:

RAW MATERIALS, PRODUCT CONTAINERS, AND CLOSURES

Written procedures describing in sufficient detail the receipt, identification, storage, handling, sampling, testing, and approval or rejection of raw materials, product containers, and closures should be provided.

Raw materials, product containers, and closures at all times should be handled and stored in a manner to prevent contamination.

Raw agricultural materials that contain soil or other contaminants shall be washed or cleaned as necessary. Water used for washing, rinsing, or conveying raw agricultural materials shall be safe and of adequate sanitary quality. Notwithstanding the general requirement for potable water, water may be reused for washing, rinsing, or conveying raw agricultural materials if it does not increase the level of contamination of such materials.

Bagged or boxed raw materials of product containers or closures should be stored off the floor and suitably spaced to permit cleaning and inspection.

Each lot should be appropriately identified as to its status (i.e., quarantined, approved, or rejected).

Receipt and Storage of Untested Raw Materials, Product Containers, and Closures

Written procedures shall be established and followed describing the receipt, identification, examination, handling, and sampling of raw materials. Upon receipt and before acceptance, each container or grouping of containers of raw materials, product containers, and closures should be examined visually for appropriate labeling as to contents, container damage, or broken seals, and for contamination. They are then stored under quarantine until they have been tested or examined, as appropriate, and released.

Raw materials shall be held in bulk, or in containers designed and constructed so as to protect against adulteration, and shall be held at such temperature and relative humidity and in such a

manner as to prevent a dietary ingredient or dietary supplement from becoming adulterated. Frozen raw materials and other ingredients shall be kept frozen. If thawing is required before use, it shall be done in a manner that prevents the raw materials and other ingredients from becoming adulterated within the meaning of the Act.

Testing and Approval or Rejection

Each lot of raw materials, product containers, and closures should be sampled, tested, or examined, as appropriate, and released for use by the quality control unit. On the basis of adequate process verification, in-process controls, and statistical confidence, a skip-lot testing plan is an alternative to fully testing every batch provided that at least one identity test is conducted. An appropriate amount of each lot of raw materials should be reserved for ~~3 years~~

■ 1 year ■ 2S (USP39)

beyond the shelf life appearing on the label of finished dietary supplements in which the raw materials were used. If adverse event reports are received (see the subsection *Adverse Event Reports*), the reserved raw materials should be kept for 6 years (serious events) or 3 years (nonserious events) from the date the first report is received.

Representative samples should be collected for testing or examination. Sampling of botanicals should be in compliance with the provisions set in *Articles of Botanical Origin* (561). The number of containers sampled, and the amount of material taken from each container, should be based upon appropriate criteria such as statistical criteria for raw material variability, confidence levels, and degree of precision desired, the past quality history of the supplier, and the quantity needed for analysis and reserve where required. The following procedures should be used to collect the samples:

- The containers of raw materials selected should be cleaned, where necessary, by adequate means.
- The containers should be opened, sampled, and resealed in a manner designed to prevent contamination of their contents and contamination of other raw materials, product containers, or closures.
- These containers should be identified so that the following information can be determined: name of the material sampled, the lot number, the container from which the sample was taken, the date on which the sample was taken, and the name of the person who collected the sample.

Use the following procedure to examine and test the samples:

- At least one test should be conducted to verify the identity of each raw material of a product even in cases where skip-lot testing is used. Such tests may include any appropriate test with established sufficient specificity to determine identity, including chemical and laboratory tests, gross organoleptic analysis, microscopic identification, or analysis of constituent markers.
- Each raw material should be tested for conformity with all appropriate written specifications for purity, strength, and quality. However, a report of analysis may be accepted from the supplier of a raw material, provided that the manufacturer establishes the reliability of the supplier's analyses and provided that at least one identity test is conducted on such raw material by the manufacturer.
- Containers and closures should be tested for conformance with all appropriate written procedures. However, a certificate of testing may be accepted from the supplier, provided that at least a visual identification is conducted on such containers or closures

by the manufacturer.

- Each lot of a raw material, rework, product container, or closure that is liable to contamination with filth, insect infestation, or other extraneous adulterant should be examined against established specifications for such contamination and shall comply with any applicable Food and Drug Administration regulations and guidelines. Skip-lot examination should not apply in such cases.
- Each lot of a raw material that is liable to microbiological contamination that is objectionable in view of its intended use shall be subjected to microbiological tests before use. Raw materials either shall not contain levels of microorganisms that may produce food poisoning or other disease in humans, or shall be otherwise treated during manufacturing operations so that they no longer contain levels that would cause the product to be adulterated within the meaning of the Act. In lieu of such testing by the manufacturer, a guarantee or certification of analysis may be accepted from the supplier of a component provided that the manufacturer establishes the reliability of the supplier's analysis.
- Raw materials and other ingredients susceptible to adulteration with aflatoxin, other natural toxins, pesticides, or heavy metals shall comply with current Food and Drug Administration regulations, guidelines, and action levels for poisonous or deleterious substances and the requirements in (561), or in each monograph, before these materials or ingredients are incorporated into a finished dietary ingredient or dietary supplement. Compliance with this requirement may be accomplished by analyzing these materials and ingredients for aflatoxins and other natural toxins; or, in lieu of such testing by the manufacturer, a guarantee or certification of analysis may be accepted from the supplier of a raw material provided that the manufacturer establishes the reliability of the supplier's analysis.
- Any lot of raw material, product container, or closure that meets the appropriate written specifications of identity, strength, quality, and purity and related tests may be approved and released for use. Any lot of such material that does not meet such specifications should be rejected.

Use of Approved Raw Materials, Product Containers, and Closures

Raw materials, product containers, and closures approved for use should be rotated so that the oldest approved stock is used first. Deviation from the requirement is permitted if such deviation is temporary and adequate.

Retesting of Approved Raw Materials, Product Containers, and Closures

Raw materials, product containers, and closures should be retested or reexamined, as appropriate, for identity, strength, quality, and purity and approved or rejected by the quality control unit after a specified time in storage or as necessary, e.g., after exposure to air, heat, or other conditions that might adversely affect the raw material, product container, or closure or after storage of active and inactive ingredients and in-process materials for long periods of time.

Rejected Raw Materials, Product Containers, and Closures

Rejected raw materials, product containers, and closures should be identified and controlled

under a quarantine system that prevents their use in manufacturing or processing operations for which they are unsuitable.

PRODUCTION AND PROCESS CONTROLS

Written Procedures

Written procedures should be provided for production and process controls designed to ensure that the dietary ingredients and dietary supplements have the identity, strength, quality, and purity that they are represented to possess. These procedures should be drafted, reviewed, and approved by the appropriate organizational units and reviewed and approved by the quality control unit. These production and process control procedures should be followed in the execution of the various production and process control functions and should be documented at the time of performance. Any deviation from the written procedures should be recorded and justified.

- All operations in the receiving, inspecting, transporting, segregating, preparing, manufacturing, packaging, and storing of dietary ingredients and dietary supplements shall be conducted in accordance with adequate sanitation principles.
- All reasonable precautions shall be taken to ensure that production procedures do not contribute adulteration from any source. Chemical, microbial, or extraneous-material testing procedures shall be used where necessary to identify sanitation failures or possible product adulteration.
- All product that has become contaminated to the extent that it is adulterated within the meaning of the Act shall be rejected, or if permissible, treated or processed to eliminate the contamination.
- All product manufacturing, including packaging and storage, shall be conducted under such conditions and controls as are necessary to minimize the potential for the growth of microorganisms, or for the adulteration of raw materials, in-process materials, and finished product.
- Measures taken to destroy microorganisms, reduce the microbial load, or prevent the growth of undesirable microorganisms, particularly those of public health significance, shall be adequate under the conditions of manufacture, handling, and distribution to prevent dietary supplements and ingredients from being adulterated within the meaning of the Act. These measures shall also comply with current regulations affecting dietary supplement products and ingredients.
- Work-in-process shall be handled in a manner that protects against adulteration.
- In-process material must be held under appropriate conditions of temperature, humidity, and light.
- Effective measures shall be taken to protect finished dietary ingredients and dietary supplements from adulteration by raw materials, in-process materials, or refuse. When raw materials, in-process materials, or refuse are unprotected, they shall not be handled simultaneously in a receiving, loading, or shipping area if that handling could result in adulterated dietary ingredients and dietary supplements. Dietary ingredients and dietary supplements transported by conveyor shall be protected against adulteration as necessary.
- Effective measures shall be taken as necessary to protect against the inclusion of metal or other extraneous material in product. Compliance with this requirement may be

accomplished by using sieves, traps, magnets, electronic metal detectors, or other suitable effective means.

- Mechanical manufacturing steps such as cutting, sorting, inspecting, shredding, drying, grinding, blending, and sifting shall be performed so as to protect dietary ingredients and dietary supplements against adulteration. Compliance with this requirement may be accomplished by providing adequate physical protection of dietary ingredients and dietary supplements from contact with adulterants. Protection may be provided by adequate cleaning and sanitizing of all processing equipment between each manufacturing step.
- Heat blanching, when required in the preparation of a dietary ingredient or a dietary supplement, should be effected by heating the product to the required temperature, holding it at this temperature for the required time, and then either rapidly cooling the material or passing it to subsequent manufacturing without delay. Thermophilic growth and contamination in blanchers should be minimized by the use of adequate operating temperatures and by periodic cleaning. Where the blanched product is washed before filling, potable water shall be used.
- Intermediate of dehydrated dietary ingredients and dietary supplements that rely on the control of water (a_w) for preventing the growth of undesirable microorganisms shall be processed to and maintained at a safe moisture level. Compliance with this requirement may be accomplished by any effective means, including employment of one or more of the following practices:
 - Monitoring the water activity (a_w) of the material;
 - Controlling the soluble solids–water ratio in finished product; and
 - Protecting finished product from moisture pickup, by use of a moisture barrier or by other means, so that the water activity (a_w) of the product does not increase to an unsafe level.
- Dietary ingredients and dietary supplements that rely principally on the control of pH for preventing the growth of undesirable microorganisms shall be monitored and maintained at an appropriate pH. Compliance with this requirement may be accomplished by any effective means, including employment of one or more of the following practices:
 - Monitoring the pH and water activity, if appropriate, of raw materials, in-process material, and finished product; and
 - Controlling the amount of acid added to the product.
- When ice is used in contact with dietary ingredients and dietary supplements, it shall be made from potable water, and shall be used only if it has been manufactured in accordance with current good manufacturing practice in manufacturing, packing, or holding human food as outlined in 21 CFR Part 110.

Charge-In of Raw Materials

Written production and control procedures should include the following, which are designed to ensure that the dietary supplements have the identity, strength, quality, and purity that they are represented to possess:

- The batch should be formulated with the intent to provide NLT 100% of the labeled or established amount of dietary ingredient.
- Raw materials for product manufacturing should be weighed, measured, or subdivided as appropriate and the appropriate signatures recorded in the batch record.
- Actual yields and percentages of theoretical yield should be determined at appropriate

phases of processing.

Material scheduled for rework shall be identified as such.

Equipment Identification

All compounding and storage containers, processing lines, and major equipment used during the production of a batch of a product should be properly identified to indicate their contents and, when necessary, the phase of processing of the batch.

Sampling and Testing of In-Process Materials, Dietary Ingredients, and Dietary Supplements

To ensure batch uniformity and integrity of dietary supplements, written procedures should be established and followed that describe the in-process controls and tests or examinations to be conducted on appropriate samples of in-process materials. On the basis of process verification, in-process controls, and statistical confidence, a skip-lot testing plan is an alternative to testing every batch of finished products provided that at least one representative measure is performed. Control procedures should be established to monitor the output of those manufacturing processes that may be responsible for causing variability in the characteristics of in-process material and the finished product. Such control procedures may include, but are not limited to, the following, where appropriate:

- Friability
- Weight variation
- Disintegration time
- Dissolution time
- Clarity, completeness, or pH of solutions
- Blend uniformity

In-process specifications for such characteristics should be consistent with finished product specifications. Examination and testing of samples should ensure that the in-process material and dietary supplement conform to the established specifications.

In-process materials should be tested for identity, strength, quality, and purity as adequate, and approved or rejected by the quality control unit during the production process, e.g., at commencement or completion of significant phases or after storage for long periods.

Rejected or adulterated in-process materials should be identified and controlled under a quarantine system designed to prevent their use in manufacturing or processing operations for which they are unsuitable and to prevent the adulteration of other products.

LABELING AND PACKAGING

Materials Examination and Usage Criteria

Written procedures should be provided describing in sufficient detail the receipt, identification, storage, handling, sampling, examination, testing of labeling and packaging materials, or products received for packaging or labeling. Each immediate container or grouping of immediate containers in a shipment of product received for packaging or labeling, or of packaging and labeling materials, must be visually examined for appropriate content label, container damage,

or broken seals to determine whether the container condition may have resulted in contamination or deterioration of the received product. The supplier's invoice, guarantee, or certification in a shipment of the received product must be visually examined to ensure that the received product is consistent with the purchase order. Labeling and packaging materials or products received for packaging or labeling should be quarantined until:

- Representative samples of each unique shipment, and of each unique lot within each unique shipment, of received product for packaging or labeling, or of packaging and labeling materials, are collected;
- The quality control unit reviews and approves the documentation to determine whether the received product for packaging or labeling, or packaging and labeling materials, meets the specifications; and
- The quality control unit approves the received product for packaging or labeling, or packaging and labeling materials, and releases for use from quarantine.

Those that do not meet such specifications should be identified and rejected to prevent their use in operations for which they are unsuitable.

A record should be kept of each shipment received of each different labeling and packaging material, or each different received product for packaging or labeling, indicating receipt, date of examination or testing, and whether accepted or rejected.

Each unique lot within each unique shipment of received product for packaging or labeling, or of packaging and labeling materials, must be identified in a manner that allows the recipient to trace the lot to the supplier, the date received, the name of the received product, the status of the received product (e.g., quarantined, approved, or rejected), and to the product that was packaged or labeled and distributed.

This unique identifier must be used whenever the disposition of each unique lot within each unique shipment of the received product for packaging or labeling, or of packaging and labeling materials, is recorded.

Labels and other labeling materials for each different product, strength, product type, or quantity of contents should be stored separately under conditions that will protect against contamination and deterioration and avoid mixups. Only authorized personnel should have access to the storage area.

Packaging and labels must be held under appropriate conditions so that the packaging and labels are not adversely affected (e.g., contamination or deterioration).

Gang printing of labeling to be used for different products or different strengths of the same product (or labeling of the same size and identical or similar format or color schemes) should be minimized. If gang printing is employed, packaging and labeling operations should provide for special control procedures, taking into consideration sheet layout, stacking, cutting, and handling during and after printing.

Printing devices on, or associated with, manufacturing lines used to imprint labeling upon the product unit label or case should be monitored to ensure that all imprinting conforms to the print specified in the batch production record.

Obsolete and outdated labels, labeling, other packaging materials, and products received for packaging or labeling should be destroyed and documented.

Labeling Issuance

Strict control should be exercised over labeling issued for use in product labeling operations. The control procedures employed should be in writing with sufficient detail.

Labeling materials issued for a batch should be carefully examined for identity and conformity to the labeling specified in the master and batch production records.

Procedures should be used to reconcile the quantities of labeling issued, used, and returned, and should require evaluation of discrepancies found. If discrepancies are found between the quantity of product finished and the quantity of labeling issued and are outside preset limits based on historical operating data, such discrepancies should be investigated.

Returned labeling should be maintained and sorted in a manner to prevent mixups and provide proper identification.

All excess labeling bearing lot or control numbers should be destroyed and documented.

Operations

Written procedures designed to ensure that correct labels, labeling, and packaging materials are used for dietary supplements should incorporate the following features:

- Prevention of mixups and cross-contamination by physical or spatial separation from operations on other products;
- Identification of the product with a lot or control number;
- Examination of packaging and labeling materials for suitability and correctness before packaging operations, and documentation of such examination in the batch production record; and
- Inspection of the packaging and labeling facilities immediately before use to ensure that all products have been removed from previous operations. Inspection should also be made to ensure that packaging and labeling materials not suitable for subsequent operations have been removed. Results of the inspection should be documented in the batch production records.

Relabeling and Repackaging

- Dietary ingredients and dietary supplements may be repackaged or relabeled only after the quality control unit has approved such repackaging or relabeling.
- A representative sample of each batch of repackaged or relabeled dietary ingredients and dietary supplements must be examined to determine whether the repackaged or relabeled dietary ingredients and dietary supplements meet all established specifications.
- The quality control unit must approve or reject each batch of repackaged or relabeled dietary ingredients and dietary supplements before its release for distribution.

Tamper-Resistant Packaging

REQUIREMENTS

Each manufacturer and packer who packages a dietary supplement for retail sale shall package the product in a tamper-resistant package, if this product is accessible to the public while held for sale. A tamper-resistant package is one having an indicator or barrier to entry which, if breached or missing, can reasonably be expected to provide visible evidence to consumers that tampering has occurred. To reduce the likelihood of substitution of a tamper-resistant feature after tampering, the indicator or barrier to entry is required to be distinctive by design or by the use of an identifying characteristic (e.g., a pattern, name, registered trademark, logo, or picture). For purposes of this section, the term "distinctive by design" means that the packaging cannot be duplicated with commonly available materials or through commonly

available processes. A tamper-resistant package may involve an immediate-container and closure system, or secondary-container or carton system, or any combination of systems intended to provide a visual indication of package integrity. The tamper-resistant feature should be designed to remain intact when handled in a reasonable manner during manufacture, distribution, and retail display.

LABELING

Each retail package of a dietary supplement covered by this section shall bear a statement that is prominently placed so that consumers are alerted to the specific tamper-resistant feature of the package. The labeling statement should be so placed that it will be unaffected if the tamper-resistant feature of the packaging is breached or missing. If the tamper-resistant feature chosen to meet the requirement above is one that uses an identifying characteristic, that characteristic should be referred to in the labeling statement. For example, the labeling statement on a bottle with a shrink band could say "For your protection, this bottle has an imprinted seal around the neck."

Dietary Supplement Examination

Packaged and labeled products should be examined during finishing operations to ensure that containers and packages in the lot have the correct label. A representative sample of units should be collected at the completion of finishing operations and visually examined for correct labeling. Results of these examinations should be recorded in the batch production or control records.

Contact Information

The manufacturer, packer, or distributor of dietary supplements is required to comply with the current labeling requirements in the law that also include a domestic address or phone number through which an adverse event report for a dietary supplement may be received.

Shelf Life

Dietary supplements should bear a date indicative of their shelf life, determined by appropriate testing, to ensure that they meet applicable standards of identity, strength, quality, and purity at or before the labeled shelf-life date.

Shelf life should be related to any storage conditions stated on the labeling.

HOLDING AND DISTRIBUTION

Warehousing Procedures

Storage and transportation of the finished product shall be under conditions that will protect the product against physical, chemical, and microbial adulteration as well as against deterioration of the product and the container.

Written procedures describing the warehousing of dietary supplements should be established and followed and should include the following:

- Quarantine of finished products before disposition by the quality control unit; and

- Storage of finished products under appropriate conditions of temperature, humidity, and light so that the identity, strength, quality, and purity of the products are not affected.

Distribution Procedures

Written procedures describing the distribution of dietary supplements shall be established and followed and should include the following:

- A procedure whereby the oldest approved stock of a product is distributed first. (Deviation from this requirement is permitted if such deviation is temporary and adequate.)
- A system by which the distribution of each lot of product can be readily determined to facilitate its recall if necessary.

QUALITY CONTROL OPERATIONS

The establishment of any specifications, standards, sampling plans, test procedures, or other laboratory control mechanisms required by this chapter, including any change in such specifications, standards, sampling plans, test procedures, or other laboratory control mechanisms, shall be drafted by the appropriate organizational unit and reviewed and approved by the quality control unit. The requirements in this section should be followed and documented at the time of performance. Any deviation from the written specifications, standards, sampling plans, test procedures, or other laboratory control mechanisms shall be recorded and justified. Quality control operations include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to ensure that raw materials, product containers, closures, in-process materials, labeling, products received for labeling and packaging operations as dietary supplements, and finished products conform to adequate standards of identity, strength, quality, and purity. These controls include the following:

- Determination of conformance to appropriate written specifications for the acceptance of each lot within each shipment of raw materials, product containers, closures, and labeling used in the manufacture of dietary ingredients and dietary supplements, and of products received for labeling and packaging operations as dietary supplements. (The specifications include a description of the sampling and testing procedures used. Samples should be representative and adequately identified. Such procedures also require appropriate retesting of any raw material, product container, or closure that is subject to deterioration.) On the basis of adequate process verification, in-process controls, and statistical confidence, a skip-lot testing plan is an alternative to testing every batch, excluding raw materials, which require 100% identity testing.
- Determination of conformance to written specifications and a description of sampling and testing procedures for in-process materials. (Such samples should be representative and properly identified.)
- Determination of conformance to written descriptions of sampling procedures and appropriate specifications for finished products. (Such samples should be representative and properly identified.)
- The calibration of instruments, at suitable intervals, in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event that accuracy and/or precision

limits are not met. Instruments not meeting established specifications shall not be used until repaired.

Testing and Release for Distribution

There should be appropriate laboratory determination of satisfactory conformance to specifications for the finished product, including the identity and strength before release. On the basis of adequate process verification, in-process controls, or statistical confidence, a skip-lot or composite testing plan is an alternative to testing every batch.

There should be appropriate laboratory testing, as necessary, of each batch of dietary supplement required to be free of objectionable microorganisms. The accuracy, linearity, sensitivity, specificity, and precision of test methods employed by the firm, when they differ from compendial methods, should be established and documented.

Written procedures should describe any sampling and testing plans, which should include the method of sampling and the number of units per batch to be tested.

Products failing to meet established standards or specifications and any other relevant quality control criteria should be rejected. Rejected or adulterated dietary ingredients and dietary supplements shall be identified, stored, and disposed of in a manner that protects against the adulteration of the other products. Reprocessing may be performed. Before acceptance and use, reprocessed material must meet established standards, specifications, and any other relevant criteria. Written procedures shall be established and followed prescribing the method for reprocessing batches or operations start-up materials that do not conform to finished goods standards or specifications. Finished goods manufactured using such materials shall meet all established purity, composition, and quality standards.

Stability Testing

There should be a written protocol designed to assess the stability characteristics of dietary supplements. The results of such testing should be used in determining appropriate storage conditions and shelf life. This protocol should include the following:

- Sample size and test intervals based on statistical criteria for each attribute should be examined to ensure valid estimates of stability;
- Storage conditions for samples retained for testing;
- Reliable, meaningful, and specific test methods should be used; and
- The dietary supplement should be tested in the same type of container-closure system as that in which the dietary supplement is marketed.

An adequate number of batches of each dietary supplement should be tested to determine an adequate shelf life, and a record of these data should be maintained. Accelerated studies combined with basic stability information on the raw materials, dietary supplements, and container-closure systems may be used to support tentative shelf life if full shelf-life studies are not available. Simplified stability testing procedures may be used where data from similar product formulations are available to support a shelf-life estimation of a new product. Where data from accelerated studies are used to project a tentative shelf-life date that is beyond a date supported by actual shelf-life studies, stability studies should be conducted, including dietary supplement testing at appropriate intervals, until the tentative shelf life is verified or the adequate shelf life is determined.

Reserve Samples

An appropriately identified reserve sample that is representative of each lot or batch of dietary supplement should be retained and stored under conditions consistent with product labeling until at least 3 years after the shelf life of the product. The reserve sample should be stored in the same immediate container–closure system in which the finished product is marketed or in one that has essentially the same characteristics. The reserve sample consists of at least twice the quantity necessary to perform all of the required tests. If an adverse event report is received, the reserve samples of dietary supplements and dietary ingredients from the same lot or batch must be analyzed by an appropriate procedure to confirm their identity and determine any adulteration or contamination. The recovered samples associated with adverse event reports from consumers, distributors, or both should also be analyzed, following the same method used for the reserved samples, if available. The results should be reported with other required information to the federal authority, using the required form. The reserve samples from a particular lot or batch associated with an adverse event report should be held for 6 years (serious events) or 3 years (nonserious events) from the date when the first adverse event report is received by the manufacturer, packer, or distributor.

Change to read:

RECORDS AND REPORTS

Any record for production, control, quality control operations, or distribution that is required to be maintained and is specifically associated with a batch of a product should be retained for at least 3 years after the shelf life of the batch.

Records should be maintained for all raw materials, product containers, closures, and labeling for at least 3 years after the shelf life of the last lot of product incorporating the raw material or using the container, closure, or labeling.

Master Production and Control Records

To ensure uniformity from batch to batch, master production and control records for each product should be prepared, dated, and signed by one person and independently checked, dated, and signed by a second person from the quality control unit.

Master production and control records should include the following:

- The name and strength of the product;
- The name and weight or measure of each dietary ingredient per unit or portion or per unit of weight or measure of the product, and a statement of the total weight or measure of any dosage unit;
- A complete list of raw materials designated by names or codes sufficiently specific to indicate any special quality characteristic;
- An accurate statement of the weight or measure of each raw material, using the same weight system (metric, avoirdupois, or apothecary) for each raw material;
- A statement concerning any calculated excess of raw material;
- A statement of theoretical weight or measure at appropriate phases of processing;
- A statement of theoretical yield, including the maximum and minimum percentages of theoretical yield beyond which investigation is required;

- A description of the product containers, closures, and packaging materials, including a specimen or copy of each label and all other labeling signed and dated by the person or persons responsible for approval of such labeling or, in lieu of specimens or copies of each label or other labeling, a positive identification of all labeling used; and
- Complete manufacturing and control instructions, testing procedures, acceptance limits, special notations, and precautions to be followed.
- Specific actions necessary to perform and verify points, steps, or stages in the manufacturing process where control is necessary to ensure the quality of dietary ingredients and dietary supplements, and to ensure that dietary ingredients and dietary supplements are packaged and labeled as specified in the master production record.
 - Such specific actions must include verifying the weight or measure of any component and verifying the addition of any component; and
 - For manual operations, such specific actions must include:
 - One person weighing or measuring a component and another person verifying the weight or measure; and
 - One person adding the component and another person verifying the addition.
- Corrective action plans for use when a specification is not met.

Batch Production and Control Records

Batch production and control records should be prepared for each batch of product produced and should include complete information relating to the production and control of each batch. These records should be reviewed and signed by a second person from the quality control unit. These records should include accurate reproduction of the appropriate master production or control record and documentation that each significant step in the manufacture, processing, packing, or holding of the batch was accomplished, including the following:

- Dates;
- Identity of individual major equipment and lines used;
- Specific identification of each batch of raw material or in-process material used;
- Weights and measures of raw materials used in the course of processing;
- In-process and laboratory control results;
- Inspection of the packaging and labeling areas before and after use;
- A statement of the actual yield and a statement of the percentage of theoretical yield at appropriate phases of processing;
- Description of product containers and closures used;
- Complete labeling control records, including:
 - The unique identifier assigned to packaging and labels used, the quantity of the packaging and labels used, and, when label reconciliation is required, reconciliation of any discrepancies between issuance and use of labels; and
 - An actual or representative label, or a cross-reference to the physical location of the actual or representative label specified in the master manufacturing record;
- Any sampling performed;
- Identification of the persons performing and directly supervising or checking any step in the operation;
- Any investigation made;
- The results of any tests or examinations conducted on packaged and labeled dietary

- supplements (including repackaged or relabeled dietary supplements), or a cross-reference to the physical location of such results;
- Documentation at the time of performance that quality control unit:
 - Reviewed the batch production record, including:
 - Review of any required monitoring operation, and
 - Review of the results of any tests and examinations, including tests and examinations conducted on components, in-process materials, finished batches of dietary supplements, and packaged and labeled dietary ingredients and dietary supplements;
 - Approved or rejected any reprocessing or repackaging;
 - Approved and released, or rejected, the batch for distribution, including any reprocessed batch; and
 - Approved and released, or rejected, the packaged and labeled dietary supplement, including any repackaged or relabeled dietary supplement;
 - Documentation at the time of performance of any required material review and disposition decision; and
 - Documentation at the time of performance of any reprocessing.

Records for Raw Materials, Packaging, and Labels and for Product Received for Packaging or Labeling as a Dietary Supplement

The following records must be made and retained:

- Written procedures for fulfilling the requirements for raw materials, packaging, and labels and for product received for packaging or labeling;
- Receiving records (including records such as certificates of analysis, suppliers' invoices, and suppliers' guarantees) for components, packaging, and labels and for products received for packaging or labeling; and
- Documentation that the requirements of *Raw Materials, Product Containers, and Closures* were met:
 - The person who performs the required operation must document, at the time of performance, that the required operation was performed; and
 - The documentation must include:
 - The date of receipt of the raw materials, packaging, labels, or products received for packaging or labeling as a dietary supplement;
 - The initials of the person performing the required operation;
 - The results of any tests or examinations conducted on raw materials, packaging, or labels, and of any visual examination of product received for packaging or labeling as a dietary supplement; and
 - Any material review and disposition decision conducted on raw materials, packaging, labels, or products received for packaging or labeling as a dietary supplement.

Laboratory Records

Laboratory records should include complete data derived from all tests necessary to ensure compliance with established specifications and standards, including examinations and assays, as follows:

- A description of the sample received for testing with identification of source (that is,

location from where sample was obtained), quantity, lot number or other distinctive code, and date sample was taken.

- A statement of each method used in the testing of the sample.
- A statement of the weight or measure of sample used for each test, where appropriate.
- A complete record of all data secured in the course of each test, including all graphs, charts, and spectra from laboratory instrumentation, properly identified to show the specific raw material, product container, closure, in-process material, or finished product, and lot tested.
- A record of all calculations performed in connection with the test, including units of measure, conversion factors, and equivalency factors.
- A statement of the results of tests and how the results compare with established standards of identity, strength, quality, and purity for the raw material, product container, closure, in-process material, or finished product tested.
- The initials or signature of the person who performs each test and the date(s) the tests were performed.

Complete records should be maintained of any modification of an established method employed in testing. Such records should include the reason for the modification and data to verify that the modification produced results that are at least as accurate and reliable for the material being tested as the established method.

Complete records should be maintained of any testing and standardization of laboratory reference standards, reagents, and standard solutions, the periodic calibration of laboratory instruments, and all stability testing should be performed. Any deviation should be reviewed and signed by the management of the quality control unit.

Quality Control Operation Records

The following records must be made and retained:

- Written procedures for the responsibilities of the quality control operations, including written procedures for conducting a material review and making a disposition decision and written procedures for approving or rejecting any reprocessing;
- Written documentation, at the time of performance, that quality control unit performed the review, approval, or rejection requirements, by recording the following:
 - Date on which the review, approval, or rejection was performed; and
 - Signature of the person performing the review, approval, or rejection; and
- Documentation of any material review and disposition decision and follow-up. Such documentation must be included in the appropriate batch production record and must include:
 - Description of the investigation into the cause of the deviation from the specification or the unanticipated occurrence;
 - Evaluation of whether the deviation or unanticipated occurrence has resulted in, or could lead to, a failure to ensure the quality of the dietary supplement or a failure to package and label the dietary supplement as specified in the master manufacturing record;
 - Identification of the action(s) taken to correct, and prevent a recurrence of, the deviation or the unanticipated occurrence;
 - Explanation of the actions taken with the raw material, dietary supplement, packaging, or label;

- A scientifically valid reason for any reprocessing of a dietary supplement that is rejected or any treatment or in-process adjustment of a component that is rejected; and
- The signatures of 1) the individual(s) designated to perform the quality control operation, who have conducted the material review and made the disposition decision; and in addition, 2) each qualified individual who has provided information relevant to that material review and disposition decision.

Distribution Records

Distribution records should contain the name and strength of the product, name and address of the consignee, date and quantity shipped, and lot or control number of the finished product.

Record Keeping

The manufacturer, packer, or distributor of dietary supplements must keep all required records, as shown in this chapter, for ~~3 years~~

■ 1 year ■ 2S (USP39)

beyond the shelf life of dietary supplements associated with those records. If adverse event reports are received, those records must be kept for additional 6 years

■ (serious events) or for 3 year (nonserious events) ■ 2S (USP39)

from the date when the first report is received. All records must be accessible by the regulatory authority when requested.

Records must be kept as original records, as true copies (such as photocopies, microfilm, microfiche, or other accurate reproductions of the original records), or as electronic records. All electronic records must comply with part 11 of Code of Federal Regulations Title 21 (21 CFR Part 11).

If reduction techniques are used, such as microfilming, suitable reader and photocopying equipment must be readily available to auditors and inspectors.

Complaint Files

Written procedures describing the handling of all written and oral complaints regarding a dietary supplement shall be established and followed. These procedures should include provisions for review by the quality control unit of any complaint involving the possible failure of a product to meet any of its specifications and a determination as to the need for an investigation. Each complaint should be recorded in a file designed especially for dietary supplement complaints. Written records should be maintained until at least 3 years after the shelf life of the product, or 3 years after the date when the complaint was received, whichever is longer. The written record should include the following information, where known: the name and strength of the product, lot number, name of complainant, nature of complaint, and reply to complainant.

If an investigation is necessary, the written record should include the findings of the investigation and follow-up.

The review and investigation of the product complaint by a qualified person, the review by quality control unit about whether to investigate a product complaint, and the findings and follow-up action of any investigation performed must extend to all relevant batches and records.

Adverse Event Reports

Adverse event reports include reports on any health-related adverse event associated with the use of a dietary supplement that is adverse. It includes both nonserious and serious adverse event reports.

The manufacturer, packer, or distributor of a dietary supplement (called the "responsible person") whose name appears on the label shall be responsible for keeping reports of all nonserious adverse events along with any related records (e.g., records of communications with the person who reported the nonserious event). All such records of nonserious adverse events should be kept for 6 years.

The responsible person whose name appears on the label shall also be responsible for reporting any serious adverse event reported to it, and associated with a dietary supplement that is marketed and used in the same country, to the regulatory authority as soon as appropriate, but NLT 15 business days after receipt of the report, using the appropriate form as defined by the regulation

(<http://www.fda.gov/food/dietarysupplements/reportadverseevent/ucm111110.htm>). A serious adverse event is an event that results in any of following:

- Death,
- A life-threatening experience,
- Inpatient hospitalization,
- A persistent or significant disability or inability,
- A congenital anomaly or birth defect, or
- A condition that requires, according to reasonable medical judgment, a medical or surgical intervention to prevent one of the five outcomes listed above.

A retailer whose name appears on the label as a distributor may, by agreement, authorize the manufacturer or packer to submit the required reports to the regulatory authority, as long as the retailer directs all received adverse event reports to the manufacturer or packer. Each serious adverse event report should include a copy of the product's label, the information described in the preceding section *Complaint Files*, and if possible, the contact information of the complainant; daily intake; alcohol consumption and amount; use of prescription medicine and OTC medicine, including a daily dose; and other medical information. The information associated with personal identification and medical records should be obtained only for the reports and kept safe from disclosure. Any new medical information that is related to an already submitted serious adverse event report that is received within 1 year of the initial report shall be submitted to the regulatory authority as soon as appropriate, but NLT 15 business days after receipt of the information. The records related to each report of a serious adverse event received by the manufacturer, packer, or retailer should be maintained for 6 years. The authorized person who is designated by the regulatory authority should be permitted access to those records.

RETURNED AND SALVAGED PRODUCTS

Returned Dietary Supplements

Returned products should be identified as such and held. If the conditions under which returned dietary ingredients and dietary supplements have been held, stored, or shipped before or during

their return, or if the condition of the product, its container, carton, or labeling, as a result of storage or shipping, casts doubt on the safety, identity, strength, quality, or purity of the product, the returned product should be destroyed unless examination, testing, or other investigations prove the product meets appropriate standards of safety, identity, strength, quality, or purity. The returned products associated with adverse events must be destroyed after a sufficient sample of products is stored for the purpose of further investigation only. The products related to the adverse event that have been returned should be kept for 6 years (serious events) or for 3 years (nonserious events) from the date when the first report is received. A product may be reprocessed provided that the subsequent product meets adequate standards, specifications, and characteristics. Records of returned products should be maintained and should include the name and label potency of the product, lot number (or control number or batch number), reason for the return, quantity returned, date of disposition, and ultimate disposition of the returned product. If the reason for a product being returned implicates associated batches, an appropriate investigation is necessary.

Dietary Supplement Salvaging

Products that have been involved in adverse events or subjected to improper storage conditions, including extremes in temperature or humidity, smoke, fumes, pressure, age, or radiation due to natural disasters, fires, accidents, or equipment failures should not be salvaged and returned to the marketplace. Whenever there is a question whether products have been subjected to such conditions, salvaging operations may be conducted only if there is *a*) evidence from laboratory tests and assays that the products meet all applicable standards of identity, strength, quality, and purity; and *b*) evidence that the products and their associated packaging were not subjected to improper storage conditions as a result of the disaster or accident. Organoleptic examinations should be accepted only as supplemental evidence that the dietary supplement meets appropriate standards of identity, strength, quality, and purity. Records including name, lot number, and disposition should be maintained for salvaged products. If the products are involved in adverse events, the instructions described in the preceding section *Records and Reports* should be followed.

Defect Action Levels

Some dietary ingredients and dietary supplements, even when produced under current good manufacturing practice, contain natural or unavoidable defects that at low levels are not hazardous to health. The Food and Drug Administration establishes maximum levels for these defects in dietary ingredients and dietary supplements produced under current good manufacturing practice and uses these levels in deciding whether to recommend regulatory action.

Defect action levels are established for dietary ingredients and dietary supplements whenever it is necessary and feasible to do so. These levels are subject to change upon the development of new technology or the availability of new information.

Compliance with defect action levels does not excuse violation of the requirement in section 402(a)(4) of the Act that dietary ingredients and dietary supplements shall not be prepared, packed, or held under unsanitary conditions or the requirements in this part that manufacturers, distributors, and holders of both dietary ingredients and dietary supplements shall observe current good manufacturing practice. Evidence indicating that such a violation exists causes a dietary ingredient and a dietary supplement to be adulterated within the

meaning of the Act, although the amounts of natural or unavoidable defects are lower than the currently established defect action levels. The manufacturer, distributor, and holder of a dietary ingredient or a dietary supplement shall, at all times, utilize quality control operations that reduce natural or unavoidable defects to the lowest level currently feasible.

The mixing of a dietary ingredient or dietary supplement containing defects above the current defect action level with another lot of dietary ingredient or dietary supplement is not permitted and renders the final product adulterated within the meaning of the Act, regardless of the defect level of the final product.

A compilation of the current defect action levels for natural or unavoidable defects in dietary ingredients and dietary supplements that present no health hazard may be obtained upon request from the Industry Activities Staff (HFS-565), Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 5100 Paint Branch Parkway, College Park, MD 20740-3835.

GLOSSARY

Acceptance criteria: The product specifications and acceptance or rejection criteria, such as acceptable quality level and unacceptable quality level, with an associated sampling plan, that are necessary for making a decision to accept or reject a lot or batch (or any other convenient subgroups of manufactured units).

Act: Federal Food, Drug and Cosmetic Act [United States Code (U.S.C.) Title 21, Chapter 9].

Adequate: That which is needed to accomplish the intended purpose in keeping with good public health practice.

Adverse event: Any health-related event that is adverse and that is associated with the use of a dietary supplement.

Adverse event report: A report of an adverse event (see definition above). (See also *Serious adverse event report*.)

Batch: A specific quantity of a finished product or other material that is intended to have uniform character and quality, within specified limits, and is produced according to a single manufacturing order during the same cycle of manufacture.

Blanching: A prepackaging heat treatment of a dietary ingredient and a dietary supplement for a sufficient time and at a sufficient temperature to partially or completely inactivate the naturally occurring enzymes and to effect other physical or biochemical changes in the product.

Composition: 1) The identity of a dietary ingredient or dietary supplement, and 2) the concentration of a dietary ingredient (e.g., weight or other unit of use/weight or volume), or the potency or activity of one or more dietary ingredients, as indicated by appropriate procedures.

Dietary ingredient: An ingredient intended for use or used in a dietary supplement that is:

- A vitamin;
- A mineral;
- An herb or other botanical;
- An amino acid;
- A dietary substance for use by humans to supplement the diet by increasing the total dietary intake, or a concentrate, metabolite, constituent, extract; or
- A combination of any of the foregoing ingredients.

Dietary supplement: A product (other than tobacco) that is intended to supplement the diet

and that bears or contains one or more of the following dietary ingredients: a vitamin, a mineral, an herb or other botanical, an amino acid, a dietary substance for use by humans to supplement the diet by increasing the total daily intake, or a concentrate, metabolite, constituent, extract or combination of these ingredients, that is intended for ingestion in a pill, capsule, tablet or liquid form, that is not represented for use as a conventional food or as the sole item of a meal or diet, and that is labeled as a dietary supplement, and includes products such as a new drug, certified antibiotic, or licensed biologic that was marketed as a dietary supplement or food before approval, certification, or license unless a sanitary authority waives this provision.

Inactive ingredient: Any raw material other than a dietary ingredient.

In-process material: Any material fabricated, compounded, blended, ground, extracted, sifted, sterilized, or processed in any other way that is produced for, and used in, the preparation of the dietary supplement.

Lot: A batch, or a specific identified portion of a batch, having uniform character and quality within specified limits.

Lot number, control number, or batch number: Any distinctive combination of letters, numbers, or symbols, or any combination of them from which the complete history of the manufacture, processing, packing, holding, and distribution of a batch or lot of finished dietary ingredient, dietary supplement, or other material can be determined.

Manufacture or manufacturing: Includes all operations associated with the production of dietary ingredients and dietary supplements, including packaging and labeling operations, testing, and quality control of a dietary ingredient or dietary supplement.

Microorganisms: Yeast, molds, bacteria, and viruses and includes, but is not limited to, species having public health significance. The term "undesirable microorganisms" includes those microorganisms that are of public health significance, that subject a dietary ingredient or a dietary supplement to decomposition, that indicate that a dietary ingredient or dietary supplement is contaminated with filth, or that otherwise may cause a dietary ingredient or a dietary supplement to be adulterated within the meaning of the Act. Occasionally in these regulations, the adjective "microbial" is used instead of an adjectival phrase containing the word "microorganism".

Pest: Any objectionable animals or insects including, but not limited to, bird, rodents, flies, and larvae.

Plant: The building or facility or parts thereof, used for or in connection with the manufacturing, packaging, labeling, or holding of a dietary ingredient and a dietary supplement.

Process evaluation: A set of tests performed on a process intended to evaluate its capacity to consistently produce the results that it is intended for.

Quality control operation: A planned and systematic procedure for taking all actions necessary to prevent a dietary ingredient and a dietary supplement from being adulterated.

Quality control unit: Any person or organizational element designated by the firm to be responsible for the duties relating to quality control operations.

Raw material: Any ingredient intended for use in the manufacture of a dietary ingredient or dietary supplement, including those that may not appear in such finished product. (A dietary ingredient is a raw material when considering the manufacture of a dietary supplement.)

Representative sample: A sample that consists of a number of units that are drawn based on rational criteria, such as random sampling, and is intended to ensure that the sample accurately portrays the material being sampled.

Rework: A clean, unadulterated material that has been removed from processing for reasons other than unsanitary conditions or that has been successfully reconditioned by reprocessing

and that is suitable for use in the manufacture of a dietary ingredient or a dietary supplement.

Sanitizing: To adequately treat equipment, containers, or utensils by a process that is effective in destroying vegetative cells of microorganisms of public health significance and in substantially reducing other undesirable microorganisms but without affecting the product or its safety for the consumer.

Serious adverse event report: A report of an adverse event that is termed "serious" because it meets certain criteria (see the subsection *Adverse Event Reports*). The Dietary Supplement and Nonprescription Drug Consumer Protection Act requires manufacturers and distributors of dietary supplements and OTC drugs to report all serious adverse events to the Secretary of the Food and Drug Administration (FDA). This is an entirely new requirement for dietary supplements.

Shall: Used to state requirements that must be met under the provisions of this guideline.

Shelf life: The period of time after manufacturing in which the dietary supplement is ensured to meet applicable standards of identity, strength, quality, and purity.

Shelf-life (Use by) date: The date beyond which the dietary supplement is no longer ensured to meet applicable standards of identity, strength, quality, and purity.

Should: Used to state recommended or advisory procedures or identify recommended equipment.

Skip-lot testing (or sampling): A reduced level of testing (or sampling) for a particular specified parameter(s) based upon one or more of the following:

- Statistical analysis of an adequate quantity of historical test data;
- Statistical confidence in the capability of the manufacturing process as determined by suitable verification; or
- Ongoing monitoring of the process using recognized statistical process control (SPC) techniques.

Strength: The concentration of the active substance (weight/weight, weight/volume, or unit of use/volume or weight basis); and/or the potency, i.e., the activity of the product as indicated by appropriate laboratory tests.

Water activity (a_w): A measure of the free moisture in a dietary ingredient or dietary supplement and is the quotient of the water vapor pressure of the substance divided by the vapor pressure of pure water at the same temperature.

BRIEFING

2,2,2-Trichloroethanol. It is proposed to add this new reagent used in the test for content of *Chlorobutanol* in the revision made to *Antimicrobial Agents—Content* (341), published in *PF* 41(2).

(HDQ: M. Marques.)

Correspondence Number—C99464

Comment deadline: November 30, 2015

Add the following:

■ 2,2,2-Trichloroethanol (*Trichloroethyl Alcohol*), $C_2H_3OCl_3$ —**149.40** [115-20-8]—Use a suitable grade with a content of NLT 99%. ■2S (*USP39*)

BRIEFING

Acetonitrile, Chromatographic. It is proposed to add this new reagent.

(HDQ: M. Marques.)

Correspondence Number—C161640

Comment deadline: November 30, 2015

Add the following:

■ Acetonitrile, Chromatographic—Use ACS reagent grade, which meets the requirements for liquid chromatography suitability. ■ 2S (USP39)

BRIEFING

Ascorbic Acid. It is proposed to add this new reagent.

(HDQ: M. Marques.)

Correspondence Number—C161905

Comment deadline: November 30, 2015

Add the following:

■ Ascorbic Acid (Vitamin C), $C_6H_8O_6$ —**176.13** [50-81-7]—Use ACS reagent grade. ■ 2S (USP39)

BRIEFING

Citric Acid, *USP 38* page 1831. It is proposed to update the specification of this reagent.

Additionally, minor editorial changes have been made to update the reagent to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C162133

Comment deadline: November 30, 2015

Change to read:

Citric Acid, Use *Citric Acid Monohydrate* (USP monograph):

■ $C_6H_8O_7 \cdot H_2O$ —**210.14**[5949-29-1]—Use ACS reagent grade. ■ 2S (USP39)

BRIEFING

Citric Acid, Anhydrous, *USP 38* page 1831. It is proposed to update the specification of this reagent.

Additionally, minor editorial changes have been made to update the reagent to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C162217

Comment deadline: November 30, 2015

Change to read:

Citric Acid, Anhydrous,

■ $C_6H_8O_7$ —**192.13** ■ 2S (USP39)

[77-92-9]—Use ~~Anhydrous Citric Acid~~ (USP monograph).

- Use ACS reagent grade. ■ 2S (USP39)
-

BRIEFING

4-Cyanopyridine. It is proposed to add this new reagent used for peak identification/system suitability in the test for *Organic Impurities* in the monograph for *Isoniazid*.

(HDQ: M. Marques.)

Correspondence Number—C161526

Comment deadline: November 30, 2015

Add the following:

- 4-Cyanopyridine (*4-Pyridinecarbonitrile; Isonicotinic Acid Nitrile; Isonicotinonitrile*), $C_6H_4N_2$ —**104.11** [100-48-1]—Use a suitable grade with a content of NLT 98%. ■ 2S (USP39)
-

BRIEFING

2-Isoniazid. It is proposed to add this new reagent used for peak identification/system suitability in the test for *Organic Impurities* in the monograph for *Isoniazid*.

(HDQ: M. Marques.)

Correspondence Number—C161524

Comment deadline: November 30, 2015

Add the following:

- 2-Isoniazid (*2-Pyridinecarboxylic acid hydrazide; Picolinic acid hydrazide; 2-Picolinyl hydrazide*), $C_6H_7N_3O$ —**137.14** [1452-63-7]—Use a suitable grade with a content of NLT 98.0%. [Note—A suitable grade is available as catalog number P0426 from www.tcichemicals.com.] ■ 2S (USP39)
-

BRIEFING

Isonicotinamide. It is proposed to add this new reagent used for peak identification/system suitability in the test for *Organic Impurities* in the monograph for *Isoniazid*.

(HDQ: M. Marques.)

Correspondence Number—C161525

Comment deadline: November 30, 2015

Add the following:

- Isonicotinamide (*Pyridine-4-carboxylic Acid Amide*), $C_6H_6N_2O$ —**122.12** [1453-82-3]—Use a suitable grade with a content of NLT 99%. ■ 2S (USP39)
-

BRIEFING

Methanol, Chromatographic. It is proposed to add this new reagent.

(HDQ: M. Marques.)

Correspondence Number—C161641

Comment deadline: November 30, 2015

Add the following:

■ Methanol, Chromatographic—Use ACS reagent grade, which meets the requirements for liquid chromatography suitability. ■2S (USP39)

BRIEFING

L-Phenylalanine. It is proposed to add this new reagent.

(HDQ: M. Marques.)

Correspondence Number—C162310

Comment deadline: November 30, 2015

Add the following:

■ L-Phenylalanine ((*S*)-2-Amino-3-phenylpropionic acid), C₉H₁₁NO₂—**165.19** [63-91-2]—Use a suitable grade with a content of NLT 98%. ■2S (USP39)

BRIEFING

Polyethylene Glycol Standards with Molecular Weights of 1000, 2000, 3000, 4000, and 6000 Daltons (g/mol). It is proposed to add this new reagent used in the monograph for *Polyethylene Glycol 3350*, published in PF 41(4) [July–Aug. 2015].

(HDQ: M. Marques.)

Correspondence Number—C161035

Comment deadline: November 30, 2015

Add the following:

■ Polyethylene Glycol Standards with Molecular Weights of 1000, 2000, 3000, 4000, and 6000 Daltons (g/mol) (*Poly(oxy-1,2-ethanediyl), α-hydroxy-Ω-hydroxy-; 1,2-Ethandiol, homopolymer*), H(OCH₂CH₂)_nOH, in which the average values of *n* are 22, 45, 69, 92, and 149—average molecular weight (*M_w*), 1020, 2010, 3060, 4080, and 6550 [25332-68-3]—Use molecular weight standard grades. [Note—Suitable grades are available from www.polymer.de.] ■2S (USP39)

BRIEFING

Silver Sulfate. It is proposed to add this new reagent.

(HDQ: M. Marques.)

Correspondence Number—C162122

Comment deadline: November 30, 2015

Add the following:

■ Silver Sulfate, Ag₂SO₄—**311.80** [10294-26-5]—Use ACS reagent grade. ■2S (USP39)

BRIEFING

10% Ascorbic Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161906

Add the following:

■ **10% Ascorbic Acid TS**—Transfer 10 g of *ascorbic acid* to a 100-mL volumetric flask. Dissolve in and dilute with water to volume. ■ 2S (USP39)

BRIEFING

0.001 N Hydrochloric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161847

Add the following:

■ **0.001 N Hydrochloric Acid TS**—Transfer 1.0 mL of *1 N hydrochloric acid VS* to a 1000-mL volumetric flask and dilute with water to volume. ■ 2S (USP39)

BRIEFING

0.06 N Hydrochloric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C162048

Add the following:

■ **0.06 N Hydrochloric Acid TS**—Transfer 20.0 mL of *3 N hydrochloric acid TS* to a 1000-mL volumetric flask. Dilute with water to volume. ■ 2S (USP39)

BRIEFING

0.36 N Hydrochloric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C162068

Add the following:

■ **0.36 N Hydrochloric Acid TS**—Transfer 120 mL of *3 N hydrochloric acid TS* to a 1000-mL volumetric flask. Dilute with water to volume. ■ 2S (USP39)

BRIEFING

2 N Hydrochloric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161902

Add the following:

■ **2 N Hydrochloric Acid TS**—Transfer 17.0 mL of *hydrochloric acid* to a 100-mL volumetric flask containing about 50 mL of water. Cool and dilute with water to volume. ■ 2S (USP39)

BRIEFING

3 N Hydrochloric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C162040

Add the following:

■ **3 N Hydrochloric Acid TS**—Transfer 246 mL of *hydrochloric acid* to a 1000-mL volumetric

flask containing about 500 mL of water. Cool and dilute with water to volume. ■ 2S (USP39)

BRIEFING

6 N Hydrochloric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161647

Add the following:

■ **6 N Hydrochloric Acid TS**—Slowly transfer 49.8 mL of *hydrochloric acid* to a 100-mL volumetric flask containing about 45 mL of water. Cool and dilute with water to volume.

■ 2S (USP39)

BRIEFING

0.01 N Nitric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161904

Add the following:

■ **0.01 N Nitric Acid TS**—Transfer 10.0 mL of *1 N nitric acid TS* to a 1000-mL volumetric flask. Dilute with water to volume. ■ 2S (USP39)

BRIEFING

0.2 N Nitric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C162121

Add the following:

■ **0.2 N Nitric Acid TS**—Slowly transfer 12.7 mL of *nitric acid* to a 1000-mL volumetric flask containing about 250 mL of water. Cool and dilute with water to volume. ■ 2S (USP39)

BRIEFING

1 N Nitric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161903

Add the following:

■ **1 N Nitric Acid TS**—Transfer 63.7 mL of *nitric acid* to a 1000-mL volumetric flask containing about 250 mL of water. Cool and dilute with water to volume. ■ 2S (USP39)

BRIEFING

2 N Nitric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161659

Add the following:

■ **2 N Nitric Acid TS**—Transfer 12.74 mL of *nitric acid* slowly to a 100-mL volumetric flask containing about 40 mL of water. Cool and dilute with water to volume. ■ 2S (USP39)

BRIEFING

10% Phosphoric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161846

Add the following:

■ **10% Phosphoric Acid TS**—Transfer 7 mL of *phosphoric acid* to a 100-mL volumetric flask containing about 50 mL of water. Cool and dilute with water to volume. ■2S (USP39)

BRIEFING

2 N Potassium Hydroxide TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161593

Add the following:

■ **2 N Potassium Hydroxide TS**—Dissolve 112.2 g of *potassium hydroxide* in about 800 mL of water. Cool and dilute with water to 1000 mL. ■2S (USP39)

BRIEFING

20% Potassium Iodide TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161907

Add the following:

■ **20% Potassium Iodide TS**—Transfer 20 g of *potassium iodide* to a 100-mL volumetric flask. Dissolve in and dilute with water to volume. ■2S (USP39)

BRIEFING

0.2 N Sodium Hydroxide TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161850

Add the following:

■ **0.2 N Sodium Hydroxide TS**—Transfer 10.0 mL of *2 N sodium hydroxide TS* to a 100-mL volumetric flask and dilute with water to volume. ■2S (USP39)

BRIEFING

5 N Sodium Hydroxide TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161804

Add the following:

■ **5 N Sodium Hydroxide TS**—Transfer 20 g of *sodium hydroxide* to a 100-mL volumetric flask. Dissolve in about 80 mL of water. Cool and dilute with water to volume. ■2S (USP39)

BRIEFING

0.02 N Sulfuric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161934

Add the following:

■ **0.02 N Sulfuric Acid TS**—Transfer 2.9 mL of 7 N sulfuric acid TS to a 1000-mL volumetric flask and dilute with water to volume. ■2S (USP39)

BRIEFING

6 N Sulfuric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161909

Add the following:

■ **6 N Sulfuric Acid TS**—Slowly transfer 168.5 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume. ■2S (USP39)

BRIEFING

7 N Sulfuric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C161910

Add the following:

■ **7 N Sulfuric Acid TS**—Slowly transfer 196.5 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume. ■2S (USP39)

BRIEFING

0.07 N Ferrous Ammonium Sulfate VS. It is proposed to add this new volumetric solution.

(HDQ: M. Marques.)

Correspondence Number—C162130

Comment deadline: November 30, 2015

Add the following:

■ **0.07 N Ferrous Ammonium Sulfate VS**

$\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, **392.14**

27.5 g in 1000 mL

In a 100-mL volumetric flask, dissolve 27.5 g of ferrous ammonium sulfate in 500 mL of water. Add 20 mL of sulfuric acid. Cool and dilute with water to volume.

Standardize this solution on the day of use as follows. Dilute 25 mL of 0.025 N potassium dichromate VS with water to 100 mL. Add 30 mL of sulfuric acid and cool to room temperature. Add 3 drops of ferroin TS and titrate with 0.07 N ferrous ammonium sulfate VS. The color change is sharp, going from blue-green to reddish brown.

$$N = \frac{\text{mL K}_2\text{Cr}_2\text{O}_7 \times N\text{K}_2\text{Cr}_2\text{O}_7}{\text{mL Fe}(\text{NH}_4)_2\text{SO}_4)_2}$$

■ 2S (USP39)

BRIEFING

0.1 N Hydrochloric Acid VS. It is proposed to add this new volumetric solution.

(HDQ: M. Marques.)

Correspondence Number—C161529

Comment deadline: November 30, 2015

Add the following:

■ **0.1 N Hydrochloric Acid VS**

HCl, **36.46**

3.646 g in 1000 mL

Dilute 8.5 mL of *hydrochloric acid* with water to 1000 mL.

Standardize the solution as follows.

Accurately weigh about 2.5 g of *tromethamine*, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. Dissolve in 50 mL of water, and add 2 drops of *bromocresol green TS*. Titrate with 0.1 N hydrochloric acid to a pale yellow endpoint. Each 12.114 mg of *tromethamine* is equivalent to 1 mL of 0.1 N hydrochloric acid.

[Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL HCl}}$$

■ 2S (USP39)

BRIEFING

0.025 N Potassium Dichromate VS. It is proposed to add this new volumetric solution.

(HDQ: M. Marques.)

Correspondence Number—C162123

Comment deadline: November 30, 2015

Add the following:

■ **0.025 N Potassium Dichromate VS**

$K_2Cr_2O_7$ **294.18**

1.2259 g in 1000 mL

Transfer 12.259 g of *potassium dichromate* primary standard, previously dried according to the label instructions or, if this information is not available, dried at 103° for 2 h, to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Transfer 100.0 mL of this solution to a 1000-mL volumetric flask and dilute with water to volume. ■2S (USP39)

BRIEFING

L90. It is proposed to add this new column packing used in the test for *Enantiomeric Purity* in the monograph for *Frovatriptan Succinate*, published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C154837

Comment deadline: November 30, 2015

Add the following:

■ L90—Amylose tris-[(S)- α -methylbenzyl]carbamate coated on porous, spherical silica particles, 3–10 μ m in diameter. ■2S (USP39)

BRIEFING

L91. It is proposed to add this new column used in the chromatographic procedure in the test for *Purity* in general chapters *Collagenase I* (89.1) and *Collagenase II* (89.2), published elsewhere in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C153137

Comment deadline: November 30, 2015

Add the following:

■ L91—Strong anion-exchange resin consisting of monodisperse polystyrene/divinyl benzene beads coupled with quaternary amine. Bead size is 10 μ m. [Note—A suitable column is available as Mono Q 5/50 GL from <http://www.gelifesciences.com/webapp/wcs/stores/servlet/productById/en/GELifeSciences-us/17516601>.] ■2S (USP39)

CONTAINERS FOR DISPENSING CAPSULES AND TABLETS

BRIEFING

Container Specifications for Capsules and Tablets, page 7697 of the *Second Supplement to USP 38*.

(HDQ.)

Correspondence Number—C109140;

C130330;C130331;C133990;C136127;C137138;C137240;C143061;C143427;C151124;C153117;C16

The following table is provided as a reminder for the pharmacist engaged in the typical

dispensing situation who already is acquainted with the *Packaging and Storage* requirements set forth in the individual monographs. It lists the capsules and tablets that are official in the *United States Pharmacopeia* and indicates the relevant tight (T), well-closed (W), and light-resistant (LR) specifications applicable to containers in which the drug that is repackaged should be dispensed.

This table is not intended to replace, nor should it be interpreted as replacing, the definitive requirements stated in the individual monographs.

Container Specifications for Capsules and Tablets

Monograph Title	Container Specification
Change to read: Chlorothiazide Tablets	W, ■ LR _{2S} (USP39)
Change to read: Codeine Sulfate Tablets	W, ■ LR _{2S} (USP39)
Delete the following: ■ Doxasetron Mesylate Tablets	W, LR_{2S} (USP39)
Add the following: ■ Eleuthero Root and Rhizome Dry Extract Capsules	W, LR _{2S} (USP39)
Add the following: ■ Eleuthero Root and Rhizome Dry Extract Tablets	W, LR _{2S} (USP39)
Add the following: ■ Frovatriptan Tablets	T _{2S} (USP39)
Add the following: ■ Imipramine Pamoate Capsules	T, LR _{2S} (USP39)
Add the following: ■ Metronidazole Extended-Release Tablets	W _{2S} (USP39)
Add the following: ■ Nebivolol Tablets	T, LR _{2S} (USP39)
Add the following: ■ Quetiapine Extended-Release Tablets	W _{2S} (USP39)
Add the following: ■ Ropinirole Extended-Release Tablets	W _{2S} (USP39)
Add the following: ■ Ziprasidone Capsules	W _{2S} (USP39)

BRIEFING

Description and Relative Solubility of USP and NF Articles, page 7708 of the *Second Supplement to USP 38*.

(HDQ.)

Correspondence Number—C154240;

C162763;C155480;C154837;C125865;C162939;C161670;C151320;C161930;C162489

Add the following:

■ **Acamprosate Calcium:** A white or almost-white powder. Freely soluble in water; practically insoluble in alcohol and in methylene chloride. ■ 2S (USP39)

Change to read:

Desoxycholic Acid:

■ [(Title for this monograph is not to change until December 1, 2021.) Prior to December 1, 2021, the current practice of labeling the article of commerce with the name Desoxycholic Acid may be continued. Use of the name Deoxycholic Acid will be permitted as of December 1, 2016; however, the use of this name will not be mandatory until December 1, 2021. The 60-month extension will provide the time needed by manufacturers and users to make necessary changes.] **Deoxycholic Acid:** ■ 2S (NF34)

Occurs as a white, crystalline powder. Freely soluble in alcohol; soluble in acetone and in solutions of alkali hydroxides and carbonates; slightly soluble in chloroform and in ether; practically insoluble in water. *NF category:* Emulsifying agent.

Change to read:

Estradiol: White or creamy white, small crystals or crystalline powder. Is odorless, and is stable in air. Is hygroscopic. Soluble in alcohol, in acetone, in dioxane, ~~in chloroform,~~

■ ■ 2S (USP39)

and in solutions of fixed alkali hydroxides;

■ slightly soluble in chloroform; ■ 2S (USP39)

sparingly soluble in vegetable oils; practically insoluble in water.

Add the following:

■ **Frovatriptan Succinate:** A white to off-white powder. Soluble in water; very slightly soluble in methanol. ■ 2S (USP39)

Add the following:

■ **Methylnaltrexone Bromide:** White to almost-white crystalline powder. Freely soluble in dimethylsulfoxide; soluble in dimethylformamide and in water; slightly soluble in methanol; very slightly soluble in ethanol; practically insoluble in toluene. ■ 2S (USP39)

Change to read:

Naphazoline Hydrochloride: White, crystalline powder. ~~Is odorless and has a bitter taste. Melts at a temperature of about 255°, with decomposition. Freely soluble in water and in alcohol; very slightly soluble in chloroform; practically insoluble in ether.~~

■ Freely soluble in water; soluble in alcohol. ■ 2S (USP39)

Add the following:

■ **Nebivolol Hydrochloride:** White to off-white powder. Soluble in dimethyl sulfoxide; practically insoluble in acetone, in purified water, and in 0.1 N hydrochloric acid. ■ 2S (USP39)

Add the following:

■ **Sodium Succinate:** White crystals or white powder. Freely soluble in water. *NF category:* Flavors and fragrance. ■2S (NF34)

Add the following:

■ **Solifenacin Succinate:** White to pale, yellowish-white crystal or crystalline powder. Freely soluble in water and in methanol. ■2S (USP39)

Change to read:

Thalidomide: White to off-white powder. Very soluble in ~~dimethylformamide, in dioxane, and in pyridine~~

■ dimethyl sulfoxide; ■2S (USP39)

sparingly soluble in ~~acetone, in butyl acetate, in ethanol, in ethyl acetate, in glacial acetic acid, in methanol, and in water; practically insoluble in benzene, in chloroform, and in ether~~

■ in ethanol, and in water. ■2S (USP39)

BRIEFING**Excipients, USP and NF Excipients, Listed by Functional Category, USP 38 page 6493.**

It is proposed to change the *Emulsifying Agent* category for *Desoxycholic Acid* to complement the proposed title change to the *Desoxycholic Acid* monograph, which also appears in this issue of *PF*. It is also proposed to add Sodium Succinate to the *Flavors and Fragrance* category to complement the proposed *Sodium Succinate* monograph, which also appears in this issue of *PF*.

(HDQ: G. Holloway, H. Wang.)

Correspondence Number—C151320; C162763

In the following reference table, the grouping of excipients by functional category is intended to summarize commonly identified purposes that these excipients serve in drug product formulations. The association of a functional category with a particular dosage form in this table is not absolute and does not limit the use of an excipient to a single type of dosage form or delivery system.

Change to read:**Emulsifying Agent**

Desoxycholic Acid

■ [(Title for this monograph—not to change until December 1, 2021.) (Prior to December 1, 2021, the current practice of labeling the article of commerce with the name Desoxycholic Acid may be continued. Use of the name Deoxycholic Acid will be permitted as of December 1, 2016; however, the use of this name will not be mandatory until December 1, 2021. The 60-month extension will provide the time needed by manufacturers and users to make necessary changes.)]

Deoxycholic Acid

■2S (NF34)

Change to read:**Flavors and Fragrance**

■ Sodium Succinate ■2S (NF34)

BRIEFING

Acamprosate Calcium. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods of analysis, is proposed.

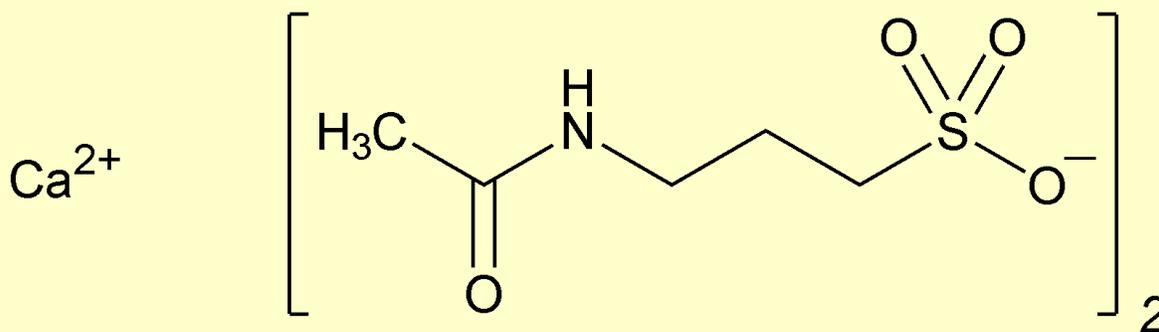
1. The liquid chromatographic procedures used in the proposed *Assay* and the test for *Organic Impurities* are based on analyses performed with the Cosmosil C18-PAQ brand of L1 column manufactured by Nacalai. The retention time for acamprosate is about 9.5 min.
2. The liquid chromatographic procedure within the proposed *Limit of Acamprosate Related Compound A* test is based on analyses conducted using the Hypersil ODS brand of L1 column manufactured by Thermo Fisher with a 5- μ m particle size. The retention time for acamprosate related compound A is about 10 min. A Discovery HS C18 brand of L1 column manufactured by Supelco may also be suitable.

All interested parties are encouraged to submit their FDA-approved specifications to USP if they are wider than those proposed.

(CHM4: H. Joyce.)

Correspondence Number—C142092

Comment deadline: November 30, 2015

Add the following:**■ Acamprosate Calcium**

$C_{10}H_{20}CaN_2O_8S_2$ 400.48

1-Propanesulfonic acid, 3-(acetylamino)-, calcium salt (2:1);
Calcium 3-(acetylamino)propane-1-sulfonate [77337-73-6].

DEFINITION

Acamprosate Calcium contains NLT 98.0% and NMT 102.0% of acamprosate calcium ($C_{10}H_{20}CaN_2O_8S_2$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General** (191), *Calcium*: Meets the requirements for the oxalate precipitation test

ASSAY

• Procedure

Mobile phase: Add 5.0 mL of *triethylamine* per 1 L of water and adjust with *phosphoric acid* to a pH of 4.0.

System suitability solution: 10 mg/mL of USP Acamprosate Calcium RS and 0.005 mg/mL each of USP Acamprosate Related Compound B RS and *glacial acetic acid* in water. Sonication may be used to aid in dissolution.

Standard solution: 0.3 mg/mL of USP Acamprosate Calcium RS in water. Sonication may be used to aid in dissolution.

Sample solution: 0.3 mg/mL of Acamprosate Calcium in water. Sonication may be used to aid in dissolution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5- μ m packing L1

Flow rate: 0.7 mL/min

Injection volume: 20 μ L

Run time: NLT 2 times the retention time of the acamprosate peak

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between acetic acid and acamprosate related compound B; NLT 1.3 between acamprosate related compound B and acamprosate, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acamprosate calcium ($C_{10}H_{20}CaN_2O_8S_2$) in the portion of Acamprosate Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Acamprosate Calcium RS in the *Standard solution* (mg/mL)

C_U = concentration of Acamprosate Calcium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• Limit of Acamprosate Related Compound A

Solution A: 5 g/L of *fluorescamine* in acetonitrile. Use within 24 h of preparation.

Buffer: 13.8 g/L of *monobasic sodium phosphate* prepared as follows. Transfer a suitable amount of *monobasic sodium phosphate* to a volumetric flask. Dissolve in 90% of the final flask volume of water. Adjust with 10 M *sodium hydroxide* or *phosphoric acid* to a pH of 6.5. Dilute with water to volume.

Mobile phase: *Acetonitrile*, *methanol*, and *Buffer* (10:10:80)

Diluent: 24.6 g/L of *boric acid* prepared as follows. Transfer a suitable amount of *boric acid* to an appropriate volumetric flask. Dissolve in 90% of the final flask volume of water. Adjust with 10 M *sodium hydroxide* to a pH of 10.4. Dilute with water to volume.

Standard stock solution A: 250 µg/mL of USP Acamprosate Related Compound A RS in water

Standard stock solution B: 1 µg/mL of USP Acamprosate Related Compound A RS from *Standard stock solution A* in *Diluent*

Standard solution: Transfer 3.0 mL of *Standard stock solution B* to an appropriate container. Add 0.15 mL of *Solution A* and shake vigorously for 30 s. Heat in a water bath at 50° for 30 min. Cool under a stream of cold water, centrifuge, and pass the supernatant through a suitable membrane filter.

Sample stock solution A: 20 mg/mL of Acamprosate Calcium in water

Sample stock solution B: 2 mg/mL of Acamprosate Calcium from *Sample stock solution A* in *Diluent*

Sample solution: Transfer 3.0 mL of *Sample stock solution B* to an appropriate container. Add 0.15 mL of *Solution A* and shake for 30 s. Heat in a water bath at 50° for 30 min. Cool under a stream of cold water, centrifuge, and pass the supernatant through a suitable membrane filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 261 nm

Column: 4.6-mm × 15-cm; 3-µm or 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: NLT 2 times the retention time of acamprosate related compound A

System suitability

Sample: *Standard solution*

[Note—The relative retention times for fluorecamine and acamprosate related compound A are about 0.5 and 1.0, respectively. Acamprosate calcium is not detected by this chromatographic system.]

Suitability requirements

Resolution: NLT 2.0 between fluorecamine and acamprosate related compound A

Relative standard deviation: NMT 5.0% for acamprosate related compound A

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acamprosate related compound A in the portion of Acamprosate Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Acamprosate Calcium Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Acamprosate Calcium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.05%

• Organic Impurities

Mobile phase: Add 5.0 mL of *triethylamine* per 1 L of water and adjust with *phosphoric acid* to a pH of 4.0.

System suitability solution: 10 mg/mL of USP Acamprosate Calcium RS and 0.005 mg/mL each of USP Acamprosate Related Compound B RS and *glacial acetic acid* in water. Sonication may be used to aid in dissolution.

Standard solution: 0.005 mg/mL of USP Acamprosate Calcium RS in water. Sonication may be used to aid in dissolution.

Sample solution: 10 mg/mL of Acamprosate Calcium in water. Sonication may be used to aid in dissolution.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 0.7 mL/min

Injection volume: 20 μ L

Run time: NLT 6 times the retention time of the acamprosate peak

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between acetic acid and acamprosate related compound B; NLT 1.3 between acamprosate related compound B and acamprosate, *System suitability solution*

Tailing factor: NMT 1.5 for acamprosate, *Standard solution*

Relative standard deviation: NMT 15.0% for acetic acid, *System suitability solution*; NMT 5% for acamprosate, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Acamprosate Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response of acamprosate from the *Standard solution*

C_S = concentration of USP Acamprosate Calcium RS in the *Standard solution* (mg/mL)

C_U = concentration of Acamprosate Calcium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Calcium ^a	0.4	—
Acetic acid	0.7	0.10
Acamprosate related compound B	0.8	0.05
Acamprosate	1.0	—
<i>N</i> -Methyl acamprosate ^b	1.9	0.05
Any individual, unspecified impurity	—	0.10
Total impurities ^c	—	0.30

^a Included for identification only. This peak is due to the calcium counterion and hence is not an impurity.

^b 3-(*N*-Methylacetamido)propane-1-sulfonate.

^c The sum of acamprosate related compound A from the *Limit of Acamprosate Related Compound A* test and all impurities from the test for *Organic Impurities*; do not include acetic acid in the total impurities.

SPECIFIC TESTS

- **pH** (791)

Sample solution: 0.05 g/mL of Acamprosate Calcium in *carbon dioxide-free water*

Acceptance criteria: 5.5–7.0

- **Loss on Drying** (731)

Analysis: Dry at 105° for 3h.

Acceptance criteria: NMT 0.4%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store in tight containers.

- **USP Reference Standards** (11)

USP Acamprosate Calcium RS

USP Acamprosate Related Compound A RS

3-Aminopropane-1-sulfonic acid.

C₃H₉NO₃S 139.17

USP Acamprosate Related Compound B RS

Calcium 3-formamidopropane-1-sulfonate.

C₈H₁₆CaN₂O₈S₂ 372.42

■ 2S (USP39)

BRIEFING

Acetaminophen Suppositories, *USP 38* page 2008. As part of the USP monograph modernization initiative, the following revisions are proposed:

1. A new HPLC procedure for monitoring 4-aminophenol, based on *4-Aminophenol in Acetaminophen-Containing Drug Products* (227), is proposed. This liquid chromatographic procedure is based on analyses performed with the Dionex Acclaim Mixed Mode WCX-1 brand of L85 column. The proposed limit for 4-aminophenol is 0.15%.
2. USP 4-Aminophenol RS is added to the *USP Reference Standards* section to support the proposed revision.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM6: C. Anthony.)

Correspondence Number—C128008

Comment deadline: November 30, 2015

Acetaminophen Suppositories

DEFINITION

Acetaminophen Suppositories contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen (C₈H₉NO₂).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. Thin-Layer Chromatographic Identification Test** (201)

Sample solution: Transfer the equivalent of 20 mg of acetaminophen from a portion of Suppositories to a beaker. Add 20 mL of methanol and heat on a steam bath until melted. Remove the beaker from the steam bath, allow to cool with occasional stirring, and filter. Use the clear filtrate.

Chromatographic system

Developing solvent system: Methylene chloride and methanol (4:1)

Acceptance criteria: Meet the requirements

ASSAY**Change to read:**

- **Procedure**

Mobile phase: Methanol and water (1:3)

Standard solution: 0.01 mg/mL of USP Acetaminophen RS in *Mobile phase*

Sample stock solution: Nominally 0.5 mg/mL of acetaminophen prepared as follows. Tare a small dish and a glass rod, place NLT 5 Suppositories in the dish, heat gently on a steam bath until melted, stir, cool while stirring, and weigh. Transfer a weighed portion of the mass, equivalent to 100 mg of acetaminophen, to a separator, •add 30 mL of solvent hexane, •(ERR 1-Jun-2014) and dissolve. Add 30 mL of water, shake gently, and allow the phases to separate. If an emulsion forms, allow sufficient time for it to separate. Transfer the aqueous layer to a 200-mL volumetric flask, •wash the solvent hexane •(ERR 1-Jun-2014) in the separator with three 30-mL portions of water, adding the washings to the volumetric flask. Dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.01 mg/mL of acetaminophen in *Mobile phase* from the *Sample stock solution*. Pass a portion of this solution through a filter of 0.5- μ m or finer pore size, discarding the first 10 mL of the filtrate. Use the clear filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 243 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

■ **2S (USP39)**

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Suppositories taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

■ ● **4-Aminophenol in Acetaminophen-Containing Drug Products** <227>

Buffer: 4.0 g/L of sodium citrate dihydrate and 1.5 g/L of anhydrous citric acid, in water

Diluent: *Buffer* and acetonitrile (9:1)

Sample stock solution: Approximately 12–13 mg/mL of acetaminophen prepared as follows. Transfer an appropriate number of whole Suppositories to a suitable volumetric flask. Add *Diluent* until the flask is about half filled and sonicate for 1 h with frequent swirling. Allow to cool and then dilute with *Diluent* to volume.

Sample solution: Approximately 4.8–5.2 mg/mL of acetaminophen in *Diluent* from the *Sample stock solution* prepared as follows. Pipet 20.0 mL of the *Sample stock solution* into a 50-mL volumetric flask and dilute with *Diluent* to volume.

Acceptance criteria: Meet the requirements ■ **2S (USP39)**

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature or in a cool place.

Change to read:

- **USP Reference Standards** <11>

USP Acetaminophen RS

■ **USP 4-Aminophenol RS**

■ **2S (USP39)**

BRIEFING

Albuterol Tablets, *USP 38* page 2072. As a part of USP monograph modernization efforts,

the following revisions are proposed:

1. Replace the TLC procedure in *Identification* test *A* with a retention time agreement from the liquid chromatographic procedure in the *Assay*.
2. Delete the identification test for *Sulfate* because the counter ion is controlled in the drug substance.
3. Add *Identification* test *B* based on a diode-array spectra agreement from the chromatographic procedures in the *Assay*.
4. Add the use of a diode-array detector in the *Assay* to support the proposed *Identification* test *B*.
5. Specify the particle size of the columns used in the *Assay* and *Dissolution* tests.
6. Remove *Column efficiency* from the system suitability requirements in the *Assay* and *Dissolution* tests. The remaining criteria are adequate to evaluate system suitability.
7. Add an equation to the *Dissolution* test for consistency with current *USP* style.
8. Replace the TLC procedure in the test for *Organic Impurities* with a stability-indicating liquid chromatographic procedure. The LC procedure is based on validated analyses performed with the Waters Acquity UPLC BEH C18 brand of L1 column. The typical retention time for albuterol is about 2.7 min.
9. Add the new Reference Standards to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

Interested parties are encouraged to submit validated dissolution procedures, which test individual units, along with supporting data to further improve the monograph.

(CHM5: R.-H. Yeh.)

Correspondence Number—C137787

Comment deadline: November 30, 2015

Albuterol Tablets

DEFINITION

Albuterol Tablets contain an amount of albuterol sulfate $[(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4]$ equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of albuterol ($C_{13}H_{21}NO_3$).

IDENTIFICATION

Change to read:

- **A.** ~~The R_f value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*, obtained as directed in the *Procedure for Organic Impurities*.~~
 - The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■2S (*USP39*)

Delete the following:

■ ● **B. Identification Tests—General, Sulfate** (191)

Sample solution: Shake a quantity of powdered Tablets equivalent to 4 mg of albuterol with 10 mL of water, and filter. Use the filtrate.

Acceptance criteria: Meet the requirements ■ 2S (USP39)

Add the following:

- ● **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

ASSAY

Change to read:

● **Procedure**

Solution A: 10 mL/L of *glacial acetic acid* in water

Solution B: 1.13 g of *sodium 1-hexanesulfonate* in 1200 mL of water. Add 12 mL of *glacial acetic acid*.

Diluent: *Methanol* and water (40:60)

Mobile phase: *Methanol* and *Solution B* (40:60)

Standard stock solution: 0.12 mg/mL of USP Albuterol Sulfate RS, prepared as follows.

Transfer USP Albuterol Sulfate RS to a suitable volumetric flask and add 60% of the final flask volume of *Solution A*. Sonicate for 5 min and dilute with *methanol* to volume.

Standard solution: 0.03 mg/mL of USP Albuterol Sulfate RS from the *Standard stock solution* in *Diluent*

Sample solution: Nominally 0.025 mg/mL of albuterol, prepared as follows. Transfer a number of whole Tablets, equivalent to 50 mg of albuterol, to a suitable volumetric flask. Add 60% of the final flask volume of *Solution A*, shake by mechanical means for 45 min, sonicate for 10 min, allow to cool to room temperature, and dilute with *methanol* to volume. Pass through a suitable filter of 0.45- μ m or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 276 nm.

- For *Identification* test B, use a diode-array detector in the range of 200–400 nm.

■ 2S (USP39)

Column: 4.6-mm \times 15-cm;

- 5- μ m ■ 2S (USP39)

packing L1

Flow rate: 1.5 mL/min

Injection volume: 25 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: ~~NLT 800 theoretical plates~~

■ 2S (USP39)

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of albuterol ($C_{13}H_{21}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times M \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Albuterol Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of albuterol in the *Sample solution* (mg/mL)

M = number of moles of albuterol per mole of albuterol sulfate, 2

M_{r1} = molecular weight of albuterol, 239.31

M_{r2} = molecular weight of albuterol sulfate, 576.70

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **Dissolution** <711>

Procedure for a pooled sample

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 30 min

Diluent, Mobile phase, and Standard stock solution: Prepare as directed in the *Assay*.

Standard solution: 0.03 mg/mL of USP Albuterol Sulfate RS from the *Standard stock solution* in *Diluent*. If necessary, dilute with *Diluent* to a concentration corresponding to the *Sample solution*.

Sample solution: Pass a portion of the solution under test through a nylon filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 276 nm

Column: 4.6-mm \times 15-cm;

■ 5- μ m ■ 2S (USP39)

packing L1

Flow rate: 1.5 mL/min

Injection volume: 100 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 800 theoretical plates

■ **2S (USP39)**

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of albuterol ($C_{13}H_{21}NO_3$) dissolved:

■

$$\text{Result} = (r_U/r_S) \times C_S \times V \times M \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Albuterol Sulfate RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

M = number of moles of albuterol per mole of albuterol sulfate, 2

M_{r1} = molecular weight of albuterol, 239.31

M_{r2} = molecular weight of albuterol sulfate, 576.70

L = label claim (mg/Tablet) ■ **2S (USP39)**

Acceptance criteria: NLT 80% (Q) of the labeled amount of albuterol ($C_{13}H_{21}NO_3$) is dissolved.

- **Uniformity of Dosage Units** (905): Meet the requirements

IMPURITIES

Change to read:

- **Organic Impurities**

Standard solution A: ~~0.580 mg/mL of USP Albuterol Sulfate RS in water, equivalent to 0.483 mg/mL of albuterol~~

Standard solution B: ~~0.218 mg/mL of USP Albuterol Sulfate RS in water, equivalent to 0.183 mg/mL of albuterol~~

Standard solution C: ~~0.073 mg/mL of USP Albuterol Sulfate RS in water, equivalent to 0.061 mg/mL of albuterol~~

Sample solution: ~~Place a quantity of finely powdered Tablets, equivalent to 48 mg of albuterol, into a suitable container. Add 60 mL of diluted alcohol (1 in 2), and shake by mechanical means for 30 min. Filter the mixture, and wash the filter with small portions of~~

alcohol, combining this with the filtrate. Evaporate the filtrate to dryness under reduced pressure below 40°. Dissolve the residue as completely as possible in 2 mL of water.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 µL. Apply two successive 5-µL aliquots, allowing the solvent to evaporate between applications.

Developing solvent system: Methyl isobutyl ketone, isopropyl alcohol, ethyl acetate, ammonium hydroxide, and water (50:45:35:3:18)

Spray reagent A: 3-Methyl-2-benzothiazolinone hydrazone hydrochloride TS

Spray reagent B: Ammoniacal potassium ferricyanide TS

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Air-dry the plate. Develop the chromatograms until the solvent front has moved about 17 cm. Spray the plate first with *Spray reagent A*, and then *Spray reagent B*, and finally again with *Spray reagent A*. Examine the plate and estimate the responses of any secondary spots observed in the lane of the *Sample solution* by comparison with those of *Standard solutions A*, *B*, and *C*.

Acceptance criteria

1. 2.0%; no major secondary spot is greater in size or intensity than the principal spot produced by *Standard solution A*.
2. 0.75%; no other secondary spot is greater in size or intensity than the principal spot produced by *Standard solution B*.
3. 0.25%; no more than two other secondary spots are equal in size or intensity to the principal spot produced by *Standard solution C*.
4. The sum of the intensities of all secondary spots obtained from the *Sample solution* corresponds to NMT 3.5%.

■ **Solution A:** 9.5 g/L of sodium borate in water; adjusted with a sodium hydroxide solution to a pH of 10.1

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
1	95	5
9	47	53
9.5	47	53
9.6	95	5
11	95	5

[Note—The gradient was established on an LC system with a dwell volume of 350–400 μL .]

System suitability solution: 2 $\mu\text{g}/\text{mL}$ each of USP Albuterol Related Compound A RS, USP Albuterol Related Compound C RS, USP Levalbuterol Related Compound C RS, and USP Levalbuterol Related Compound E RS in water

Standard solution: 0.0025 mg/mL of USP Albuterol Sulfate RS, 0.003 mg/mL of USP Albuterol Related Compound E RS, 0.0015 mg/mL of USP Levalbuterol Related Compound C RS, and 0.001 mg/mL of USP Levalbuterol Related Compound D RS in water

Sample solution: Nominally 1.0 mg/mL of albuterol from NLT 20 Tablets in water prepared as follows. Transfer a suitable amount of the Tablets to an appropriate volumetric flask. Add 80% of the final flask volume of water and sonicate for 15 min. Dilute with water to volume. Pass through a suitable filter of 0.2- μm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 231 nm

Column: 2.1-mm \times 10-cm; 1.7- μm packing L1

Temperatures

Autosampler: 4°

Column: 30°

Flow rate: 0.37 mL/min

Injection volume: 6 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between levalbuterol related compound C and albuterol related compound C; NLT 1.5 between albuterol related compound A and levalbuterol related compound E, *System suitability solution*

Relative standard deviation: NMT 5.0% for albuterol, albuterol related compound E, levalbuterol related compound C, and levalbuterol related compound D, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of albuterol related compound E, levalbuterol related compound C, and levalbuterol related compound D in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of albuterol related compound E, levalbuterol related compound C, or levalbuterol related compound D from the *Sample solution*

r_S = peak response of albuterol related compound E, levalbuterol related compound C, or levalbuterol related compound D from the *Standard solution*

C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

C_U = nominal concentration of albuterol in the *Sample solution* (mg/mL)

Calculate the percentage of each individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times M \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual unspecified degradation product from the *Sample solution*

r_S = peak response of albuterol from the *Standard solution*

C_S = concentration of USP Albuterol Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of albuterol in the *Sample solution* (mg/mL)

M = number of moles of albuterol per mole of albuterol sulfate, 2

M_{r1} = molecular weight of albuterol, 239.31

M_{r2} = molecular weight of albuterol sulfate, 576.70

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Albuterol	1.00	—
Levalbuterol related compound D	1.40	0.1
Albuterol related compound D ^{a,b}	1.45	—
Levalbuterol related compound H ^{a,c}	1.50	—
Albuterol related compound B ^{a,d}	1.53	—
Levalbuterol related compound C	1.82	0.15
Albuterol related compound C ^a	1.84	—
Albuterol related compound A ^a	1.99	—
Levalbuterol related compound E ^a	2.04	—
Albuterol related compound E	2.25	0.3
Levalbuterol related compound F ^{a,e}	3.19	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	3.5

^a Process impurities are included for identification only and are not to be included in the calculation of total degradation products. These impurities are controlled in the drug substance.
^b 4-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-chloro-6-(hydroxymethyl)phenol.
^c 4-[2-(*tert*-Butylamino)-1-methoxyethyl]-2-(hydroxymethyl)phenol.
^d 2-(*tert*-Butylamino)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone.
^e 1-[4-(Benzyloxy)-3-(hydroxymethyl)phenyl]-2-(*tert*-butylamino)ethanol.

■ 2S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

Change to read:

- **USP Reference Standards** <11>

USP Albuterol Sulfate RS

- USP Albuterol Related Compound A RS

4-{2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl}-2-methylphenol sulfate.
 $(C_{13}H_{21}NO_2)_2 \cdot H_2SO_4$ 544.70

USP Albuterol Related Compound C RS

2-(*tert*-Butylamino)-1-[3-chloro-4-hydroxy-5-(hydroxymethyl)phenyl]ethanone.
 $C_{13}H_{18}ClNO_3$ 271.74

USP Albuterol Related Compound E RS

2,2'-Oxybis(methylene)bis{4-[2-(*tert*-butylamino)-1-hydroxyethyl]phenol} diacetate.
 $C_{26}H_{40}N_2O_5 \cdot 2(C_2H_4O_2)$ 580.71

USP Levalbuterol Related Compound C RS

4-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-(methoxymethyl)phenol;
 Also known as α -{[(1,1-Dimethylethyl)amino]methyl}-4-hydroxy-3-(methoxymethyl)-benzenemethanol.

$C_{14}H_{23}NO_3$ 253.34

USP Levalbuterol Related Compound D RS

5-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-hydroxybenzaldehyde sulfate;
 Also known as 5-{2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl}-2-hydroxybenzaldehyde sulfate.

$(C_{13}H_{19}NO_3)_2 \cdot H_2SO_4$ 572.67

USP Levalbuterol Related Compound E RS

4-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-(ethoxymethyl)phenol;
 Also known as α -{[(1,1-Dimethylethyl)amino]methyl}-3-(ethoxymethyl)-4-hydroxybenzenemethanol.

$C_{15}H_{25}NO_3$ 267.36

■ 2S (USP39)

BRIEFING

Aminohippurate Sodium Injection, USP 38 page 2184. In preparation for the omission of the flame tests from *Identification Tests—General* (191), proposed in PF 41(2) [Mar.–Apr. 2015], it is proposed to delete the reference to (191) in *Identification* test C and include a complete description of the flame test in the monograph. Manufacturers are encouraged to submit a replacement wet chemistry or an instrumental procedure for consideration by the Expert Committee.

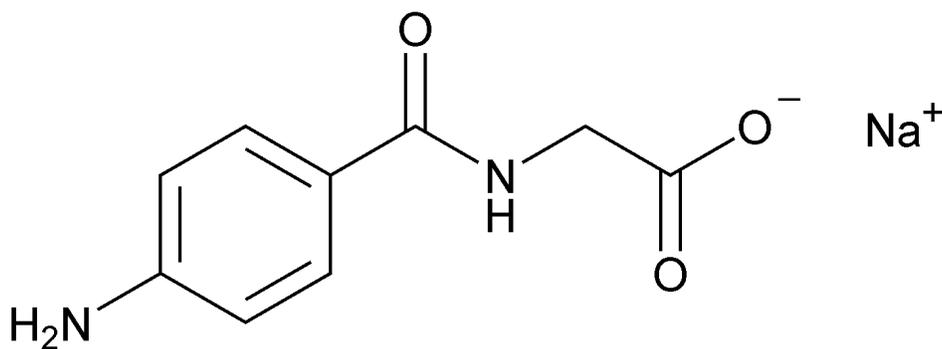
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(BIO2: M. Metta.)

Correspondence Number—C160573

Comment deadline: November 30, 2015

Aminohippurate Sodium Injection



$C_9H_9N_2NaO_3$ 216.17

Glycine, *N*-(4-aminobenzoyl)-, monosodium salt;
 Monosodium *p*-aminohippurate [94-16-6].

DEFINITION

Aminohippurate Sodium Injection is a sterile solution of Aminohippuric Acid in Water for Injection prepared with the aid of Sodium Hydroxide. It contains NLT 95.0% and NMT 105.0% of the labeled amount of aminohippurate sodium ($C_9H_9N_2NaO_3$).

IDENTIFICATION

• A.

Sample solution: A volume of Injection equivalent to 100 mg of aminohippuric acid

Analysis: Dilute the *Sample solution* with water to 50 mL. To 5 mL of this solution add 0.5 mL of 3 N *hydrochloric acid*, 0.5 mL of *sodium nitrite* solution (1 in 10), and a solution of 0.20 g of *2-naphthol* in 10 mL of 6 N *ammonium hydroxide*.

Acceptance criteria: A red color is produced.

• B.

Sample solution: A volume of Injection equivalent to about 200 mg of aminohippurate sodium

Analysis: In the order named, add to the *Sample solution* 2 mL of *potassium iodide TS*, 10 mL of water, and 5 mL of *sodium hypochlorite TS*.

Acceptance criteria: A red color is produced.

Delete the following:

- • ~~C. Identification Tests—General, Sodium (191): Meets the requirements of the flame test~~
 ■ 2S (USP39)

Add the following:

- • C. A sample imparts an intense yellow color to a nonluminous flame. ■ 2S (USP39)

ASSAY

• Procedure

Sample solution: A volume of Injection equivalent to 1 g of aminohippurate sodium

Analysis: Transfer the *Sample solution* to a 200-mL volumetric flask, and dilute with water to volume. Transfer 50.0 mL of the solution to a suitable container, and add 5 mL of *hydrochloric acid*. Proceed as directed in *Nitrite Titration* (451), beginning with "cool to about 15°". Each mL of 0.1 M *sodium nitrite* is equivalent to 21.62 mg of aminohippurate sodium ($C_9H_9N_2NaO_3$).

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

- pH (791): 6.7–7.6
- **Bacterial Endotoxins Test** (85): NMT 0.04 USP Endotoxin Units/mg of aminohippurate sodium
- **Other Requirements:** Meets the requirements in *Injections* (1)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of

Type 1 glass.

- **USP Reference Standards** (11)
USP Endotoxin RS

BRIEFING

Antithrombin III Human, *USP 38* page 2271. On the basis of comments received, the following stage one revisions are proposed:

1. The CAS number for this substance is added in the chemical information section.
2. Revise the *Definition* to remove aspects of processing and references to the USP Antithrombin Unit.
3. Revise the *Assay* to use the International Unit.
4. Revise the *Assay* in agreement with validated methods of analysis.
5. Revise the *Assay* acceptance criteria in agreement with validated methods of analysis.
6. Revise the test for *Organic Impurities, Procedure: Heparin Content* in agreement with validated methods of analysis.
7. Revise the test for *Molecular Weight Distribution* in agreement with validated methods of analysis.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BIO3: K. Carrick.)

Correspondence Number—C146225

Comment deadline: November 30, 2015

Antithrombin III Human

Add the following:

[■ 9000-94-6]. ■ 2S (*USP39*)

DEFINITION

Change to read:

Antithrombin III Human is a glycoprotein, which is the major inhibitor of thrombin and other activated clotting factors, including factors IX, X, XI, and XII. ~~and the cofactor through which heparin exerts its effect~~

■ ■ 2S (*USP39*)

It is obtained from human plasma of healthy donors who ~~must, as far as can be ascertained,~~

■ have been tested and shown to ■ 2S (*USP39*)

be free from detectable agents of infection transmissible by transfusion of blood or blood derivatives. The ~~method of manufacturing includes steps that have been~~

■ manufacturing steps are ■ 2S (*USP39*)

shown to remove or inactivate known agents of infection. If substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to an acceptable level and that any residues are such as not to compromise the safety of the preparation for patients.

~~The antithrombin III concentrate is passed through a bacteria-retentive filter, filled aseptically into its final, sterile containers, and immediately frozen. It is then freeze-dried, and the containers are closed under vacuum. No antimicrobial preservative is added at any stage of production. Antithrombin III Human complies with the requirements for *Biologics* (1041):~~

■ **2S (USP39)**

When reconstituted in the recommended volume of diluent, the potency is NLT 25 USP Antithrombin III Units

■ **International Units (IU)** ■ **2S (USP39)**

/mL.

■ **It contains 80%–120% of the potency stated on the label.** ■ **2S (USP39)**

~~[Note—One USP Antithrombin III Unit is the amount of antithrombin III that forms a complex with one unit of thrombin at 25° in the presence of heparin at a pH of 8.4.]~~

■ **2S (USP39)**

IDENTIFICATION

- Meets the requirements of the Assay

ASSAY

Change to read:

- **Procedure**

Solution A: Dissolve Tris(hydroxymethyl)aminomethane, edetic acid, and sodium chloride in water containing ~~0.1% polyethylene glycol 6000~~

■ **2S (USP39)**

to obtain a solution having concentrations of 0.050, 0.0075, and 0.175 M, respectively. Adjust with hydrochloric acid or sodium hydroxide solution to a pH of 8.4.

Solution B: 0.05% (w/v) of albumin human in *Solution A*

Solution C: 10 mg/mL of polybrene in *Solution B*

Solution D: ~~15 USP Heparin Units/mL of USP Heparin Sodium for Assays RS in *Solution B*~~

■ **Reconstitute thrombin bovine (factor IIa), and dilute with *Solution B* to obtain a solution having a concentration of 4 Thrombin IU/mL.** ■ **2S (USP39)**

Solution E: ~~Reconstitute thrombin bovine, and dilute with *Solution B* to obtain a solution having a concentration of 2.0 Thrombin Units/mL.~~

■ **Prepare a solution of chromogenic substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for thrombin (factor IIa) in *Solution C* to obtain a solution having a concentration of about 40.0 mM.** ■ **2S (USP39)**

Solution F: ~~Prepare a solution of chromogenic substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for factor II_a in water to obtain a solution having a concentration of about 5.0 mM, and dilute the solution further with *Solution C* to 1.0 mM.~~

■ **Resuspend USP Heparin Sodium for Assays RS according to the Certificate and dilute to 3 USP Heparin Units/mL in *Solution A*.** ■ **2S (USP39)**

Stopping solution: ~~20% (v/v) of acetic acid in water~~

Standard solution A: ~~USP Antithrombin III Human RS in *Solution D* to obtain a solution containing 1.0 USP Antithrombin III Unit~~

■ **Standard solutions:** Prepare seven dilutions from USP Antithrombin III Human RS within the linear range of the assay in *Solution F* (for example, 1.7, 1.5, 1.2, 1.0, 0.8, 0.6, and 0.4 IU/mL.) ■ 2S (USP39)

~~**Standard solutions B, C, D, and E:** Dilute *Standard solution A* with *Solution D* 60-, 120-, 180-, and 300-fold.~~

~~**Sample solution A:** Dissolve a quantity of Antithrombin III Human in *Solution D* to obtain a solution having the same concentration as *Standard solution A*.~~

■ **Sample solutions:** Prepare three or more dilutions in *Solution F* within the linear range of the assay.

Blank: *Solution A* ■ 2S (USP39)

~~**Sample solutions B, C, D, and E:** Dilute *Sample solution A* with *Solution D* 60-, 120-, 180-, and 300-fold.~~

■ 2S (USP39)

Analysis:

■ [Note—The procedure also can be performed using alternative platforms.] ■ 2S (USP39)
Pipet 400 μL each of *Standard solutions B, C, D, and E* and *Sample solutions B, C, D, and E* into suitable tubes placed in a water bath set at 37°. Add 200 μL of *Solution E*, prewarmed at 37° to each tube, mix, and incubate for 1 min. Add 200 μL of *Solution F* prewarmed at 37° to each tube, mix, and incubate for 60 s. Stop the reaction by adding 200 μL *Stopping solution*. To prepare a blank, add the reagents in reverse order, starting with 200 μL of *Stopping solution*, followed by the addition of 200 μL of *Solution F*, then adding 200 μL of *Solution E*, and ending with 400 μL of *Solution D*. Record the absorbance at 405 nm against the blank.

For *Standard solutions* and *Sample solutions*, calculate the regression of the absorbance against log concentrations, and calculate the activity of Antithrombin III Human in USP Antithrombin III Units, using a suitable statistical method for parallel line assays. The four independent relative activity estimates are then combined to obtain the final mean, and the confidence limits are calculated.

■ For each dilution of the *Standard solution* and *Sample solution*, at least duplicates should be tested. Label a suitable number of tubes depending on the number of replicates that will be tested. For example, if five blanks will be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, and T3 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, S4, S5, S6, and S7 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [Note—Treat the tubes in the order B1, S1, S2, S3, S4, S5, S6, S7, B2, T1, T2, T3, B3, T1, T2, T3, B4, S1, S2, S3, S4, S5, S6, S7, B5.]

Prewarm *Solution D* and *Solution E* at 37°. Pipet 50 μL each of the *Standard solutions*, *Sample solutions*, and *Blank* into suitable tubes placed in a water bath set at 37°. Add 350 μL of prewarmed *Solution D* to each tube, mix, and incubate for 1 min. Add 100 μL of prewarmed *Solution E* to each tube in the same order and mix. Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer

(see *Ultraviolet-Visible Spectroscopy* (857)). Calculate the change in absorbance/min. Plot standard concentrations against resulting absorbance values and determine potency by interpolating from the standard curve using mean sample absorbances.

System suitability

Samples: *Standard solutions and Sample solutions*

The R^2 value of the standard curve is NLT 0.99. The initial and final blanks differ by NMT 10%. The absorbances of the three dilutions of the *Sample solution* must fall within the range of absorbances of the standard curve. The three dilutions of the *Sample solution* give potency estimates that differ by NMT 10%.

■ 2S (USP39)

Acceptance criteria: 80%–120% of the potency stated on the label. The specific activity is NLT 6.0 USP Antithrombin III Units/mg of total protein. The confidence interval ($P = 0.95$) is between 90% and 110% of the estimated potency.

■ 2S (USP39)

IMPURITIES

Change to read:

Organic Impurities

● Procedure: Heparin Content

Solution A: Dissolve Tris(hydroxymethyl)aminomethane, edetic acid, and sodium chloride in water containing 0.1% polyethylene glycol 6000 to obtain a solution having concentrations of 0.050 M, 0.0075 M, and 0.175 M, respectively. Adjust with hydrochloric acid or sodium hydroxide solution to a pH of 8.4.

Solution B: Solution of chromogenic substrate for amidolytic test for factor X_a in water to obtain a solution of concentration of 2.5 mM

Solution C: Dissolve Factor X_a in *Solution A* to obtain a solution containing 20 nanokatalytic units (nkats).

Solution D: 20% (v/v) of acetic acid in water

Standard solution: Dissolve USP Antithrombin III Human RS in *Solution A* to obtain a solution containing 1.0 USP Antithrombin III Unit.

Sample solution: Dissolve Antithrombin III Human in *Solution A* to obtain a solution containing 1.0 USP Antithrombin III Unit.

Analysis: Pipet 250 μ L each of *Solution A*, the *Standard solution*, and the *Sample solution* to suitable tubes placed in a water bath set at 37°. Add 250 μ L of *Solution C* prewarmed at 37° to each tube, and incubate for 2 min. Add 250 μ L of *Solution B* prewarmed at 37° to each tube, mix, and incubate for 120 s. Stop the reaction by adding 250 μ L of *Solution D*. Record the absorbance at 405 nm, using *Solution A* as the blank.

Calculate the USP Heparin Unit per USP Antithrombin III Unit:

$$\text{Result} = P_R (A_F - A_U) / (A_F - A_S)$$

P_R = heparin content of USP Antithrombin III Human RS in USP Heparin Unit per USP Antithrombin III Unit

A_f = absorbance value from *Solution A*

A_y = absorbance value from the *Sample solution*

A_S = absorbance value from the *Standard solution*

Acceptance criteria: NMT 0.1 USP Heparin Unit per USP Antithrombin III Unit

■ • Heparin Content

Solution A: 9 g/L of sodium chloride

Sheep plasma substrate: Use sheep plasma suitable for the test procedure. If frozen, thaw at 37°.

APTT reagent: Use a suitable Activated Partial Thromboplastin Time (APTT) reagent containing phospholipid and a contact activator at a dilution giving a suitable blank recalcification time not exceeding 60 s.

Calcium chloride solution: 3.7 g/L of calcium chloride

System suitability solution: 5 IU/mL of heparin sodium for assays in USP Antithrombin III Human RS

Standard solutions: Make three or more dilutions of USP Heparin Sodium for Assays RS to known concentrations in USP Heparin Units/mL that are in the expected range of the sample (for example, 0.5–1.5 USP Heparin Units/mL)

Sample solutions: Make three or more dilutions of Antithrombin III Human in the range of the *Standard solution* dilutions.

Blank: *Solution A*

Analysis: [Note—The procedure also can be performed using alternative platforms.] For each *System suitability solution*, *Standard solution*, and *Sample solution*, at least duplicates should be tested. Label a suitable number of tubes depending on the number of replicates that will be tested. For example, if five blanks will be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, and T3 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, and S3 each at least in duplicate for the dilutions of the *Standard solutions* and SS for the *System suitability solution*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [Note—Treat the tubes in the order B1, S1, S2, S3, B2, SS, T1, T2, T3, B3, T1, T2, T3, SS, B4, S1, S2, S3, B5.] In the following order add 1.0 mL of thawed *Sheep plasma substrate* to 1.0 mL of the *Standard solution* dilutions or the *Sample solution* dilutions or the *System suitability solution*. After each addition, mix but do not allow bubbles to form. Transfer each tube to a water bath at 37°, allow to equilibrate at 37° for about 15 min, and add to each tube 1 mL of *APTT reagent* previously heated to 37°. After an appropriate time for the *APTT reagent* used, usually 2–5 min, add 1 mL of *Calcium chloride solution* previously heated to 37° and determine the clotting time. Plot standard concentrations against resulting clotting times and determine heparin content by interpolating from the standard curve using mean sample clotting times. For samples with clotting times longer than the lowest standard dilution, report the result as NMT the lowest *Standard solution* concentration.

System suitability

Samples: *Standard solutions* and *Sample solutions*

The R^2 value of the standard curve is NLT 0.99. The three dilutions of the *Sample solution* give heparin content estimates that differ by NMT 10%. The heparin content in the *System suitability solution* is in the range of 4.0–7.5 IU/mL.

Acceptance criteria: NMT 0.1 USP Heparin Unit/Antithrombin III IU

■ 2S (USP39)

SPECIFIC TESTS

- **Sterility Tests** (71), *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*: Meets the requirements
- **Water Determination** (921), *Method I*: NMT 3.0%
- **Pyrogen Test** (151): Inject 50 USP Antithrombin III Units per kg of the rabbit's weight, calculated from the activity stated on the label: meets the requirements.
- **General Safety**: Meets the requirements for biologics as set forth under *Biological Reactivity Tests, In Vivo* (88), *Safety Tests—Biologicals*
- **Osmolality and Osmolarity** (785), *Osmolality*: Reconstitute with the diluent according to the manufacturer's instruction: NLT 240 mOsm/kg for the solution.
- **pH** (791): Reconstitute with the diluent according to the manufacturer's instruction: 6.0–7.5.

Change to read:

- **Molecular Weight Distribution**

Mobile phase: ~~Solution containing 0.1 M sodium phosphate, 0.15 M sodium chloride, and 0.05% sodium azide, having a pH of 6.5. Degas and filter.~~

- 0.05 M sodium phosphate (dibasic), 0.05 M sodium phosphate (monobasic), 0.4 M arginine monohydrochloride, and 0.05% sodium azide. Adjust with 1 N sodium hydroxide to a pH of 6. Degas and filter. ■ 2S (USP39)

Solution A: 4–5 mg/mL of thyroglobulin in *Mobile phase*

Sample solution: 8–10 mg/mL of Antithrombin III Human

~~**System suitability solution:** Dilute USP Albumin Human RS, if necessary, with water to obtain a solution containing 5%.~~

■ 2S (USP39)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column

Guard: 7.5 mm × 7.5 cm guard column containing packing L59

Analytical: 7.5 mm × 30 cm analytical column containing packing L59

Temperatures

■ **Autosampler:** 7° ■ 2S (USP39)

Column: Ambient

Flow rate: 0.5 mL/min maintained constant to $\pm 1\%$

Injection volume: $\pm 10 \mu\text{L}$

■ 20 μL ■ 2S (USP39)

System suitability

Sample: ~~System suitability solution~~

■ Sample solution ■ 2S (USP39)

Suitability requirements

Column efficiency: NLT ± 500

■ 2000 ■ 2S (USP39)

theoretical plates

Tailing factor: 0.5–2.5

■ 0.9–1.3 ■ 2S (USP39)

Analysis

Samples: *Solution A* and *Sample solution*

Acceptance criteria: Note the retention times of the major peak in the *Solution A* chromatogram. The relative peak area of the high-molecular weight peak eluting at about the same retention time as the major peak in the *Solution A* chromatogram, or earlier, is NMT 13%.

• Total Protein Content

Solution A: 1000 mg/mL of trichloroacetic acid in water

Sample solution: 7.5 mg/mL of Antithrombin III Human in 0.15 M sodium chloride solution

Blank: 0.15 M solution of sodium chloride

Analysis: To each of 2.0 mL of the *Sample solution* and the *Blank* in suitable centrifuge tubes, add 1.5 mL of *Solution A*. Mix, allow to stand for at least 10 min, centrifuge for 5 min, and decant the supernatant. Resuspend the precipitates in 1.5 mL of *Solution A*, centrifuge for 5 min, decant the supernatant, and hold the tubes inverted on a filter paper to drain. Quantitatively transfer the residues with a minimum quantity of water to a micro-Kjeldahl flask, and determine the nitrogen content using *Method II* (see *Nitrogen Determination* (461)). Multiply the result, corrected for the *Blank*, by 6.25 to calculate the quantity of protein.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Use a Type I glass container with an appropriate stopper and seal. Store protected from light between 2° and 8°, excursions permitted up to 25°.
- **Labeling:** The labeling should state the content of antithrombin III in USP Antithrombin III Units. The diluent and the volume to be used to reconstitute the preparation are indicated.

Change to read:

- **USP Reference Standards** (11)

USP Albumin Human RS

■ ■ 2S (USP39)

USP Antithrombin III Human RS

BRIEFING

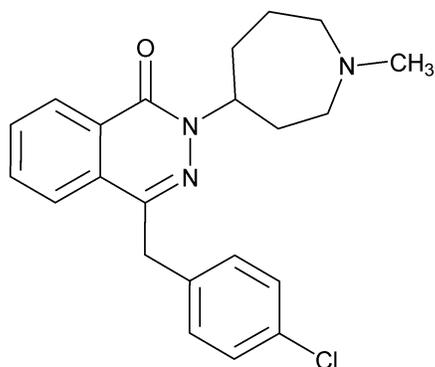
Azelastine Hydrochloride, *USP 38* page 2338. On the basis of comments and supporting data received, it is proposed to revise the relative response factor of chlorophenylacetylbenzoic acid from 1.0 to 0.45.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM5: D. A. Porter.)

Correspondence Number—C157907

Comment deadline: November 30, 2015

Azelastine Hydrochloride

$C_{22}H_{24}ClN_3O \cdot HCl$ 418.36

1(2*H*)-Phthalazinone, 4-[(4-chlorophenyl)methyl]-2-(hexahydro-1-methyl-1*H*-azepin-4-yl), monohydrochloride;

4-(*p*-Chlorobenzyl)-2-(hexahydro-1-methyl-1*H*-azepin-4-yl)-1(2*H*)-phthalazinone monohydrochloride [79307-93-0].

DEFINITION

Azelastine Hydrochloride contains NLT 99.0% and NMT 101.0% of azelastine hydrochloride ($C_{22}H_{24}ClN_3O \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Identification solution*, as obtained in the test for *Organic Impurities*.
- **C. Identification Tests—General** (191), *Chloride*: Meets the requirements

ASSAY

- **Procedure**

Sample: 300 mg

Blank: 5 mL of anhydrous formic acid and 30 mL of acetic anhydride

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the endpoint has been reached.

Dissolve the *Sample* in 5 mL of anhydrous formic acid, and add 30 mL of acetic anhydride. Titrate with *Titrant*.

Calculate the percentage of azelastine hydrochloride ($C_{22}H_{24}ClN_3O \cdot HCl$) in the portion of Azelastine Hydrochloride taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F] / W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 41.84 mg/mEq

W = weight of the *Sample* (mg)

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.2%

Delete the following:

- ~~**Heavy Metals** (231), *Method II*: NMT 20 ppm~~
- (Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

Dilute phosphoric acid: 115 g/L of phosphoric acid in water

Buffer: 2.92 g/L of octanesulfonic acid sodium salt and 0.92 g/L of monobasic potassium phosphate in water. Adjust with *Dilute phosphoric acid* to a pH of 3.0–3.1.

Mobile phase: Acetonitrile and *Buffer* (260:740)

Diluent: Acetonitrile and water (45:55)

Identification solution: 2.5 mg/mL of USP Azelastine Hydrochloride RS in *Diluent*. [Note —This solution is used for *Identification* test B.]

System suitability stock solution: 0.5 mg/mL each of USP Azelastine Related Compound B RS, USP Azelastine Related Compound D RS, and USP Azelastine Related Compound E RS in acetonitrile

System suitability solution: 50 µg/mL each of USP Azelastine Related Compound B RS, USP Azelastine Related Compound D RS, and USP Azelastine Related Compound E RS from

the *System suitability stock solution* and 2.5 mg/mL of USP Azelastine Hydrochloride RS in *Diluent*

Standard stock solution: 50 µg/mL of USP Azelastine Hydrochloride RS in acetonitrile

Standard solution: 2.5 µg/mL of USP Azelastine Hydrochloride RS in *Diluent*

Sample solution: 2.5 mg/mL of Azelastine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 10-µm packing *L10*

Column temperature: 30°

Flow rate: 2 mL/min

Injection volume: 10 µL

Run time: 2.4 times the retention time of azelastine

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between azelastine related compound B and azelastine related compound D; NLT 1.5 between azelastine related compound D and azelastine; NLT 1.5 between azelastine and azelastine related compound E, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Identification solution*, *Standard solution*, and *Sample solution*

Calculate the percentage of each impurity in the portion of Azelastine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of azelastine from the *Standard solution*

C_S = concentration of USP Azelastine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Azelastine Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. [Note—Disregard peaks that are less than 0.05% of the azelastine peak.]

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzohydrazide	0.2	0.38	0.1
Azelastine related compound B ^a	0.3	0.22	0.1
Chlorophenylacetylbenzoic acid ^b	0.4	1.0 ■ 0.45 ■ 2S (USP39)	0.1
Azelastine related compound D ^c	0.6	1.2	0.1
Azelastine	1.0	1.0	—
Azelastine related compound E ^d	1.4	0.48	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.2

^a *N*-(1-Methylazepan-4-yl)benzohydrazide; also known as 1-benzoyl-2-[(4*RS*)-1-methylhexahydro-1*H*-azepin-4-yl]diazane.
^b 2-[2-(4-Chlorophenyl)acetyl]benzoic acid.
^c 4-(4-Chlorobenzyl)phthalazin-1(2*H*)-one.
^d 3-(4-Chlorobenzylidene)isobenzofuran-1(3*H*)-one.

SPECIFIC TESTS

- **Loss on Drying** (731)

Analysis: Dry at 105° to constant weight.

Acceptance criteria: NMT 1.0%

- **Acidity or Alkalinity**

Sample solution: 10 mg/mL of Azelastine Hydrochloride in water

Analysis: Add 0.2 mL of bromothymol blue TS to 10 mL of the *Sample solution*.

Acceptance criteria: NMT 0.1 mL of 0.01 M hydrochloric acid or 0.01 M sodium hydroxide is required to produce a color change.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Protect from light and moisture. Store at controlled room temperature.

- **USP Reference Standards** (11)

USP Azelastine Hydrochloride RS

USP Azelastine Related Compound B RS

N-(1-Methylazepan-4-yl)benzohydrazide.

C₁₄H₂₁N₃O 247.34

USP Azelastine Related Compound D RS

4-(4-Chlorobenzyl)phthalazin-1(2*H*)-one.

C₁₅H₁₁ClN₂O 270.71

USP Azelastine Related Compound E RS

3-(4-Chlorobenzylidene)isobenzofuran-1(3*H*)-one.



BRIEFING

Betamethasone Valerate Cream, *USP 38* page 2423. As part of the USP monograph modernization initiative, it is proposed to make the following changes:

1. Replace the TLC procedure in *Identification* test A with a test based on the retention time agreement in the proposed *Assay*.
2. Add *Identification* test B based on the UV spectrum agreement of the major peak in the chromatogram of the sample and standard in the proposed *Assay*.
3. Replace the HPLC procedure in the *Assay*, which uses an internal standard, with a new HPLC procedure similar to the one in the proposed *Organic Impurities* test. The chromatographic procedure is based on analyses performed with the Agilent Zorbax SB-C18 brand of L1 column. The typical retention time for betamethasone valerate is about 16 min.
4. Add the test for *Organic Impurities*. The proposed procedure uses the same chromatographic system as in the proposed *Assay* to be operationally efficient.
5. Add proposed acceptance criteria for specified and unspecified degradation products to the *Organic Impurities* section.
6. Add USP Betamethasone RS and USP Betamethasone Valerate Related Compound A RS to the *USP Reference Standards* section to support the proposed revision. Remove USP Beclomethasone Dipropionate RS, which is no longer needed as an internal standard in the proposed procedures, from the *USP Reference Standards* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM5: G. Hsu.)

Correspondence Number—C143025

Comment deadline: November 30, 2015

Betamethasone Valerate Cream

DEFINITION

Betamethasone Valerate Cream contains an amount of betamethasone valerate ($\text{C}_{27}\text{H}_{37}\text{FO}_6$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ($\text{C}_{22}\text{H}_{29}\text{FO}_5$), in a suitable cream base.

IDENTIFICATION

Delete the following:

- ~~**A. Thin-Layer Chromatographic Identification Test** (201)~~

~~**Standard solution:** 1 mg/mL of USP Betamethasone Valerate RS in alcohol~~

~~**Sample solution:** Transfer an amount of Cream, equivalent to 2 mg of betamethasone, to a separator, add 20 mL of water and 2 mL of dilute hydrochloric acid (1 in 120), and mix. Extract with four 50 mL portions of chloroform, and combine the extracts. Filter through a~~

~~cotton pledget, previously layered over with anhydrous sodium sulfate. Evaporate the filtrates on a steam bath under a stream of dry nitrogen to dryness. Dissolve the residue in alcohol to obtain a solution containing about 1 mg/mL.~~

Developing solvent system: Toluene and ethyl acetate (1:1)

Application volume: 10 μ L

Analysis

Samples: *Sample solution* and *Standard solution*

~~Proceed as directed in the chapter. Spray the plate with a mixture of sulfuric acid, methanol, and nitric acid (10:10:1), and heat at 105° for 15 min.~~

■ 2S (USP39)

Add the following:

- ● **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

Add the following:

- ● **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

ASSAY

Change to read:

● Procedure

Mobile phase: ~~Acetonitrile and water (3:2)~~

Diluent: ~~Glacial acetic acid in methanol (1 in 1000)~~

Internal standard solution: ~~0.4 mg/mL of USP Beclomethasone Dipropionate RS in Diluent~~

Standard stock solution: ~~0.6 mg/mL of USP Betamethasone Valerate RS in Diluent~~

Standard solution: ~~0.2 mg/mL of USP Betamethasone Valerate RS prepared as follows. Transfer 5.0 mL of Standard stock solution to a suitable stoppered vial, add 10.0 mL of Internal standard solution, and mix.~~

Sample solution: ~~Nominally 0.17 mg/mL of betamethasone prepared as follows. Transfer a portion of Cream, equivalent to about 2.5 mg of betamethasone, to a 50 mL centrifuge tube. Add 10.0 mL of the Internal standard solution and 5.0 mL of Diluent. Insert the stopper into the tube, and place in a water bath held at 60° until the specimen melts. Remove from the bath, and shake vigorously until the specimen resolidifies. Repeat the heating and shaking two more times. Place the tube in an ice-methanol bath for 20 min, then centrifuge to separate the phases. Decant the clear supernatant into a suitable stoppered flask, and allow to warm to room temperature.~~

Chromatographic system

~~(See Chromatography (621), System Suitability.)~~

Mode: LC

Detector: UV 254 nm

Column: 4 mm \times 30 cm; packing L1

~~**Flow rate:** 1.2 mL/min~~

~~**Injection volume:** 10 μ L~~

~~**System suitability**~~

~~**Sample:** *Standard solution*~~

~~[Note—The relative retention times for betamethasone valerate and beclomethasone dipropionate are 1.0 and 1.7, respectively.]~~

~~**Suitability requirements**~~

~~**Resolution:** NLT 4.5 between betamethasone valerate and beclomethasone dipropionate~~

~~**Relative standard deviation:** NMT 2.0%~~

~~**Analysis**~~

~~**Samples:** *Standard solution and Sample solution*~~

Calculate the percentage of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of the betamethasone valerate peak to the internal standard peak from the *Sample solution*

R_S = peak response ratio of the betamethasone valerate peak to the internal standard peak from the *Standard solution*

C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of betamethasone, 392.46

M_{r2} = molecular weight of betamethasone valerate, 476.59

▪ **Solution A:** Water

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	63	37
7.0	63	37
15.0	30	70
19.0	30	70
19.1	10	90
21.0	10	90
21.1	63	37
25.0	63	37

Diluent A: Tetrahydrofuran and water (50:50)

Diluent B: Acetonitrile and water (40:60)

System suitability solution: 25 µg/mL of USP Betamethasone Valerate RS and 10 µg/mL of USP Betamethasone Valerate Related Compound A RS in *Diluent B*. Sonicate to dissolve if necessary.

Standard solution: 25 µg/mL of USP Betamethasone Valerate RS in *Diluent B*. Sonicate to dissolve if necessary.

Sample solution: Nominally 20 µg/mL of betamethasone, prepared as follows. Transfer 1.0 mg of betamethasone from a portion of Cream to a suitable glass centrifuge tube. Add 15.0 mL of *Diluent A* and mix with a vortex mixer to disperse the sample thoroughly. Add 35.0 mL of *Diluent B* and sonicate for 10 min with intermittent shaking. Centrifuge to obtain a clear supernatant. Pass through a suitable filter of 0.2-µm pore size using a glass syringe. Discard the first 1 mL.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm. For *Identification* test B, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Temperatures

Autosampler: 4°

Column: Ambient

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betamethasone (C₂₂H₂₉FO₅) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of betamethasone in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of betamethasone, 392.46

M_{r2} = molecular weight of betamethasone valerate, 476.58

■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

■ • Organic Impurities

Solution A, Solution B, Mobile phase, Diluent A, Diluent B, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 0.25 µg/mL each of USP Betamethasone RS, USP Betamethasone Valerate RS, and USP Betamethasone Valerate Related Compound A RS in *Diluent B*. Sonicate to dissolve if necessary.

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*

Relative standard deviation: NMT 5%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each specified degradation product in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified degradation product from the *Sample solution*

r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*

C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (µg/mL)

C_U = nominal concentration of betamethasone in the *Sample solution* (µg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of betamethasone valerate from the *Standard solution*

C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of betamethasone in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of betamethasone, 392.46

M_{r2} = molecular weight of betamethasone valerate, 476.58

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Betamethasone	0.30	1.0
Betamethasone valerate	1.00	—
Betamethasone valerate related compound A	1.04	1.0
Any individual unspecified degradation product	—	1.0
Total degradation products	—	2.0

■ 2S (USP39)

SPECIFIC TESTS

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **Minimum Fill** (755): Meets the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in collapsible tubes or in tight containers.

Change to read:

- **USP Reference Standards** (11)

USP Beclomethasone Dipropionate RS

- USP Betamethasone RS

■ 2S (USP39)

USP Betamethasone Valerate RS

- USP Betamethasone Valerate Related Compound A RS

9-Fluoro-11 β ,17-dihydroxy-16 β -methyl-3,20-dioxopregna-1,4-dien-21-yl valerate.

$C_{27}H_{37}FO_6$ 476.58

■ 2S (USP39)

BRIEFING

Betamethasone Valerate Lotion, USP 38 page 2424. As part of the USP monograph modernization initiative, it is proposed to make the following changes:

1. Replace the TLC procedure in *Identification* test A with a test based on the retention time agreement in the proposed *Assay*.
2. Add *Identification* test B based on the UV spectrum agreement in the proposed *Assay*.
3. Replace the LC procedure in the *Assay*, which uses an internal standard in chloroform, with a new LC procedure that uses the same chromatographic system as the *Organic Impurities* test to be operationally efficient.
4. Add the test for *Organic Impurities*. The proposed procedure is based on analyses

performed with the Agilent Zorbax SB-C18 brand of L1 column. The typical retention time for betamethasone valerate is about 16 min.

5. Add proposed acceptance criteria for specified and unspecified impurities to the monograph. Manufacturers are encouraged to submit their approved specifications to USP if they are different from those proposed in this revision.
6. Add USP Betamethasone RS and USP Betamethasone Valerate Related Compound A RS to the *USP Reference Standards* section to support the proposed revision.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM5: G. Hsu.)

Correspondence Number—C143027

Comment deadline: November 30, 2015

Betamethasone Valerate Lotion

DEFINITION

Betamethasone Valerate Lotion contains an amount of Betamethasone Valerate ($C_{27}H_{37}FO_6$) equivalent to NLT 95.0% and NMT 115.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$).

IDENTIFICATION

Delete the following:

■ ● ~~A. Thin-Layer Chromatography~~

~~**Standard solution:** 0.6 mg/mL of USP Betamethasone Valerate RS in a mixture of methanol and chloroform (2:1)~~

~~**Sample solution:** Equivalent to 0.5 mg/mL of betamethasone from Lotion, in a mixture of methanol and chloroform (2:1)~~

~~**Chromatographic system** (See *Chromatography* (621) *Thin-Layer Chromatography*.)~~

~~**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture~~

~~**Application volume:** 20 μ L~~

~~**Developing solvent system:** Chloroform and ethyl acetate (1:1)~~

~~Analysis~~

~~**Samples:** *Standard solution* and *Sample solution*~~

~~Allow the spots to dry, and develop the chromatogram in a solvent system, until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. View the spots under UV light.~~

~~**Acceptance criteria:** The R_f value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*. ■ 2S (USP39)~~

Add the following:

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

Add the following:

- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

ASSAY

Change to read:

• **Procedure**

Mobile phase: ~~Acetonitrile and water (3:2)~~

Diluent: ~~Glacial acetic acid in methanol (1 in 1000)~~

Internal standard solution: ~~2 mg/mL of beclomethasone dipropionate in chloroform~~

Standard stock solution: ~~1.6 mg/mL of USP Betamethasone Valerate RS in chloroform~~

Standard solution: ~~Pipet 2 mL of *Standard stock solution* into a 50 mL centrifuge tube, add 10 mL of 0.1 N hydrochloric acid, then add 2.0 mL of *Internal standard solution*. Insert the stopper into the tube, shake vigorously for 2 min, and centrifuge to separate the phases. Using a syringe, transfer the lower chloroform phase to a small stoppered vial. Evaporate the chloroform on a steam bath, at low heat, with the aid of a stream of nitrogen. Add 4.0 mL of *Diluent*, and swirl to dissolve the residue.~~

Sample solution: ~~Transfer an amount equivalent to 2.5 mg of betamethasone from Lotion to a stoppered, 50 mL centrifuge tube. Add 10.0 mL of 0.1 N hydrochloric acid, insert the stopper, and shake to disperse the specimen. Add 2.0 mL of chloroform and 2.0 mL of *Internal standard solution*. Insert the stopper into the tube, shake vigorously for 2 min, and centrifuge to separate the phases. Using a syringe, transfer the lower chloroform phase to a small stoppered vial. Evaporate the chloroform on a steam bath, at low heat, with the aid of a stream of nitrogen. Add 4.0 mL of *Diluent*, and swirl to dissolve the residue.~~

Chromatographic system

~~(See *Chromatography* (621), *System Suitability*.)~~

Mode: ~~LC~~

Detector: ~~UV 254 nm~~

Column: ~~4 mm × 30 cm; packing L1~~

Flow rate: ~~1.2 mL/min~~

Injection volume: ~~10 µL~~

System suitability

Sample: ~~*Standard solution*~~

~~[Note—The relative retention times for betamethasone valerate and beclomethasone dipropionate are 1.0 and 1.7, respectively.]~~

Suitability requirements

Resolution: ~~NLT 4.5 between betamethasone valerate and beclomethasone dipropionate~~

Relative standard deviation: ~~NMT 2.0%~~

Analysis

Samples: ~~Standard solution and Sample solution~~

Calculate the percentage of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio from the *Sample solution*

R_S = peak response ratio from the *Standard solution*

C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

M_{r1} = molecular weight of betamethasone, 392.46

M_{r2} = molecular weight of betamethasone valerate, 476.59

▪ **Solution A:** Water

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	63	37
7.0	63	37
15.0	30	70
19.0	30	70
19.1	10	90
21.0	10	90
21.1	63	37
25.0	63	37

Diluent: Acetonitrile and water (40:60)

System suitability solution: 0.05 mg/mL of USP Betamethasone Valerate RS and 0.01 mg/mL of USP Betamethasone Valerate Related Compound A RS in *Diluent*. Sonicate to dissolve if necessary.

Standard solution: 0.05 mg/mL of USP Betamethasone Valerate RS in *Diluent*. Sonicate to dissolve if necessary.

Sample solution: Nominally 0.04 mg/mL of betamethasone in *Diluent*, prepared as follows. Accurately weigh and transfer a portion of Lotion to a suitable volumetric flask. Add about 80% of the final flask volume of *Diluent*. Sonicate for about 5 min. Dilute with *Diluent* to volume. Pass through a suitable filter of 0.2- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm. For *Identification* test B, use a diode-array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

Temperatures

Column: Ambient

Autosampler: 4°

Flow rate: 1 mL/min

Injection volume: 50 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betamethasone (C₂₂H₂₉FO₅) in the portion of Lotion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of betamethasone, 392.46

M_{r2} = molecular weight of betamethasone valerate, 476.58

■ 2S (USP39)

Acceptance criteria: 95.0%–115.0%

IMPURITIES

Add the following:

■ ● **Organic Impurities**

Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.001 mg/mL each of USP Betamethasone RS, USP Betamethasone Valerate RS, and USP Betamethasone Valerate Related Compound A RS in *Diluent*. Sonicate to dissolve if necessary.

System suitability

Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each specified degradation product in the portion of Lotion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified degradation product from the *Sample solution*

r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*

C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

C_U = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Lotion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of betamethasone valerate from the *Standard solution*

C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of betamethasone, 392.46

M_{r2} = molecular weight of betamethasone valerate, 476.58

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Betamethasone	0.30	1.0
Betamethasone valerate	1.00	—
Betamethasone valerate related compound A	1.04	1.0
Any individual unspecified degradation product	—	1.0
Total degradation products	—	2.0

■ 2S (USP39)

SPECIFIC TESTS

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*
- **Minimum Fill** (755): Meets the requirements
- **pH** (791): 4.0–6.0

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

Change to read:

- **USP Reference Standards** (11)
 - USP Betamethasone RS
- 2S (USP39)
 - USP Betamethasone Valerate RS
 - USP Betamethasone Valerate Related Compound A RS
 - 9-Fluoro-11 β ,17-dihydroxy-16 β -methyl-3,20-dioxopregna-1,4-dien-21-yl valerate.
 - C₂₇H₃₇FO₆ 476.58

■ 2S (USP39)

BRIEFING

Betamethasone Valerate Ointment, USP 38 page 2424. As part of the USP monograph modernization initiative, it is proposed to make the following changes:

1. Replace the TLC procedure in *Identification* test A with a test based on the retention time agreement in the proposed *Assay*.
2. Add *Identification* test B based on the UV spectrum agreement in the proposed *Assay*.
3. Replace the LC procedure in the *Assay*, which uses an internal standard, with a new LC procedure which uses the same chromatographic system as the *Organic Impurities* test to be operationally efficient.
4. Add the test for *Organic Impurities*. The proposed procedure is based on analyses performed with the Agilent Zorbax SB-C18 brand of L1 column. The typical retention time for betamethasone valerate is about 16 min.
5. Add proposed acceptance criteria for specified and unspecified impurities to the monograph. Manufacturers are encouraged to submit their approved specifications to

USP if they are different from those proposed in this revision.

6. Add USP Betamethasone RS and USP Betamethasone Valerate Related Compound A RS to the *USP Reference Standards* section to support the proposed revision.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM5: G. Hsu.)

Correspondence Number—C143030

Comment deadline: November 30, 2015

Betamethasone Valerate Ointment

DEFINITION

Betamethasone Valerate Ointment contains an amount of Betamethasone Valerate ($C_{27}H_{37}FO_6$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$), in a suitable ointment base.

IDENTIFICATION

Delete the following:

- **A. Thin-Layer Chromatographic Identification Test** ~~(201)~~

Standard solution: 1 mg/mL of USP Betamethasone Valerate RS in alcohol

Sample solution: Transfer the equivalent to 2 mg of betamethasone from the Ointment to a separator. Add 20 mL of water and 2 mL of dilute hydrochloric acid (1 in 120). Extract with four 50 mL portions of chloroform, and combine the extracts. Filter through a cotton pledget, previously layered over with anhydrous sodium sulfate. Evaporate the filtrates on a steam bath under a stream of dry nitrogen to dryness. Dissolve the residue in alcohol to obtain a solution containing 1 mg/mL.

Chromatographic system (See *Chromatography* ~~(621)~~ *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Developing solvent system: *Toluene and ethyl acetate (1:1)*

Spray reagent: *A mixture of sulfuric acid, methanol, and nitric acid (10:10:1)*

Analysis

Samples: *Sample solution and Standard solution*

When the solvent front has moved three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow to air-dry. Locate the spots on the plate by spraying lightly with *Spray Reagent*, and dry at 105° for 15 min.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. ■ 2S (USP39)

Add the following:

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the

Standard solution, as obtained in the Assay. ■2S (USP39)

Add the following:

- ● **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ■2S (USP39)

ASSAY

Change to read:

● **Procedure**

~~**Mobile phase:** Acetonitrile and water (3:2)~~

~~**Diluent:** Glacial acetic acid in alcohol (1 in 1000)~~

~~**Internal standard solution:** 0.4 mg/mL of beclomethasone dipropionate in *Diluent*~~

~~**Standard stock solution:** 0.6 mg/mL of USP Betamethasone Valerate RS in *Diluent*~~

~~**Standard solution:** 0.2 mg/mL of USP Betamethasone Valerate RS in *Diluent* prepared as follows. Transfer 5.0 mL of *Standard stock solution* to a suitable stoppered vial, add 10.0 mL of the *Internal standard solution*.~~

~~**Sample solution:** Nominally 0.17 mg/mL of betamethasone from the Ointment prepared as follows. Transfer 2.5 mg of betamethasone from a portion of Ointment to a 50-mL centrifuge tube. Add 10.0 mL of the *Internal standard solution* and 5.0 mL of *Diluent*. Insert the stopper into the tube, and place in a water bath held at 70° until the specimen melts. Remove from the bath, and shake vigorously until the specimen resolidifies. Repeat the heating and shaking two more times. Place the tube in an ice-methanol bath for 20 min, then centrifuge to separate the phases. Decant the clear supernatant into a suitable stoppered flask, and allow to warm to room temperature.~~

~~**Chromatographic system**~~

~~(See *Chromatography* (621), *System Suitability*.)~~

~~**Mode:** LC~~

~~**Detector:** UV 254 nm~~

~~**Column:** 4 mm × 30 cm; packing L1~~

~~**Flow rate:** 1.2 mL/min~~

~~**Injection volume:** 10 µL~~

~~**System suitability**~~

~~**Sample:** *Standard solution*~~

~~[Note—The relative retention times for betamethasone valerate and beclomethasone dipropionate are 1.0 and 1.7, respectively.]~~

~~**Suitability requirements**~~

~~**Resolution:** NLT 4.5 between betamethasone valerate and beclomethasone dipropionate~~

~~**Relative standard deviation:** NMT 2.0%~~

~~**Analysis**~~

~~**Samples:** *Standard solution* and *Sample solution*~~

Calculate the percentage of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of the betamethasone valerate peak to the internal standard peak from the *Sample solution*

R_S = peak response ratio of the betamethasone valerate peak to the internal standard peak from the *Standard solution*

C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of betamethasone, 392.46

M_{r2} = molecular weight of betamethasone valerate, 476.59

▪ **Solution A:** Water

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	63	37
7.0	63	37
15.0	30	70
19.0	30	70
19.1	10	90
21.0	10	90
21.1	63	37
25.0	63	37

Diluent A: Tetrahydrofuran and water (50:50)

Diluent B: Acetonitrile and water (40:60)

System suitability solution: 25 µg/mL of USP Betamethasone Valerate RS and 10 µg/mL of USP Betamethasone Valerate Related Compound A RS in *Diluent B*. Sonicate to dissolve if necessary.

Standard solution: 25 µg/mL of USP Betamethasone Valerate RS in *Diluent B*. Sonicate to dissolve if necessary.

Sample solution: Nominally 20 µg/mL of betamethasone, prepared as follows. Transfer 1.0 mg of betamethasone from a portion of Ointment to a suitable glass centrifuge tube. Add 15.0 mL of *Diluent A* and mix with a vortex mixer to disperse the sample thoroughly. Add 35.0 mL of *Diluent B* and sonicate for 10 min with intermittent shaking. Centrifuge to obtain a clear supernatant and use the clear supernatant.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm. For *Identification* test B, use a diode-array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 100 μL

Autosampler temperature: 4°

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betamethasone (C₂₂H₂₉FO₅) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (μg/mL)

C_U = nominal concentration of betamethasone in the *Sample solution* (μg/mL)

M_{r1} = molecular weight of betamethasone, 392.46

M_{r2} = molecular weight of betamethasone valerate, 476.58

■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

■ ● Organic Impurities

Solution A, Solution B, Mobile phase, Diluent A, Diluent B, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.25 μg/mL each of USP Betamethasone RS, USP Betamethasone Valerate RS, and USP Betamethasone Valerate Related Compound A RS in *Diluent B*.

Sonicate to dissolve if necessary.

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each specified degradation product in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified degradation product from the *Sample solution*

r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*

C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of betamethasone in the *Sample solution* ($\mu\text{g/mL}$)

Calculate the percentage of each unspecified degradation product in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of betamethasone valerate from the *Standard solution*

C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of betamethasone in the *Sample solution* ($\mu\text{g/mL}$)

M_{r1} = molecular weight of betamethasone, 392.46

M_{r2} = molecular weight of betamethasone valerate, 476.58

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Betamethasone	0.30	1.0
Betamethasone valerate	1.00	—
Betamethasone valerate related compound A	1.04	1.0
Any individual unspecified degradation product	—	1.0
Total degradation products	—	2.0

■ 2S (USP39)

SPECIFIC TESTS

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*
- **Minimum Fill** (755): Meets the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in collapsible tubes or in tight containers, and avoid exposure to excessive heat.

Change to read:

- **USP Reference Standards** (11)

■ USP Betamethasone RS

■ 2S (USP39)

USP Betamethasone Valerate RS

■ USP Betamethasone Valerate Related Compound A RS

9-Fluoro-11 β ,17-dihydroxy-16 β -methyl-3,20-dioxopregna-1,4-dien-21-yl valerate.

C₂₇H₃₇FO₆ 476.58

■ 2S (USP39)

BRIEFING

Bismuth Subsalicylate Oral Suspension, USP 38 page 2455. On the basis of comments received, it is proposed to revise the monograph as follows:

1. Clarify requirements for *Identification* test *B* based on the supporting data.
2. Widen the upper pH limit from 5.0 to 5.5 to accommodate products with different formulations.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM6: H. Cai.)

Correspondence Number—C141882; C137544

Comment deadline: November 30, 2015

Bismuth Subsalicylate Oral Suspension

DEFINITION

Bismuth Subsalicylate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of bismuth subsalicylate ($C_7H_5BiO_4$). It may contain one or more suitable buffers, coloring agents, flavors, preservatives, stabilizers, sweeteners, and suspending agents.

IDENTIFICATION

- **A. Identification Tests—General** (191), *Bismuth*: Meets the requirements

Change to read:

- **B. Identification Tests—General** (191), *Salicylate*: ~~Meets the requirements after acidifying with nitric acid~~
 - Meets the requirements for the response to *ferric chloride TS* after acidifying with *nitric acid* ■ 2S (USP39)

ASSAY

- **Procedure**

Standard stock solution: 2.5 mg/mL of *bismuth* in *nitric acid*. Prepare by dissolving in 6% of the flask volume of *nitric acid* and diluting with 0.01 N nitric acid to volume.

Standard solution: 0.05 mg/mL of *bismuth* in 1 N nitric acid from the *Standard stock solution*

Sample solution: Transfer 10 g of Oral Suspension, previously well shaken in its original container to ensure homogeneity, to a 200-mL volumetric flask. Add about 100 mL of 1 N nitric acid, and dilute with 1 N nitric acid to volume. Mix well without shaking, transfer 10.0 mL of this mixture to a 100-mL volumetric flask, and dilute with 1 N nitric acid to volume. Centrifuge about 20 mL at 4500 rpm for at least 10 min.

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

Mode: UV-Vis

Analytical wavelength: 463 nm

Cell: 1 cm

Blank: 1 N nitric acid

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Transfer a measured volume of the *Sample solution* that contains 0.9 mg of bismuth subsalicylate and 10 mL of the *Standard solution* to separate 50-mL volumetric flasks. Add 10.0 mL of 10% ascorbic acid solution and 25.0 mL of 20% potassium iodide solution to each volumetric flask, and dilute with water to volume. Concomitantly determine the absorbances of both solutions, using the *Blank* to set the spectrophotometer. Calculate the percentage of the labeled amount of bismuth subsalicylate ($C_7H_5BiO_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of bismuth in the *Standard solution* (mg/mL)

C_U = nominal concentration of bismuth subsalicylate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of bismuth subsalicylate, 362.09

M_{r2} = molecular weight of bismuth, 208.98

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): The total aerobic microbial count is NMT 10^2 cfu/g, and the total combined molds and yeasts count is NMT 5×10^1 cfu/g. It meets the requirements of the tests for the absence of *Escherichia coli*.

Change to read:

- **pH** (791): 3.0–5.0
 - 5.5 (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Protect from freezing. Avoid excessive heat (over 40°).

BRIEFING

Bivalirudin. Because there is no existing *USP* monograph for this drug substance, a new monograph based on validated methods of analyses is being proposed. The liquid chromatographic procedure in the *Assay* is based on analyses performed with the Vydac C18 brand of *L1* column. The typical retention time for bivalirudin in the *Assay* is about 24 min. The liquid chromatographic procedures in the *Product Related Substances and Impurities* are based on analyses performed with the Vydac C18 brand of *L1* column and Waters Xbridge HILIC brand of *L3* column. The typical retention times for bivalirudin in the tests for *Product-Related Substances and Impurities* are about 24 min and between 9.4 and 14.7 min, respectively.

(BIO1: A. Szajek.)

Correspondence Number—C149943

Comment deadline: November 30, 2015

Add the following:

- **Bivalirudin**

DFPRPGGGGNG DFEEIPEEYL

$C_{98}H_{138}N_{24}O_{33}$ 2180.29 (free base)

L-Leucine, D-phenylalanyl-L-prolyl-L-arginyl-L-prolylglycylglycylglycylglycyl-L-asparaginyglycyl-L- α -aspartyl-L-phenylalanyl-L- α -glutamyl-L- α -glutamyl-L-isoleucyl-L-prolyl-L- α -glutamyl-L- α -glutamyl-L-tyrosyl-;

D-Phenylalanyl-L-prolyl-L-arginyl-L-prolylglycylglycylglycylglycyl-L-asparaginyglycyl-L- α -aspartyl-L-phenylalanyl-L- α -glutamyl-L- α -glutamyl-L-isoleucyl-L-prolyl-L- α -glutamyl-L- α -glutamyl-L-tyrosyl-L-leucine [128270-60-0].

DEFINITION

Bivalirudin is a synthetic 20 amino acid peptide, which is a specific and reversible direct thrombin inhibitor. Bivalirudin is indicated for use as an anticoagulant in patients with unstable angina undergoing percutaneous transluminal coronary angioplasty (PTCA). Bivalirudin is intended for use with aspirin and has been studied only in patients receiving concomitant aspirin. It contains NLT 96.0% and NMT 103.0% of bivalirudin ($C_{98}H_{138}N_{24}O_{33}$), calculated on the anhydrous, counter ion-free basis. Bivalirudin is a white to off-white powder. [Note—Bivalirudin is very hygroscopic. Protect from exposure to moisture.]

IDENTIFICATION

• A

Standard solution and Sample solution: Proceed as directed in the Assay.

Identity sample solution: 0.75 mg/mL each of USP Bivalirudin RS and Bivalirudin in water

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. The major peaks of the *Identity sample solution* co-elute.

• **B.** The monoisotopic mass by *Mass Spectrometry* (736) is 2179.0 ± 1.0 mass units.

• C. Amino Acid Content

For further discussion of the theory and applications, see *Biotechnology-Derived Articles—Amino Acid Analysis* (1052), which may be a helpful, but not mandatory, resource.

Standard solution: Standardize the instrument with a mixture containing equal molar per volume amounts (except for L-cystine, which is half the molar amount) of glycine and the L-form of the following amino acids: lysine, threonine, alanine, leucine, histidine, serine, valine, tyrosine, arginine, glutamic acid, methionine, phenylalanine, aspartic acid, proline, isoleucine, tryptophan, and cystine.

Sample solution: Accurately weigh out 1.0 mg of bivalirudin in glass ampuls. Add a minimum of 1.0 mL of *Hydrolysis Solution* containing 4% phenol, freeze the sample ampul, and flame seal under vacuum. Hydrolyze at 110° for about 18 h. After hydrolysis, dry the test sample under vacuum to remove any residual acid. To the ampul add 2 mL of a buffer solution that is suitable for the amino acid analyzer, and pass through a filter of 0.45- μ m pore size.

Analysis

Samples: *Standard solution and Sample solution*

First record and measure the responses for each amino acid peak in the *Standard solution*.

Express the content of each amino acid in moles.

Calculate the mean nmol of the amino acids in the *Sample solution*:

Result = (nmol found in the *Sample solution* for Asx, Glx, Gly, Leu, Phe, Ile, Arg, Pro, Tyr)/20

Divide the nmol of each amino acid by the *Result* to determine the amino acid ratios that must meet the *Acceptance criteria*.

Acceptance criteria: See *Table 1*.

Table 1

Name	Acceptance Criteria
Glycine	Between 4.5 and 5.5
Aspartic acid	Between 1.8 and 2.2
Glutamic acid	Between 3.6 and 4.4
Proline	Between 2.7 and 3.3
Leucine, isoleucine, arginine, and tyrosine	Between 0.9 and 1.1
Phenylalanine	Between 1.8 and 2.2

• D. Bioidentity

Thrombin inhibition activity

Buffer solution: 50 mM tris-HCl and 120 mM sodium chloride in water. Adjust to a pH of 7.40 ± 0.04 . Add bovine serum albumin (BSA) to obtain a 100-mg/mL concentration and pass through a filter of 0.45- μ m pore size.

Human thrombin solution: 10 μ g/mL of human thrombin¹ in *Buffer solution*

Diluted human thrombin solution: Dilute *Human thrombin solution* in *Buffer solution* to obtain a 0.5- μ g/mL solution. Thrombin from alternate sources must be standardized in the substrate reaction. Prepare six replicates by adding 910 μ L of *Buffer solution* to each sample tube followed by 30 μ L of *Chromogenic substrate solution*. Add 60 μ L of *Diluted human thrombin solution*, vortex, and begin the 20-min incubation as described below. Determine the absorbance at 405 nm for each replicate.

Calculate the standardized volume (E_C) of *Diluted human thrombin solution* from the mean absorbance as follows:

$$E_C = (0.45 \times 60 \mu\text{L}) / \text{mean absorbance}$$

Chromogenic substrate solution: 5 mM solution of H-d-cyclohexylalanyl-Ala-Arg-p-Nitroanilide diacetate salt in water. Dilute with water to obtain a 50- μ M solution.

Standard solution: Prepare a 0.6-mg/mL solution of USP Bivalirudin RS in water. Dilute with *Buffer solution* to obtain a 5- μ g/mL solution.

Sample solution: Prepare a 0.6-mg/mL solution of Bivalirudin in water. Measure the absorbance of the 0.6-mg/mL solution at 275 nm (A_{275}), using the *Blank* to auto-zero. Calculate the concentration of bivalirudin ($C_{98}H_{138}N_{24}O_{33}$) in the portion of Bivalirudin taken:

$$C_{\text{bivalirudin}} (\text{mg/mL}) = A_{275} / 0.62$$

From this concentration, dilute with *Buffer solution* to obtain a 5- μ g/mL solution. Prepare triplicate dilutions of the *Sample solution*.

Blank: Water

Stop solution: Glacial acetic acid

Preparation of Control assay solution, Sample assay solution, and Standard assay solution: In each sample tube, add the *Buffer solution* (1000 μL – E_C – S_C) first, then 30 μL of *Chromogenic substrate solution* (S_C), then the Bivalirudin (if using). Vortex and incubate at 37° for 10 min in a water bath. Add the appropriate volume of *Diluted human thrombin solution* to give a final concentration of 0.095 NIH Units/mL (E_C) and immediately after activate the chronometer. Vortex for a few s and heat to 37° for 20 min \pm 15 s in a water bath. Stop the reaction by adding 100 μL of *Stop solution*. Measure the absorbance of the six solutions at 405 nm using the *Blank* to auto-zero. Prepare the solutions for the analysis as indicated in *Table 2*.

Table 2

	Blank	Control Assay Solution	Sample Assay Solution	Standard Assay Solution
Number of replicate	1	3	3	3
Total number of UV readings	1	3	9	3
Chromogenic substrate solution	30 μL	30 μL	30 μL	30 μL
Bivalirudin	100 μL (<i>Standard solution</i>)	0	100 μL (<i>Sample solution</i>)	100 μL (<i>Standard solution</i>)
Thrombin	0	E_C	E_C	E_C
Buffer solution	1000 μL –30 μL –100 μL	1000 μL –30 μL – E_C	1000 μL –30 μL –100 μL – E_C	1000 μL –30 μL –100 μL – E_C

System suitability

Samples: *Control assay solution* and *Standard assay solution*

Suitability requirements

Mean absorbance: Between 0.428 and 0.473, *Control assay solution*

Relative standard deviation: NMT 5% for six replicates, *Control assay solution*

Percent average inhibition: 44%–50% for the three readings, *Standard assay solution*

Analysis

Samples: *Standard assay solution* and *Sample assay solutions*. Prepare three independent preparations of the *Sample assay solution* to obtain a 0.6-mg/mL concentration.

Calculations: Determine the %thrombin inhibition for each of the nine sample readings of the sample tested. The final inhibition %result is given by the average of these nine values.

$$\% \text{ Inhibition} = [1 - (r_U/r_S)] \times 100$$

r_U = absorbance response of the *Sample assay solution*

r_S = average absorbance response of the six readings of the *Control assay solution*

Acceptance criteria: 42%–52% average inhibition. Percent inhibition of each single *Sample assay solution* reading is between 41%–53%; % RSD of % average inhibition of the nine readings of the *Sample assay solution* is NMT 10%.

ASSAY

• Procedure

Buffer solution: Dissolve 13.6 g of sodium acetate trihydrate in 900 mL of water and adjust the pH to 6.5 ± 0.1 with glacial acetic acid. Dilute with water to 1000 mL and pass through a filter of 0.2- μm pore size.

Solution A: *Buffer solution* and water (1:1)

Solution B: Acetonitrile and *Buffer solution* (1:1)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	85	15
30	65	35
35	65	35
35.1	90	10
40	90	10

Standard solution A1: 1.5 mg/mL of USP Bivalirudin RS in water. Prepare in duplicate.

Standard solution A2: 0.16 mg/mL of USP [Asp⁹]-Bivalirudin RS in water. Prepare in duplicate.

System suitability solution: *Standard solution A1* and *Standard solution A2* (1:1)

Sample solutions: 1.5 mg/mL of Bivalirudin in water. Prepare in triplicate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm \times 25-cm; 5- μm packing *L1*

Temperatures

Column: 40°

Autosampler: 2°–8°

Flow rate: 1.2 mL/min

Injection volume: 40 μL

System suitability

Samples: *Standard solution A1 and System suitability solution*

Suitability requirements

Resolution: NLT 2.5 between bivalirudin and [Asp⁹]-bivalirudin peaks, *System suitability solution*

Column efficiency: NLT 15,000 theoretical plates, *Standard solution A1*

Relative standard deviation: NMT 1.5 for three replicate injections of each preparation, *Standard solution A1*

Analysis

Samples: *Standard solution A1 and Sample solutions*

Calculate the percentage of bivalirudin (C₉₈H₁₃₈N₂₄O₃₃) in the portion of Bivalirudin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of bivalirudin from the *Sample solutions*

r_S = mean peak response of bivalirudin from *Standard solution A1*

C_S = concentration of USP Bivalirudin RS in *Standard solution A1* (mg/mL)

C_U = concentration of Bivalirudin in the *Sample solutions* (mg/mL)

Take the percentage of bivalirudin for each *Sample solution*. Average the results to report the acceptance criteria.

Acceptance criteria: 96.0%–103.0% on the anhydrous and trifluoroacetic acid-free basis

● **Product-Related Substances and Impurities**

Procedure 1

[Note—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak equal to or above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]

Mobile phase and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: Prepare a solution containing 2.5 mg/mL of USP Bivalirudin RS spiked with 0.05 mg/mL of USP [Asp⁹]-Bivalirudin RS in water.

Standard stock solution: 25 mg/mL of USP Bivalirudin RS in *Buffer solution*

Standard solution: Prepare a 2.5-mg/mL solution of USP Bivalirudin RS by diluting the *Standard stock solution* with water.

Sample solution: 2.5 mg/mL of Bivalirudin in water

Blank: Water

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Sample: *System suitability solution*

Suitability requirements

Retention time: The relative retention time for [Asp⁹]-bivalirudin compared to

bivalirudin is NMT 1.0.

Resolution: NLT 2.5 between [Asp⁹]-bivalirudin and bivalirudin

Column efficiency: NLT 12,000 theoretical plates

Analysis

Sample: *Sample solution*

Record the chromatograms and measure the response of each peak from the *Sample solution* using the drop-down integration method with respect to the baseline. Exclude from the integration the peaks present in the blank. Among all the integrated peaks, only those with a signal-to-noise ratio higher than 10 shall be used for the calculation. Calculate the percentage of each impurity in the portion of Bivalirudin taken:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria

Individual impurities: See Table 4.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fragment [1-11]	0.49	0.5
Fragment [2-20]	0.60	0.5
Total fragments ^a	0.44-0.65	1.8
[Asp ⁹]-bivalirudin	0.93	0.4
Bivalirudin	1.00	—
[des-Glu ¹³]-bivalirudin	1.31-1.34	0.4
Unspecified impurities	—	0.4
^a Peptide fragment peaks resulting from degradation.		

Procedure 2

[Note—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak equal to or above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]

Solution A: Acetonitrile and water (85:15)

Solution B: 0.04% Trifluoroacetic acid (v/v) in *Solution A*

Solution C: Acetonitrile and 0.04% trifluoroacetic acid (v/v)

Mobile phase: See Table 5.

Table 5

Time (min)	Solution B (%)	Solution C (%)
0	50	50
35	50	50

System suitability solution: Prepare a solution containing 3 mg/mL of USP Bivalirudin RS spiked with 0.015 mg/mL of USP [des-Glu¹³]-Bivalirudin RS in *Solution B*.

Standard solution: 3 mg/mL of USP Bivalirudin RS in *Solution B*

Sample solution: 3 mg/mL of Bivalirudin in *Solution B*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 207 nm

Column: 4.6-mm × 25-cm; 5-μm packing L3

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 5 μL

System suitability

Sample: *System suitability solution*

[Note—See *Table 6* for relative retention times.]

Suitability requirements

Retention time: The bivalirudin peak elutes between 9.4 and 14.7 min.

Peak width: The bivalirudin peak width at half height should be NMT 0.45.

Peak-to-valley ratio: NLT 1.25 between [des-Glu¹³]-bivalirudin and bivalirudin

Analysis

Sample: *Sample solution*

Record the chromatograms and measure the response for each peak in the chromatogram of the *Sample solution*. Disregard any peak due to trifluoroacetic acid, the peaks at RRT 0.2, RRT 0.91, RRT 0.64, and any peak with an area less than 0.10% of that of Bivalirudin.

Calculate the percentage of each impurity in the portion of Bivalirudin taken:

$$\text{Result} = (r_i / r_T) \times 100$$

r_i = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria

Individual impurities: See *Table 6*.

Table 6

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
[des-Gly ⁴]-bivalirudin	0.75	0.4
Bivalirudin	1.00	—
[Endo-Gly ⁵]-bivalirudin	1.36	0.5
Unspecified impurities	—	0.4

Total impurities: The sum of all impurities from *Procedure 1* and *Procedure 2* is NMT 2.5%.

OTHER COMPONENTS

- **Trifluoroacetic Acid (TFA) in Peptides** (503.1): Between 8.0% and 12.0%

SPECIFIC TESTS

- **Microbial Enumeration Tests** (61): The total aerobic microbial count does not exceed 100 cfu/g and the total combined yeast and mold count does not exceed 50 cfu/g.
- **Bacterial Endotoxins Test** (85): NMT 1 USP Endotoxins Unit/mg
- **Water Determination** (921), *Method Ic*: NMT 6.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in an airtight, closed container protected from light at a temperature between $-20 \pm 5^\circ$.
- **Labeling:** Label it to state the strength, in mg, of bivalirudin.
- **USP Reference Standards** (11)
 - USP Bivalirudin RS
 - USP [Asp⁹]-Bivalirudin RS
 - USP [des-Glu¹³]-Bivalirudin RS
 - USP Endotoxin RS

■ 2S (USP39)

¹ A suitable human alpha-thrombin is available from Sekisui Diagnostics, LLC.

BRIEFING

Bivalirudin for Injection. Because there is no existing *USP* monograph for this drug product, a new monograph based on validated methods of analysis is being proposed. The liquid chromatographic procedures in the *Assay* and the test for *Product-Related Substances and Impurities* are based on analyses performed with the Vydac C18 brand of *L1* column. The typical retention time for bivalirudin in the *Assay* and the test for *Product-Related Substances and Impurities* is about 24 min.

(BIO1: A. Szajek.)

Correspondence Number—C152088

Comment deadline: November 30, 2015

Add the following:

- **Bivalirudin for Injection**

DEFINITION

Bivalirudin for Injection is a sterile, lyophilized powder for reconstitution. Bivalirudin used in the manufacture of Bivalirudin for Injection complies with the compendial requirements stated in the *Bivalirudin* monograph. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of bivalirudin.

IDENTIFICATION

- **A.**

Standard solution and Sample solution: Proceed as directed in the *Assay*.

Identity sample solution: 0.75 mg/mL of USP Bivalirudin RS and 0.75 mg/mL of Bivalirudin in water

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. The major peaks of the *Identity sample solution* co-elute.

- **B. Bioidentity**

Thrombin inhibition activity

Buffer solution: 50 mM Tris-HCl and 120 mM sodium chloride in water. Adjust to a pH of 7.40 ± 0.04 . Add bovine serum albumin (BSA) to obtain a 100-mg/mL concentration and pass through a filter of 0.45- μ m pore size.

Human thrombin solution: 10 μ g/mL of human thrombin¹ in *Buffer solution*

Chromogenic substrate solution: 5 mM solution of *H*-d-cyclohexylalanyl-Ala-Arg-p-nitroanilide diacetate salt in water. Dilute with water to obtain a 50- μ M solution.

Diluted human thrombin solution: Dilute *Human thrombin solution* in *Buffer solution* to obtain a 0.5- μ g/mL solution. Thrombin from alternate sources must be standardized in the substrate reaction. Prepare six replicates by adding 910 μ L of *Buffer solution* to each sample tube followed by 30 μ L of *Chromogenic substrate solution*. Add 60 μ L of *Diluted human thrombin solution*, vortex, and begin the 20-min incubation. Determine the absorbance at 405 nm for each replicate.

Calculate the standardized volume (E_C) of *Diluted human thrombin solution* from the mean absorbance:

$$E_C = (0.45 \times 60 \mu\text{L}) / \text{mean absorbance}$$

Blank: Water

Standard solution: Prepare a 0.6-mg/mL solution of USP Bivalirudin RS in water. Dilute with *Buffer solution* to obtain a 5- μ g/mL solution.

Sample solution: Prepare a 0.6-mg/mL solution of Bivalirudin for Injection in water. Measure the absorbance of the 0.6-mg/mL solution at 275 nm, using *Blank* to auto-zero. Calculate the concentration of bivalirudin in the *Sample solution*:

$$C_{\text{bivalirudin}} (\text{mg/mL}) = A_{275} / 0.62$$

From this concentration, dilute with *Buffer solution* to obtain a 5- $\mu\text{g}/\text{mL}$ solution. Prepare in triplicate.

Stop solution: Glacial acetic acid

Preparation of Control assay solution, Sample assay solution, and Standard assay solution: In each sample tube, add the *Buffer solution*, (1000 μL - E_C - S_C) first, then 30 μL of *Chromogenic substrate solution* (S_C), then the bivalirudin (if using). Vortex and incubate for 10 min at 37° in a water bath. Add the appropriate volume of *Diluted human thrombin solution* to give a final concentration of 0.095 NIH Units/mL (E_C) and immediately after activate the chronometer. Vortex for a few seconds and heat to 37° for 20 min \pm 15 sec in a water bath. Stop the reaction by adding 100 μL of *Stop solution*. Measure the absorbance of the six solutions at 405 nm using *Blank* to auto-zero.

Prepare the solutions for the analysis as indicated in *Table 1*.

Table 1

	Blank	Control Assay Solution	Sample Assay Solution	Standard Assay Solution
Number of replicates	1	3	3	3
Total number of UV readings	1	3	9	3
Chromogenic substrate solution	30 μL	30 μL	30 μL	30 μL
Bivalirudin	100 μL (<i>Standard solution</i>)	0	100 μL (<i>Sample solution</i>)	100 μL (<i>Standard solution</i>)
Thrombin	0	E_C	E_C	E_C
Buffer solution	1000 μL – 30 μL – 100 μL	1000 μL – 30 μL – E_C	1000 μL – 30 μL – 100 μL – E_C	1000 μL – 30 μL – 100 μL – E_C

System suitability

Samples: *Control assay solution* and *Standard assay solution*

Suitability requirements

Mean absorbance: Between 0.428 and 0.473, *Control assay solution*

Relative standard deviation: NMT 5% for six replicates, *Control assay solution*

Percent average inhibition: 44%–50% for the three readings of *Standard assay solution*

Analysis

Samples: *Standard assay solution* and *Sample assay solution*. Prepare three independent preparations of *Sample assay solution* to obtain a 0.6-mg/mL concentration.

Calculations: Determine % thrombin inhibition for each of the nine sample readings of

the sample tested. The final inhibition % result is given by the average of these nine values.

$$\% \text{ Inhibition} = [1 - (r_U/r_S)] \times 100$$

r_U = absorbance response, *Sample assay solution*

r_S = average absorbance response of the six readings of *Control assay solution* preparations

Acceptance criteria: 42%–52% average inhibition. Percent inhibition of each single *Sample assay solution* reading is between 41%–53%; % RSD of % average inhibition of the nine readings of the *Sample assay solutions* is NMT 10%.

ASSAY

• Procedure

Buffer solution: Dissolve 8.2 g of sodium acetate in 900 mL of water, adjust with glacial acetic acid to a pH of 6.5 ± 0.1 . Dilute with water to 1000 mL and pass through a filter of 0.2- μm pore size.

Solution A: *Buffer solution* and water (1:1)

Solution B: Acetonitrile and *Buffer solution* (1:1)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	85	15
30	65	35
35	65	35
35.1	90	10
40	90	10

Standard solution A1: 275 $\mu\text{g}/\text{mL}$ of USP Bivalirudin RS in water. Prepare in duplicate.

Standard solution A2: 300 $\mu\text{g}/\text{mL}$ of USP [Asp⁹]-Bivalirudin RS in water. Prepare in duplicate.

System suitability solution: Using *Standard solution A1* and *Standard solution A2*, prepare a solution with a concentration of 275 $\mu\text{g}/\text{mL}$ of USP Bivalirudin RS and 3 $\mu\text{g}/\text{mL}$ of USP [Asp⁹]-Bivalirudin RS.

Sample solution: Transfer the entire contents of a vial (250 mg) to a 100-mL volumetric flask. Dissolve in and dilute with water to volume. Dilute with water to 275 $\mu\text{g}/\text{mL}$. Prepare in triplicate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5- μ m packing L1

Temperatures

Column: 40°

Autosampler: 2°–8°

Flow rate: 1.2 mL/min

Injection volume: 40 μ L

System suitability

Samples: *Standard solution A1* and *System suitability solution*

Suitability requirements

Resolution: NLT 2.5 between the bivalirudin peak and the [Asp⁹]-bivalirudin peak, *System suitability solution*

Column efficiency: NLT 12,000 theoretical plates, *Standard solution A1*

Relative standard deviation: NMT 1.0% for three replicate injections of each preparation of *Standard solution A1*

Analysis

Samples: *Standard solution A1* and *Sample solution*

Analyze a single injection each of *Standard solution A1* and *Sample solution*. Calculate the percentage of the labeled amount of bivalirudin (C₉₈H₁₃₈N₂₄O₃₃) in the portion of Bivalirudin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = mean peak response from *Standard solution A1*

C_S = concentration of USP Bivalirudin RS in *Standard solution A1* (μ g/mL)

C_U = nominal concentration of bivalirudin in the *Sample solution* (μ g/mL)

Acceptance criteria: 90.0%–120.0%

● **Product-Related Substances and Impurities**

Buffer solution: Dissolve 27.2 g of sodium acetate trihydrate in 1800 mL of water, and adjust with glacial acetic acid to a pH of 6.5 \pm 0.1. Dilute with water to 2000 mL and pass through a filter of 0.2- μ m pore size.

Solution A: *Buffer solution* and water (1:1)

Solution B: Acetonitrile and *Buffer solution* (1:1)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	85	15
30	65	35
35	65	35
35.1	90	90
40	90	90

Standard stock solution: 5.0 mg/mL of USP Bivalirudin RS in water

Standard solution A1: 0.05 mg/mL of USP [Asp⁹]-Bivalirudin RS in water. Prepare in duplicate.

High load sample solution (S1): Transfer the entire contents of a vial (250 mg) to a 100-mL volumetric flask. Dissolve in and dilute with water to volume. Prepare in duplicate.

Low load sample solution (S2): Dilute 2.0 mL of *Sample solution S1* with water to 100 mL. Prepare in duplicate.

System suitability solution: *Standard stock solution* and *Standard solution A1* (1:1)

Blank: Water

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Samples: *Standard solution A1* and *System suitability solution*

Suitability requirements

Resolution: NLT 2.5 between the bivalirudin peak and the [Asp⁹]-bivalirudin peak, *System suitability solution*

Column efficiency: NLT 10,000 theoretical plates, *Standard solution A1*

Relative standard deviation: NMT 1.0% for three replicate injections of each preparation of *Standard solution A1*

Analysis

Sample: *Low load sample solution (S2)*, single injection

Record the chromatograms, and measure the response for each peak of the *Low load sample solution* using the drop-down method of integration with respect to the baseline. Exclude from the integration the peaks present in the blank. Among all the integrated peaks, only those with a signal-to-noise ratio higher than 10 shall be used for the calculation. Report the area of all peaks of the chromatogram of the *High load sample solution*. To calculate the corrected bivalirudin peak area for the *Low load sample solution*, report the area of the bivalirudin peak of the *Low load sample solution* chromatogram and multiply this value by the dilution factor (50).

For the *Low load sample solution*, calculate the corrected total area:

$$r_T = \text{total area}_{S1} - \text{bivalirudin peak area}_{S1} + \text{bivalirudin corrected peak area}_{S2}$$

r_T = corrected total peak area

total area_{S1} = total peak area from the *High load sample solution*

bivalirudin peak area_{S1} = bivalirudin peak area from the *High load sample solution*
 bivalirudin corrected peak area_{S2} = mean bivalirudin peak area from the *Low load sample solution*
 × dilution factor (50)

For the *High load sample solution*, calculate the percentage of each impurity in the portion of Bivalirudin for Injection taken by using the corrected total peak area:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = peak response of each impurity from the *High load sample solution*

r_T = corrected total peak area

Acceptance criteria

Individual impurities: See *Table 4*. NMT 1.0% of any individual impurity is found unless specified, and the sum of all impurities is NMT 5.0%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fragment [1-11]	0.49	0.7
Fragment [2-20]	0.60	0.7
Total fragments ^a	0.44-0.65	1.8
[Asp ⁹]-Bivalirudin	0.93	1.0
Bivalirudin	1.00	—
Unspecified impurities	—	1.0
Total impurities	—	5.0

^a Peptide fragment peaks resulting from degradation.

PERFORMANCE TESTS

- **Uniformity of Dosage Units** (905): Meets the requirements

SPECIFIC TESTS

- **Completeness of Solution** (641)

Sample solution: Reconstitute 1 vial of Bivalirudin for Injection with 5 mL of carbon dioxide-free water.

Acceptance criteria: After 3 min, the solution is clear and free from undissolved solids.

- **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products (1)*, *Product Quality Tests Common to Parenteral Dosage Forms*, *Specific Tests*, *Completeness and clarity of solutions*.
- **Bacterial Endotoxins Test** (85): NMT 50.0 USP Endotoxin Units/mL
- **Sterility Tests** (71), *Test for Sterility of the Product to Be Examined*, *Membrane Filtration:* Meets the requirements

- **Water Determination** (921), *Method Ic*: NMT 4.0%
- **Particulate Matter in Injections** (788): Meets the requirements for small-volume injections
- **pH** (791)
Sample solution: Use the *Sample solution* prepared in the test for *Completeness of Solution*.
Acceptance criteria: 5.0–6.0
- **Other Requirements:** Meets the requirements in *Labeling* (7)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I glass. Store at controlled room temperature.
 - **Labeling:** Label it to indicate its synthetic origin.
 - **USP Reference Standards** (11)
 - USP Bivalirudin RS
 - USP [Asp⁹]-Bivalirudin RS
 - USP Endotoxin RS
- 2S (USP39)

¹ A suitable human alpha-thrombin is available from Sekisui Diagnostics LLC.

BRIEFING

Castor Oil, *USP 38* page 2633. As part of the USP monograph modernization effort, and on the basis of comments and data received, it is proposed to make the following revisions:

1. Add a CAS number and chemical structure.
2. Update the monograph *Definition*.
3. Add an *Identification* test *A, Identity by Fatty Acid Composition*. The added procedure is based on a GC method of analysis performed with the Agilent DB-225 or Restek Rtx-225 brand of G7 column. The typical retention times for methyl palmitate, methyl stearate, methyl oleate, methyl *cis*-11-octadecenoate, methyl linoleate, methyl linolenate, methyl *cis*-11-eicosenoate, and methyl ricinoleate are 7.25, 11.51, 11.75, 11.94, 12.69, 13.98, 19.20, and 31.49 min, respectively.
4. Move the test for *Distinction from Most Other Fixed Oils* from *Specific Tests* to the *Identification* section as test *B*.
5. In the *Assay*, add a test for *Triglyceride Composition*, which is based on analyses performed using the Phenomenex Prodigy ODS-3 brand or Phenomenex Ultremex C18 brand of column that contains 5- μ m packing L1. The typical retention times for RRR, RRL, RRO, and RRS are 7.6, 12.2, 13.6, and 15.4 min, respectively.
6. Replace the specification for *Free Fatty Acids* with a specification for *Acid Value*, and modify the test for *Hydroxyl Value* as the specification for *Acid Value* is known.
7. Add a specification for *Peroxide Value*.
8. Delete the test for *Iodine Value*.
9. Add a specification for *Water Determination*.
10. Add a specification for *Unsaponifiable Matter*.
11. Update the *Packaging and Storage* section.

12. Add a *Labeling* section with a statement accommodating a special grade that is used in injectable dosage forms. The specific grade must meet the *Other Requirements* to be added under the *Additional Requirements* section.
13. Add a *USP Reference Standards* section including USP Castor Oil RS, USP Methyl Linoleate RS, USP Methyl Linolenate RS, USP Methyl Ricinoleate RS, USP Methyl Palmitate RS, and USP Methyl Stearate RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC1: H. Wang.)

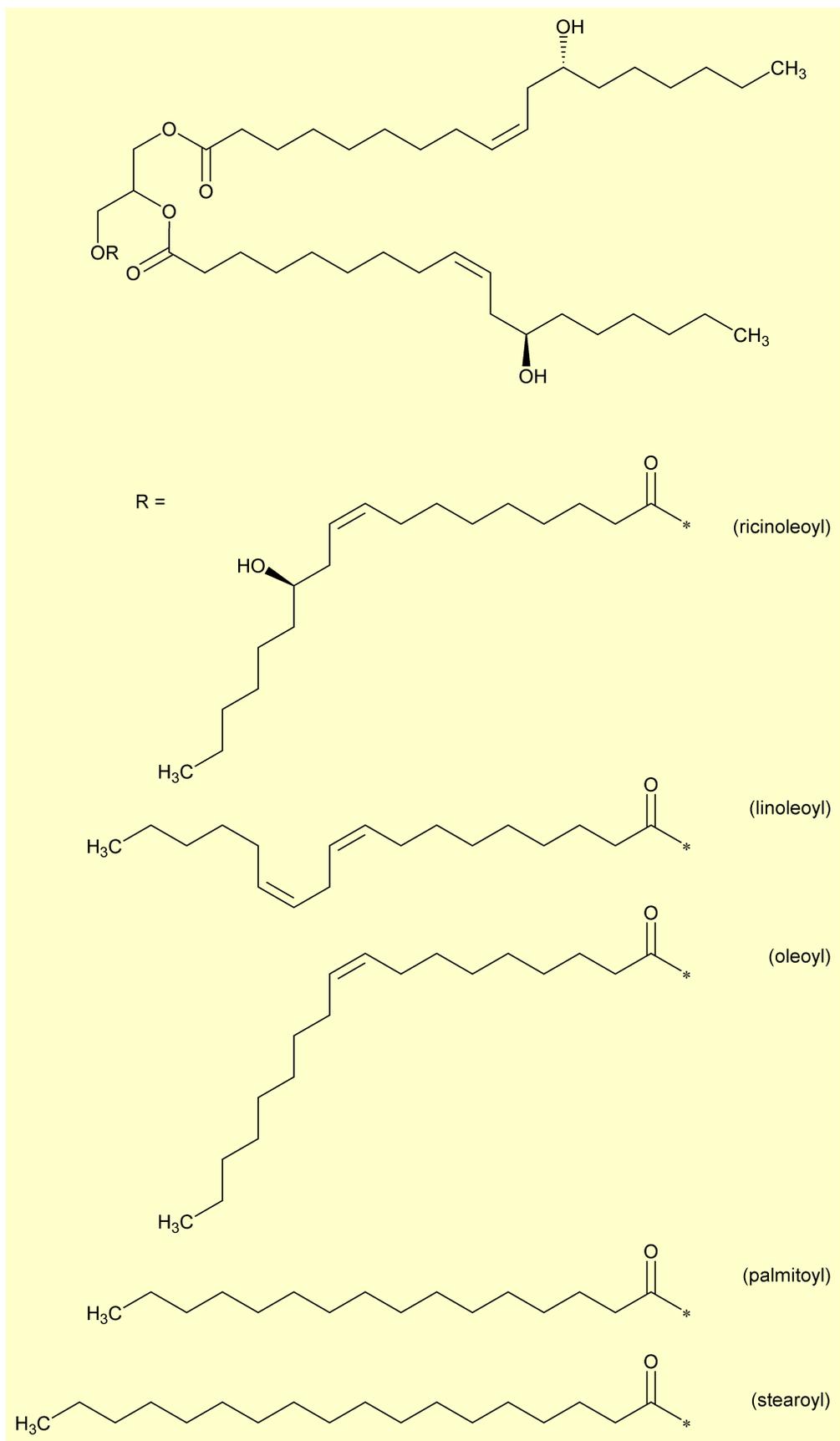
Correspondence Number—C109769

Comment deadline: November 30, 2015

Castor Oil

Add the following:





Triricinolein (glyceryl triricinoleate or triricinoleoyl-glycerol) predominates [8001-79-4].

■ 2S (USP39)

DEFINITION

Change to read:

Castor Oil is the

■ refined ■ 2S (USP39)

fixed oil obtained from the seed of *Ricinus communis* L. (Fam. Euphorbiaceae).

■ Castor oil consists of NLT 90.0% of the triglyceride of ricinoleic acid. ■ 2S (USP39)

It contains no added substances.

IDENTIFICATION

Add the following:

■ ● A. Identity by Fatty Acid Composition

Diluent: *n*-Heptane

Standard solution 1: 0.2 mg/mL each of methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, methyl linolenate, methyl *cis*-11-eicosenoate, and methyl ricinoleate from USP Methyl Palmitate RS, USP Methyl Stearate RS, USP Methyl Oleate RS, USP Methyl Linoleate RS, USP Methyl Linolenate RS, methyl *cis*-11-eicosenoate, and USP Methyl Ricinoleate RS in *Diluent*

Standard solution 2: 4 mg/mL each of methyl stearate and methyl ricinoleate from USP Methyl Stearate RS and USP Methyl Ricinoleate RS in *Diluent*

Sample solution: Transfer 140 mg of Castor Oil to a 10-mL screw-cap test tube, add 3.0 mL of *Diluent*, and mix well. Add 0.5 mL of 0.5 M sodium methoxide in methanol,¹ and mix with the sample. Allow the reaction to proceed at room temperature for 2 h. After 2 h, add 5 mL of water, and mix. Separate the organic layer (the upper layer), and remove the lower layer. Place an aliquot of the organic layer into an autosampler vial.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 15-m fused silica capillary bonded with a 0.25-μm layer of phase G7

Temperatures

Injection port: 240°

Detector: 250°

Column: See *Table 1* for oven program.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)	Total Time (min)
80	0	80	1	1
80	30	140	0	3
140	3	150	0	6.3
150	1	155	0	11.3
155	2	165	0	16.3
165	3	220	10	45

Column mode: See Table 2 for pressure program.

Table 2

Pressure (psi)	Pressure Ramp (psi/min)	Hold Time (min)	Total Time (min)
10	0	16	16
4	5	9 or 0 ^a	26.2 or 17.2
3	10	19 or 28	45

^a If considerable discrimination of late-eluting compounds is observed, the hold time can be adjusted from 9 to 0 min. Thus the total time should be 17.2 min. The next step of the hold time should be 28 min.

Carrier gas: Hydrogen

Injection volume: 0.5 µL

Injection type: Split ratio 60:1

Liner: Single taper, low-pressure drop liner with deactivated wool

Run time: 45 min

System suitability

Sample: Standard solution 1

[Note—See Table 3 for relative retention times.]

Table 3

Component	Relative Retention Time
Methyl palmitate (C16:0)	0.62
Methyl stearate (C18:0)	0.98
Methyl oleate (C18:1)	1.00
Methyl linoleate (C18:2)	1.08
Methyl linolenate (C18:3)	1.19
Methyl <i>cis</i> -11-eicosenoate (C20:1)	1.63
Methyl ricinoleate	2.68

Suitability requirements

Resolution: NLT 1.5 between the methyl stearate and methyl oleate peaks

Relative standard deviation: NMT 2.0% for the peak area ratio of methyl ricinoleate to methyl linoleate

Analysis

Samples: *Standard solution 1, Standard solution 2, and Sample solution*

The peak of methyl *cis*-11-octadecenoate, which is an isomer of methyl oleate, can be resolved from the methyl oleate peak with a resolution of about 1 and a relative retention time of 1.01 with respect to methyl oleate.

Calculate the relative response factor, F , for methyl ricinoleate:

$$F = (r_S/r_R) \times (C_R/C_S)$$

r_S = peak area of methyl stearate from *Standard solution 2*

r_R = peak area of methyl ricinoleate from *Standard solution 2*

C_R = concentration of USP Methyl Ricinoleate RS in *Standard solution 2* (mg/mL)

C_S = concentration of USP Methyl Stearate RS in *Standard solution 2* (mg/mL)

Correct the peak area of methyl ricinoleate in the *Sample solution* by multiplying by F . Calculate the percentage of each fatty acid component in the portion of sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual fatty acid methyl ester except for the uncorrected peak area of methyl ricinoleate (or corrected peak area of methyl ricinoleate) in the *Sample solution*

r_T = sum of all of the peak areas, excluding the solvent and methyl ricinoleate peaks and including the corrected peak area of methyl ricinoleate in the *Sample solution*

Acceptance criteria: Castor Oil exhibits the composition profile of fatty acids shown in *Table 4*.

Table 4

Component	Percentage (%)
Palmitic acid (C16:0)	≤2.0
Stearic acid (C18:0)	≤2.5
Oleic acid (C18:1)	2.5–6.0
Linoleic acid (C18:2)	2.5–7.0
Linolenic acid (C18:3)	≤1.0
<i>cis</i> -11-Eicosenoic acid (C20:1)	≤1.0
Ricinoleic acid	85.0–92.0
<i>cis</i> -11-Octadecenoic acid or any other unidentified fatty acid	≤1.0

■ 2S (USP39)

Add the following:

- ● **B. Distinction from Most Other Fixed Oils:** It is only slightly soluble in *solvent hexane* (distinction from most other fixed oils), but it yields a clear liquid with an equal volume of *alcohol* (foreign fixed oils). ■ 2S (USP39)

ASSAY**Add the following:****Triglyceride Composition**

[Note—The fatty acid radicals are designated as linoleic (L), oleic (O), palmitic (P), ricinoleic (R), and stearic (S), and the common abbreviations for triglycerides used are as follows: triricinolein (glyceryl triricinoleate or triricinoleoyl-glycerol) (RRR), diricinoleoyl-linoleoyl-glycerol (RRL), diricinoleoyl-oleoyl-glycerol (RRO), diricinoleoyl-palmitoyl-glycerol (RRP), and diricinoleoyl-stearoyl-glycerol (RRS).]

Solution A: *Methanol*

Solution B: *2-Propanol*

Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	50	50
23	0	100
25	100	0
35	100	0

Diluent: *2-Propanol*

System suitability solution: 2.0 mg/mL of USP Castor Oil RS in *Diluent*

Sample solution: 2.0 mg/mL of Castor Oil in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Evaporative light-scattering

Column: 4.6-mm × 25-cm; 5- μ m packing *L1*

Temperatures

Column: 25°

Detector: 40°

Flow rate: 1.0 mL/min

Injection volume: 5 μ L

Run time: 35 min

[Note—Depending on the different settings of the detector, the *Temperature* and *Flow rate* can be adjusted as long as system suitability requirements are met.]

System suitability

Sample: *System suitability solution*

[Note—See *Table 6* for relative retention times.]

Table 6

Component	Relative Retention Time
RRR	1.0
RRL	1.6
RRO ^a	1.8
RRS	2.0

^a RRP coelutes with RRO, and the percentage of RRP is about 10 times less than that of RRO.

Suitability requirements

Resolution: NLT 5.0 between the RRR and RRL peaks, NLT 2.0 between the RRL and RRO peaks, NLT 3.0 between the RRO and RRS peaks

Tailing factor: 0.8–1.8 for the RRR peak

Relative standard deviation: NMT 2% for the RRR peak

Analysis

Samples: *System suitability solution and Sample solution*

Calculate the percentage of each of the triglycerides in the portion of sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual triglyceride

r_T = sum of the areas of all the peaks, excluding the solvent peak

Acceptance criteria: Castor Oil exhibits the composition profile of triglycerides shown in *Table 7*.

Table 7

Component	Percentage (%)
RRR	≥ 90.0
RRL	2.0–4.0
RRO	2.5–5.0
RRS	0.2–0.8

■ 2S (USP39)

IMPURITIES

Delete the following:

- **Heavy Metals** (231), *Method II*: NMT 10 ppm (Official 1-Dec-2015)

SPECIFIC TESTS

- **Specific Gravity** (841): 0.957–0.961

Delete the following:

- ● **Distinction from Most Other Fixed Oils:** It is only partly soluble in solvent hexane (distinction from most other fixed oils), but it yields a clear liquid with an equal volume of alcohol (foreign fixed oils). ■ 2S (USP39)

■

$$\text{Result} = (V_B - V_T) \times [(M_r \times N)/W] + A \quad \blacksquare_{2S} \text{ (USP39)}$$

V_B = volume of *Titrant* consumed by the *Blank* (mL)

~~V_A = volume of *Titrant* consumed by the *Sample* in the free acid determination (mL)~~

~~W_A = sample weight from the free acid determination (g)~~

■ \blacksquare_{2S} (USP39)

V_T = volume of *Titrant* consumed by the *Sample* in the hydroxyl value determination (mL)

M_r = milliequivalent weight of potassium hydroxide, 56.11 mg/mEq

N = actual normality of the *Titrant*

W = sample weight from the hydroxyl value determination (g)

= acid value from the test for *Fats and Fixed Oils, Acid Value* ■ \blacksquare_{2S} (USP39)

■

A

Acceptance criteria: 160–168

Add the following:

- ● **Fats and Fixed Oils** (401), *Peroxide Value*: NMT 5.0 ■ \blacksquare_{2S} (USP39)

Delete the following:

- ● ~~**Fats and Fixed Oils**, *Iodine Value* (401): 83–88 ■ \blacksquare_{2S} (USP39)~~
- **Fats and Fixed Oils** (401), *Saponification Value*: 176–182

Add the following:

- ● **Fats and Fixed Oils** (401), *Unsaponifiable Matter*: NMT 0.8% ■ \blacksquare_{2S} (USP39)

Add the following:

- ● **Water Determination** (921): NMT 0.3% ■ \blacksquare_{2S} (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers, avoid exposure to excessive heat,
 - and protect from light. ■ \blacksquare_{2S} (USP39)

Add the following:

- ● **Labeling:** Where Castor Oil is intended for use in the manufacture of injectable dosage forms, it is so labeled. ■ \blacksquare_{2S} (USP39)

Add the following:

- ● **Other Requirements:** For Castor Oil intended for use in injectable dosage forms, which is specified in the *Labeling*, the water content ((921)) is NMT 0.2%, and the acid value ((401)) is NMT 0.8. ■ \blacksquare_{2S} (USP39)

Add the following:

■ ● USP Reference Standards (11)

USP Castor Oil RS

USP Methyl Linoleate RS USP Methyl Linolenate RS USP Methyl Oleate RS USP Methyl Palmitate RS

USP Methyl Ricinoleate RS

USP Methyl Stearate RS **■ 2S (USP39)**

■ ¹ 0.5 M sodium methoxide in methanol is available from Sigma-Aldrich (www.sigmaaldrich.com), product #403067. Any other equivalent reagent can be used as well. ■ 2S (USP39)

BRIEFING

Chlorhexidine Gluconate Topical Gel. Because there is no existing *USP* monograph for this dosage form, a new monograph, based on validated methods of analysis, is proposed. There has been an increasing use of chlorhexidine gluconate solution and gel for umbilical cord care in Nepal, Nigeria, and other countries. A 2012 UN Commission Report on Life-Saving Commodities for Women and Children listed this product as potentially saving 422,000 lives over five years,¹ and it subsequently was added to the 2013 World Health Organization (WHO) List of Essential Medicines for Children specifically for umbilical cord care.² Although the *USP-NF* currently includes monographs for the Chlorhexidine Gluconate drug substance and other dosage forms, it does not include a monograph for Chlorhexidine Gluconate Topical Gel because this product is not marketed in the U.S. USP developed this monograph as a result of its involvement with the Chlorhexidine Working Group, which comprises 18 global health organizations.

USP is consulting key global stakeholders to help define and implement a program to develop monographs for high-impact drugs legally marketed outside the U.S. The initiative targets critical medicines approved outside the U.S. for which no relevant standards exist and would be conducted in collaboration with the WHO, other pharmacopeias, and key stakeholders in ongoing consultation with the U.S. Food and Drug Administration. The proposed approach would use the existing *USP-NF* revision processes and resulting monographs would be published in *USP-NF* and designated to indicate their non-U.S. status. This could occur through a note within the monograph itself (see the proposed *Note* in the *Definition* section) or, alternatively, by placing these monographs in a separate section in *USP-NF*. USP welcomes and encourages comments and feedback on the proposed monograph and the broader concept for non-U.S. monographs presented here, including the need for a separate section within the compendia for these monographs to avoid confusion about their regulatory status in the U.S.

The chromatographic procedures in the *Assay* and the *Limit of p-Chloroaniline* test are based on analyses performed with the Phenomenex Luna C18 brand of L1 column. The typical retention time for chlorhexidine is about 8 min.

Correspondence should be addressed to: Behnam Davani, PhD, Director, United States Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; phone: 1-301-816-8394; email: bd@usp.org

(CHM6: K.K. Seela, B. Davani.)

Correspondence Number—C153598

Comment deadline: November 30, 2015

Add the following:

▪ **Chlorhexidine Gluconate Topical Gel**

DEFINITION

Chlorhexidine Gluconate Topical Gel is prepared from Chlorhexidine Gluconate Solution. It contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$). [Note—The U.S. Food and Drug Administration has not reviewed the safety and efficacy of Chlorhexidine Gluconate Topical Gel and it is not approved for marketing in the United States.]

IDENTIFICATION

• **A. Ultraviolet Absorption** (197U)

Wavelength range: 200–400 nm

Sample solution: Nominally 0.01 mg/mL of chlorhexidine gluconate from Topical Gel, prepared as follows. Transfer a suitable amount of Topical Gel to an appropriate volumetric flask and dilute with water to volume.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits two maxima at 231 and 255 nm and two minima at 222 and 242 nm.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **C. Thin-Layer Chromatographic Identification Test** (201)

Diluent: *Acetonitrile* and water (1:1)

Standard solution: 10 mg/mL of USP Potassium Gluconate RS in *Diluent*

Sample solution: Nominally 20 mg/mL of chlorhexidine gluconate from Topical Gel, prepared as follows. Transfer a suitable amount of Topical Gel, equivalent to 500 mg of chlorhexidine gluconate, to a 25-mL volumetric flask. Add a sufficient quantity of *Diluent*, sonicate with intermittent shaking for 30 min, and dilute with *Diluent* to volume. Centrifuge the solution for 5 min at 3000 rpm.

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 μ L

Developing solvent system: *Alcohol*, *ethyl acetate*, *ammonium hydroxide*, and water (5:1:1:3)

Spray reagent: Dissolve 2.5 g of *ammonium molybdate* in 50 mL of 2 N *sulfuric acid* in a 100-mL volumetric flask. Add 1.0 g of *ceric sulfate*, swirl to dissolve, and dilute with 2 N *sulfuric acid* to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram in a solvent system until the solvent front has moved 10 cm from the point of spotting. Remove the plate from the chamber and dry at 110° for 20 min. Allow to cool and spray with *Spray reagent*. Heat the plate at 110° for 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_f value to that of the *Standard solution*.

ASSAY

• Procedure

Buffer: Dissolve 27.6 g of *sodium dihydrogen phosphate* and 10 mL of *triethylamine* in 1.5 L of water. Adjust with *phosphoric acid* to a pH of 3.0 and dilute with water to 2000 mL.

Solution A: *Acetonitrile* and *Buffer* (30:70)

Solution B: *Acetonitrile*

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

Standard solution: 50 µg/mL of USP Chlorhexidine Acetate RS in *Solution A*

Sample stock solution: Nominally 0.4 mg/mL of chlorhexidine gluconate from Topical Gel, prepared as follows. Transfer a suitable amount of Topical Gel, equivalent to 40 mg of chlorhexidine gluconate, to a 100-mL volumetric flask. Add about 70 mL of *Solution A*, sonicate with intermittent shaking for 30 min, and dilute with *Solution A* to volume.

Sample solution: Nominally 80 µg/mL of chlorhexidine gluconate from the *Sample stock solution* with *Solution A*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 25-cm; 5-µm packing *L1*

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements**Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$) in the portion of Topical Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak area of chlorhexidine from the *Sample solution* r_S = peak area of chlorhexidine from the *Standard solution* C_S = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* ($\mu\text{g/mL}$) C_U = nominal concentration of chlorhexidine gluconate in the *Sample solution* ($\mu\text{g/mL}$) M_{r1} = molecular weight of chlorhexidine gluconate, 897.76 M_{r2} = molecular weight of chlorhexidine acetate, 625.55**Acceptance criteria:** 90.0%–110.0%**IMPURITIES**• **Limit of *p*-Chloroaniline****Solution A, Solution B, Mobile phase, and Chromatographic system:** Proceed as directed in the *Assay*.**System suitability solution:** 50 $\mu\text{g/mL}$ of USP Chlorhexidine Acetate RS and 1 $\mu\text{g/mL}$ of USP *p*-Chloroaniline RS in *Solution A***Standard solution:** 1.0 $\mu\text{g/mL}$ of USP *p*-Chloroaniline RS in *Solution A***Sample solution:** Nominally 0.4 mg/mL of chlorhexidine gluconate from Topical Gel, prepared as follows. Transfer a suitable amount of Topical Gel, equivalent to 40 mg of chlorhexidine gluconate, to a 100-mL volumetric flask. Add about 70 mL of *Solution A*, sonicate with intermittent shaking for 30 min, and dilute with *Solution A* to volume. Centrifuge the solution.**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 3.0 between chlorhexidine and *p*-chloroaniline, *System suitability solution***Relative standard deviation:** NMT 5.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of *p*-chloroaniline in the portion of Topical Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of *p*-chloroaniline from the *Sample solution*

r_S = peak response of *p*-chloroaniline from the *Standard solution*

C_S = concentration of USP *p*-Chloroaniline RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of chlorhexidine gluconate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.25%

SPECIFIC TESTS

- **pH** <791>

Sample solution: Nominally 1% of chlorhexidine gluconate from Topical Gel with water

Acceptance criteria: 5.0–7.0

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light. Store at controlled room temperature.

- **USP Reference Standards** <11>

USP Chlorhexidine Acetate RS

USP *p*-Chloroaniline RS

USP Potassium Gluconate RS

- **2S** (USP39)

¹ UN Commission on Life-Saving Commodities for Women and Children, *Commissioners' Report*, September 2012.

² *Chlorhexidine for Umbilical Cord Care: A new, low cost intervention to reduce newborn mortality*, Chlorhexidine Working Group, PATH (Secretariat).

BRIEFING

Chlorothiazide Oral Suspension, USP 38 page 2773. As part of the USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Replace the UV-based *Assay* procedure with a stability-indicating HPLC procedure. The procedure has the same HPLC parameters as those proposed in the test for *Organic Impurities*. The liquid chromatographic procedure is validated using the Waters XBridge C18 brand of L1 column. The typical retention time for chlorothiazide is about 7.2 min.
2. Replace *Identification* test *A* based on UV spectrum match with a retention time agreement in the proposed *Assay*.
3. Add an orthogonal *Identification* test *B* based on UV spectrum match from the proposed chromatographic procedure in the *Assay*.
4. Add a stability-indicating HPLC procedure to the test for *Organic Impurities*.
5. Add a new USP Reference Standard used in the test for *Organic Impurities* to the *USP Reference Standards* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM2: D. Min.)

Correspondence Number—C136881

Comment deadline: November 30, 2015

Chlorothiazide Oral Suspension

DEFINITION

Chlorothiazide Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$).

IDENTIFICATION

Change to read:

- **A.** ~~The UV absorption spectrum of the solution of chlorothiazide prepared from Oral Suspension as directed in the Assay exhibits maxima and minima at the same wavelengths as that of a solution of USP Chlorothiazide RS, prepared as directed in the Assay, concomitantly measured.~~
 - The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ■ 2S (USP39)

Add the following:

- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ■ 2S (USP39)

ASSAY

Change to read:

- **Procedure**

Standard solution: ~~10 µg/mL of USP Chlorothiazide RS in sodium hydroxide solution (1 in 250)~~

Sample stock solution: ~~Transfer an accurately measured volume of Oral Suspension, equivalent to about 250 mg of chlorothiazide, to a 250 mL volumetric flask. Dilute in sodium hydroxide solution (1 in 250) to volume, and mix. Transfer 10.0 mL of this solution to a 100 mL volumetric flask, add dilute hydrochloric acid (1 in 100) to volume, and mix. Transfer 50.0 mL of the resulting solution to a 125 mL separator, and wash with two 25 mL portions of chloroform, discarding the washings.~~

Sample solution: ~~Nominally 10 µg/mL of chlorothiazide from Oral Suspension prepared as follows. Transfer 10.0 mL of *Sample stock solution* to a 100 mL volumetric flask, and dilute with sodium hydroxide solution (1 in 250) to volume, and mix.~~

Spectrometric conditions

Mode: ~~UV~~

Analytical wavelength: ~~292 nm~~

Cell: ~~1 cm~~

Blank: ~~Sodium hydroxide solution (1 in 250)~~

Analysis

Samples: ~~Standard solution and Sample solution~~

~~Determine the absorbances of the Standard solution and the Sample solution at 292 nm.~~

~~Calculate the percentage of labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$) in the portion of Oral Suspension taken:~~

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

~~A_U = absorbance of the Sample solution~~

~~A_S = absorbance of the Standard solution~~

~~C_S = concentration of the Standard solution ($\mu\text{g/mL}$)~~

~~C_U = nominal concentration of chlorothiazide in the Sample solution ($\mu\text{g/mL}$)~~

▪ **Solution A:** 0.1% Formic acid in water

Solution B: Methanol

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	94	6
4	94	6
14	80	20
18	40	60
25	40	60
26	94	6
30	94	6

Diluent: Acetonitrile and Solution A (30:70)

Standard solution: 0.1 mg/mL of USP Chlorothiazide RS in Diluent

Sample stock solution: 0.5 mg/mL of chlorothiazide in Diluent prepared as follows.

Transfer a suitable amount of Oral Suspension, equivalent to 25 mg of chlorothiazide, to a 50-mL volumetric flask. Add 40 mL of Diluent and sonicate to dissolve. Dilute with Diluent to volume. Centrifuge and use the supernatant.

Sample solution: Nominally 0.1 mg/mL of chlorothiazide in Diluent, from Sample stock solution

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 275 nm. For Identification test B, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm \times 15-cm; 5- μm packing L1

Temperatures

Autosampler: 5°

Column: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chlorothiazide from the *Sample solution*

r_S = peak response of chlorothiazide from the *Standard solution*

C_S = concentration of USP Chlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of chlorothiazide in the *Sample solution* (mg/mL)

■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Uniformity of Dosage Units** (905)

For single-unit containers

Acceptance criteria: Meets the requirements

- **Deliverable Volume** (698)

For multiple-unit containers

Acceptance criteria: Meets the requirements

IMPURITIES

Add the following:

- ● **Organic Impurities**

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 10 µg/mL of USP Benzothiadiazine Related Compound A RS and 2 µg/mL of USP Chlorothiazide RS in *Diluent*

Sample solution: Nominally 1 mg/mL of chlorothiazide in *Diluent* prepared as follows.

Transfer a suitable amount of Oral Suspension, equivalent to 25 mg of chlorothiazide, to a 25-mL volumetric flask. Add 20 mL of *Diluent* and sonicate to dissolve. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.

System suitability**Sample:** *Standard solution*[Note—See *Table 2* for relative retention times.]**Suitability requirements****Resolution:** NLT 4.0 between the benzothiadiazine related compound A and chlorothiazide peaks**Relative standard deviation:** NMT 5.0% for chlorothiazide**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified degradation product in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of each unspecified degradation product from the *Sample solution* r_S = peak response of chlorothiazide from the *Standard solution* C_S = concentration of USP Chlorothiazide RS in the *Standard solution* ($\mu\text{g/mL}$) C_U = nominal concentration of chlorothiazide in the *Sample solution* ($\mu\text{g/mL}$)**Acceptance criteria:** See *Table 2*. Disregard any peak below 0.05%.**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A ^a	0.7	—
Chlorothiazide	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	2.0
^a Not included in the total degradation products.		

■ 2S (USP39)

SPECIFIC TESTS

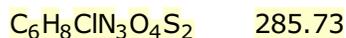
- pH (791): 3.2–4.0

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in tight containers.
 - Protect from freezing and store at controlled room temperature. ■ 2S (USP39)

Change to read:

- **USP Reference Standards** (11)
 - USP Benzothiadiazine Related Compound A RS
 - 4-Amino-6-chloro-1,3-benzenedisulfonamide.



■ 2S (USP39)

USP Chlorothiazide RS

BRIEFING

Chlorothiazide Tablets, *USP 38* page 2773. As part of the USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Add a test for *Organic Impurities* with a stability-indicating HPLC procedure. The validation of the proposed HPLC procedure is based on analyses performed with the Waters XBridge C18 brand of L1 column. The typical retention time for chlorothiazide is about 7.2 min.
2. Replace the current HPLC procedure in the *Assay* with an HPLC procedure that has the same parameters as proposed in the test for *Organic Impurities*.
3. Replace *Identification* test *B* for sulfite with the UV spectrum agreement from the proposed *Assay*.
4. Add additional storage requirements in the *Packaging and Storage* section based on the information in the drug product package insert.
5. Add a new USP Reference Standard, USP Benzothiadiazine Related Compound A RS, used for *Organic Impurities*, to the *USP Reference Standards* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM2: D. Min.)

Correspondence Number—C137138

Comment deadline: November 30, 2015

Chlorothiazide Tablets

DEFINITION

Chlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of chlorothiazide ($\text{C}_7\text{H}_6\text{ClN}_3\text{O}_4\text{S}_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

B. Identification Tests—General, Sulfite (191): Powder 1 Tablet, and fuse it with a pellet of sodium hydroxide: the ammonia fumes produced turn moistened red litmus paper blue, and the residue responds to the test.

- The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

ASSAY

Change to read:● **Procedure**

Mobile phase: Prepare a solution of 0.08 M monobasic sodium phosphate (adjusted with phosphoric acid to a pH of 2.9 ± 0.1) and methanol (95:5).

Standard solution: 0.5 mg/mL of USP Chlorothiazide RS prepared as follows. Transfer 25 mg of USP Chlorothiazide RS to a 50 mL volumetric flask, add 5 mL of 0.05 M monobasic sodium phosphate solution, followed by 10 mL of acetonitrile to the flask, and sonicate with occasional shaking for 3 min. Dilute with water to volume and filter.

Sample solution: Nominally 0.5 mg/mL of chlorothiazide from the Tablets prepared as follows. Transfer a portion of the powder, equivalent to 250 mg of chlorothiazide, from finely powdered Tablets (NLT 20) to a 500 mL volumetric flask. Add 50 mL of 0.05 M monobasic sodium phosphate solution, and shake by mechanical means for 15 min, followed by sonication for 2 min. Add 100 mL of acetonitrile, sonicate for 3 min, dilute with water to volume, and filter. [Note—Prepare fresh daily.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9 mm × 30 cm; packing L1, and fitted with a guard column

Flow rate: 2 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor: NLT 4.3

Tailing factor: NMT 2.0

Column efficiency: NLT 1300 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Chlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of chlorothiazide in the *Sample solution* (mg/mL)

■ **Solution A:** 0.1% *Formic acid* in water

Solution B: *Methanol*

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	94	6
4	94	6
14	80	20
18	40	60
25	40	60
26	94	6
30	94	6

Diluent: *Acetonitrile* and *Solution A* (30:70)

Standard solution: 0.1 mg/mL of USP Chlorothiazide RS in *Diluent*

Sample stock solution: 0.5 mg/mL of chlorothiazide in *Diluent* prepared as follows.

Transfer a suitable amount of finely powdered Tablets (NLT 20), equivalent to 25 mg of chlorothiazide, to a 50-mL volumetric flask. Add 40 mL of *Diluent* and sonicate. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.

Sample solution: Nominally 0.1 mg/mL of chlorothiazide in *Diluent* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 275 nm. For *Identification test B*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 5-μm packing L1

Temperatures

Autosampler: 5°

Column: 30°

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlorothiazide (C₇H₆ClN₃O₄S₂) in the

portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chlorothiazide from the *Sample solution*

r_S = peak response of chlorothiazide from the *Standard solution*

C_S = concentration of USP Chlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of chlorothiazide in the *Sample solution* (mg/mL)

■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution (711)

Medium: 0.05 M pH 8.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 75 rpm

Time: 60 min

Standard solution: Known concentration of USP Chlorothiazide RS in *Medium*

Sample solution: Filtered portions of the solution under test, suitably diluted with *Medium* to a concentration that is similar to the *Standard solution*

Instrumental conditions

Mode: UV

Analytical wavelength: 294 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 75% (Q) of the labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$) is dissolved.

• Uniformity of Dosage Units (905): Meet the requirements

IMPURITIES

Add the following:

■ • Organic Impurities

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 10 µg/mL of USP Benzothiadiazine Related Compound A RS and 2 µg/mL of USP Chlorothiazide RS in *Diluent*

Sample solution: Nominally 1 mg/mL of chlorothiazide in *Diluent* prepared as follows. Transfer a suitable amount of finely powdered Tablets (NLT 20) to an appropriate volumetric flask. Add *Diluent* to 80% of the final flask volume and sonicate. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 4.0 between the benzothiadiazine related compound A and chlorothiazide peaks

Relative standard deviation: NMT 5.0% for chlorothiazide

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of chlorothiazide from the *Standard solution*

C_S = concentration of USP Chlorothiazide RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of chlorothiazide in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A ^a	0.7	—
Chlorothiazide	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	2.0

^a Not included in the total degradation products.

■ 2S (USP39)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in well-closed,
 - light-resistant ■ 2S (USP39) containers.
 - Store at controlled room temperature. ■ 2S (USP39)

Change to read:

- **USP Reference Standards** <11>
 - USP Benzothiadiazine Related Compound A RS

4-Amino-6-chloro-1,3-benzenedisulfonamide.

$\text{C}_6\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2$ 285.73

■ 2S (USP39)

USP Chlorothiazide RS

BRIEFING

Ciprofloxacin, *USP 38* page 2816; and *PF 40(3)* [May–June 2014]. The revision proposal in *PF 40(3)* is being cancelled and replaced with the following proposal based on the comments received:

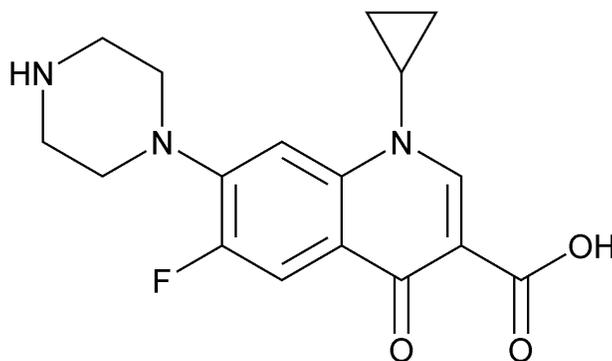
1. The nonselective TLC procedure in *Organic Impurities, Procedure 1* is replaced with a stability-indicating liquid chromatography procedure. The proposed HPLC procedure is based on the analysis performed with the YMC-Pack ODS-AQ brand of L1 column. The typical retention time for ciprofloxacin is about 9.5 min.
2. The *Organic Impurities, Procedure 2* is deleted as the proposed HPLC procedure is sufficient to monitor all the impurities.
3. The tests for *Chloride* and *Sulfate* are deleted, as they are not needed.
4. The *Column efficiency* criteria in the *Assay* is deleted as other system suitability criteria are sufficient.
5. In the *USP Reference Standards* section, chemical information is updated for the USP impurity Reference Standards that support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(SHM1: S. Shivaprasad.)

Correspondence Number—C109903; C150762

Comment deadline: November 30, 2015

Ciprofloxacin

$C_{17}H_{18}FN_3O_3$ 331.34

3-Quinolinecarboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-; 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid [85721-33-1].

DEFINITION

Ciprofloxacin contains NLT 98.0% and NMT 102.0% of ciprofloxacin ($C_{17}H_{18}FN_3O_3$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

Buffer: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 3.0 ± 0.1 .

Mobile phase: Acetonitrile and *Buffer* (13:87)

Standard solution: 0.5 mg/mL of USP Ciprofloxacin RS prepared as follows. Transfer 12.5 mg of USP Ciprofloxacin RS to a 25-mL volumetric flask. Add 0.1 mL of 7% phosphoric acid, and dilute with *Mobile phase* to volume.

System suitability stock solution: 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*

System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.

Sample solution: Transfer 25 mg of Ciprofloxacin to a 50-mL volumetric flask. Add 0.2 mL of 7% phosphoric acid, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm \times 25-cm; packing L1

Column temperature: $30 \pm 1^\circ$

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6 between ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*

Column efficiency: NLT 2500 theoretical plates from the ciprofloxacin peak, *Standard solution*

■ **2S (USP39)**

Tailing factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) in the portion of Ciprofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Ciprofloxacin RS in the *Standard solution* (mg/mL)

C_U = concentration of Ciprofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%, except that where it is intended for use in preparing Ciprofloxacin for Oral Suspension, it is NMT 0.2%.

Delete the following:

● Chloride

Standard solution: 8.2 µg/mL of sodium chloride (5 µg/mL of chloride)

Sample solution: Add 30.0 mL of water to 0.5 g of Ciprofloxacin, shake for 5 min, and pass through chloride-free filter paper. Use the filtrate as the *Sample solution*.

Analysis

Samples: *Standard solution* and *Sample solution*

Transfer 15.0 mL of the *Sample solution* to a 50 mL color-comparison tube. Transfer 10.0 mL of the *Standard solution* to a second matched 50 mL color-comparison tube, add 5.0 mL of water, and mix. To each tube add 1 mL of 2 N nitric acid, mix, add 1 mL of silver nitrate TS, and mix.

Acceptance criteria: The turbidity exhibited by the *Sample solution* does not exceed that of the *Standard solution* (0.02%). ■ 2S (USP39)

Delete the following:

● Sulfate

Standard solution: 18.1 µg/mL of potassium sulfate in 30% alcohol (10 µg/mL of sulfate)

Sample solution: Dissolve 0.5 g of Ciprofloxacin in 5.0 mL of 2 N acetic acid and 15.0 mL of water.

Analysis

Samples: *Standard solution* and *Sample solution*

To each of two 50 mL matched color-comparison tubes transfer 1.50 mL of the *Standard solution*. To each tube add, successively and with continuous shaking, 1.0 mL of 250 mg/mL barium chloride solution, and allow to stand for 1 min. To one of the tubes transfer 15.0 mL of the *Standard solution* and 0.5 mL of 30% acetic acid, and mix. To the second tube add 15.0 mL of the *Sample solution* and 0.5 mL of 30% acetic acid, and mix.

Acceptance criteria: The turbidity exhibited in the tube containing the *Sample solution* does not exceed that of the tube containing the *Standard solution* (0.04%). ■ 2S (USP39)

Delete the following:

- **Heavy Metals** (231), *Method II*: NMT 20 ppm • (Official 1-Dec-2015)

Delete the following:

■ ● **Procedure 1: Limit of Fluoroquinolonic Acid**

Standard stock solution: Transfer 5.0 mg of USP Fluoroquinolonic Acid RS to a 50-mL volumetric flask containing 0.05 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Standard solution: Dilute 2.0 mL of the *Standard stock solution* with water to 10.0 mL.

Sample solution: 10.0 mg/mL of Ciprofloxacin in 0.1 N acetic acid

Developing solvent system: Methylene chloride, methanol, acetonitrile, and ammonium hydroxide (4:4:1:2)

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of silica gel mixture

Application volume: 5 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Place the plate in a suitable chamber in which is placed a beaker containing 50 mL of ammonium hydroxide. After 15 min, transfer the plate to a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for about 15 min. Examine the plate under short-wavelength UV light.

Acceptance criteria: Any spot from the *Sample solution*, at an R_f value corresponding to the principal spot from the *Standard solution*, is not greater in size or intensity than the principal spot from the *Standard solution* (0.2%). ■ 2S (USP39)

Delete the following:

■ ● **Procedure 2**

Solution A, Mobile phase, System suitability stock solution, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Ciprofloxacin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each impurity peak

~~r_T = sum of the responses of all the peaks~~

~~Acceptance criteria~~

~~**Ciprofloxacin ethylenediamine analog or any other individual impurity peak:** NMT 0.2%~~

~~**Total impurities:** NMT 0.5%~~

■ 2S (USP39)

Add the following:

■ ● Organic Impurities

Buffer: Dilute 3.4 mL of phosphoric acid with water to 2000 mL. Adjust with triethylamine to a pH of 3.0 ± 0.1 .

Solution A: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Solution A (%)
0	87	13
10	87	13
11	50	50
16	50	50
16.1	87	13
20	87	13

Diluent: *Solution A* and *Buffer* (13:87)

System suitability solution: 7.5 µg/mL each of USP Ciprofloxacin Ethylenediamine Analog RS and USP Ciprofloxacin RS in *Diluent*

Standard stock solution: 0.1 mg/mL each of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin RS prepared as follows. Add suitable amounts of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin RS to a suitable volumetric flask. Add 0.1% of the flask volume of 6 M ammonium hydroxide and dilute with water to volume.

Standard solution: 0.7 µg/mL each of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin RS from *Standard stock solution* in *Diluent*

Sample solution: 0.35 mg/mL of Ciprofloxacin prepared as follows. Transfer 35 mg of Ciprofloxacin to a 100-mL volumetric flask, add 0.2 mL of 7% phosphoric acid, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 263 and 278 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 30 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6.0 between ciprofloxacin ethylenediamine analog and ciprofloxacin at 278 nm, *System suitability solution*

Tailing factor: NMT 2.0 for the ciprofloxacin peak at 278 nm, *Standard solution*

Relative standard deviation: NMT 5.0% for ciprofloxacin at 278 nm; NMT 5.0% for fluoroquinolonic acid at 263 nm, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fluoroquinolonic acid in the portion of Ciprofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluoroquinolonic acid at 263 nm from the *Sample solution*

r_S = peak response of fluoroquinolonic acid at 263 nm from the *Standard solution*

C_S = concentration of USP Fluoroquinolonic Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Ciprofloxacin in the *Sample solution* (mg/mL)

Calculate the percentage of the ciprofloxacin ethylenediamine analog and any individual unspecified impurity in the portion of Ciprofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity at 278 nm from the *Sample solution*

r_S = peak response of ciprofloxacin at 278 nm from the *Standard solution*

C_S = concentration of USP Ciprofloxacin RS in the *Standard solution* (mg/mL)

C_U = concentration of Ciprofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Wavelength (nm)	Acceptance Criteria, NMT (%)
Ciprofloxacin ethylenediamine analog	0.70	278	0.2
Ciprofloxacin	1.00	278	—
Fluoroquinolonic acid	1.89	263	0.2
Any individual unspecified impurity	—	278	0.2
Total impurities ^a	—	—	0.5
^a Total impurities does not include the fluoroquinolonic acid impurity.			

■ 2S (USP39)

SPECIFIC TESTS● **Clarity of Solution**

Sample solution: Dissolve 0.25 g in 10 mL of 0.1 N hydrochloric acid.

Acceptance criteria: A clear to slightly opalescent solution is obtained.

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): Where it is intended for use in preparing Ciprofloxacin for Oral Suspension, the total microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g. It also meets the requirements for absence of *Salmonella* species and *Escherichia coli*.

● **Loss on Drying** (731)

Analysis: Dry under vacuum at 120° for 6 h.

Acceptance criteria: NMT 1.0%, except that where it is labeled as intended for use in preparing Ciprofloxacin for Oral Suspension, 10%–20%

- **Sterility Tests** (71), *Test for Sterility of the Product to Be Examined, Membrane Filtration:* Where the label states that it is sterile, it meets the requirements.
- **Bacterial Endotoxins Test** (85): Where the label states that it is sterile or where the label states that Ciprofloxacin must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.50 USP Endotoxin Units/mg of ciprofloxacin.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at 25°, excursion permitted between 15° and 30°, and avoid excessive heat.
- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Where it is intended for use in preparing Ciprofloxacin for Oral Suspension, it is so labeled.

Change to read:● **USP Reference Standards** (11)

USP Ciprofloxacin RS

USP Ciprofloxacin Ethylenediamine Analog RS

~~1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.~~

~~C₁₅H₁₆FN₃O₃·HCl 341.77~~

■ 7-(2-Aminoethylamino)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

C₁₅H₁₆FN₃O₃ 305.30 ■ 2S (USP39)

USP Endotoxin RS

USP Fluoroquinolonic Acid RS

■ 7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

C₁₃H₉ClFNO₃ 281.67 ■ 2S (USP39)

Ciprofloxacin Hydrochloride, *USP 38* page 2818 and *PF 40(3)* [May–June 2014]. The revision proposal in *PF 40(3)* is being cancelled and replaced with the following proposal based on the comments received:

1. The chemical structure, chemical formula, molecular weight, and chemical name are revised to include various hydrated forms of ciprofloxacin hydrochloride.
2. The *Definition* section is updated to indicate various hydrate forms and to be consistent with the *European Pharmacopoeia* monograph for ciprofloxacin hydrochloride.
3. The nonselective TLC procedure in *Organic Impurities, Procedure 1* is replaced with a stability-indicating liquid chromatography procedure. The proposed HPLC procedure is based on the analysis performed with the YMC-Pack ODS-AQ brand of L1 column. The typical retention time for ciprofloxacin is about 9.5 min.
4. The *Organic Impurities, Procedure 2* is deleted as the proposed HPLC procedure is sufficient to monitor all the impurities.
5. The test for *Chloride and Sulfate (221), Sulfate* is deleted, as it is not needed.
6. The *Column efficiency* criteria in the *Assay* is deleted as other system suitability criteria are sufficient.
7. In the *USP Reference Standards* section, chemical information is updated for the USP impurity Reference Standards that support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

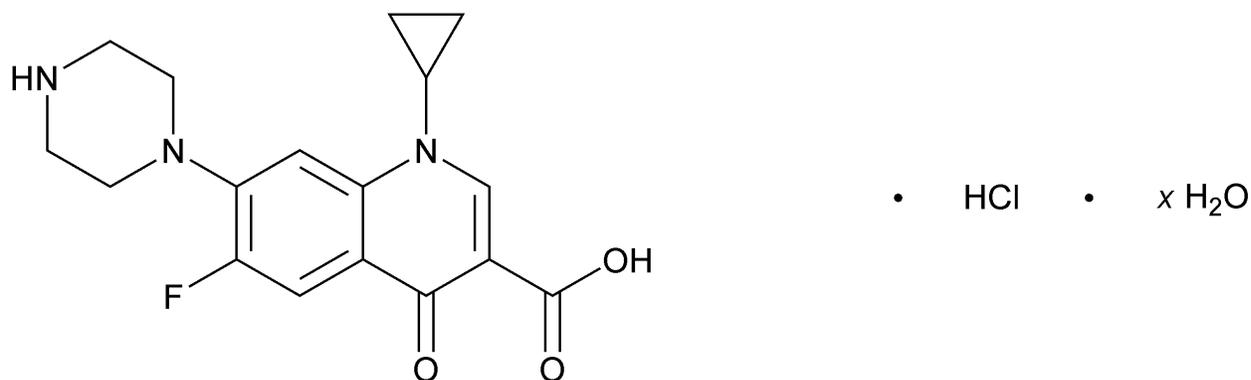
(CHM1: S. Shivaprasad.)

Correspondence Number—C120728; C109903; C148753

Comment deadline: November 30, 2015

Ciprofloxacin Hydrochloride

Change to read:



$C_{17}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$

■ $C_{17}H_{18}FN_3O_3 \cdot HCl \cdot xH_2O$

Sesquihydrate 394.83

Monohydrate 385.82

Anhydrous 367.81

■ 2S (USP39)

3-Quinolinecarboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-, monohydrochloride; ~~monohydrate~~

■ 2S (USP39)

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, monohydrochloride; ~~monohydrate~~

■ Monohydrate[86393-32-0]. ■ 2S (USP39)

DEFINITION**Change to read:**

Ciprofloxacin Hydrochloride contains NLT 98.0% and NMT 102.0% of ciprofloxacin hydrochloride ($C_{17}H_{18}FN_3O_3 \cdot HCl$), calculated on the anhydrous basis.

- It contains a variable quantity of water. ■ 2S (USP39)

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General** (191), *Chloride*

ASSAY**Change to read:**● **Procedure**

Buffer: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 3.0 ± 0.1 .

Mobile phase: Acetonitrile and *Buffer* (13:87)

Standard solution: 0.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase*

System suitability stock solution: 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*

System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.

Sample solution: 0.5 mg/mL of Ciprofloxacin Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: $30 \pm 1^\circ$

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6 between ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*

Column efficiency: ~~NLT 2500 theoretical plates from the ciprofloxacin peak, *Standard solution*~~

■ **2S (USP39)**

Tailing factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ciprofloxacin hydrochloride ($C_{17}H_{18}FN_3O_3 \cdot HCl$) in the portion of Ciprofloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area response from the *Sample solution*

r_S = peak area response from the *Standard solution*

C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Ciprofloxacin Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

Delete the following:

- ● **Chloride and Sulfate** (221), *Sulfate*: A 375-mg portion shows no more sulfate than corresponds to 0.15 mL of 0.020 N sulfuric acid (0.04%). ■ 2S (USP39)

Delete the following:

- ● **Heavy Metals** (231), *Method II*: NMT 20 ppm. (Official 1-Dec-2015)

Delete the following:

- ● **Procedure 1: Limit of Fluoroquinolonic Acid**

Standard solution: Transfer 5.0 mg of USP Fluoroquinolonic Acid RS to a 50-mL volumetric flask containing 0.05 mL of 6 N ammonium hydroxide, add water to volume, and mix. Transfer 2.0 mL of this solution to a 10.0-mL volumetric flask, and dilute with water to volume.

Sample solution: 10 mg/mL of Ciprofloxacin Hydrochloride in water

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of silica gel mixture

Application volume: 5 µL

Developing solvent system: Methylene chloride, methanol, acetonitrile, and ammonium hydroxide (4:4:1:2)

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter. Place a beaker containing 50 mL of ammonium hydroxide in a suitable chamber, and then place the plate in the chamber. After 15 min, transfer the plate to a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air dry for about 15 min. Examine the plate under short-wavelength UV light.

Acceptance criteria: Any spot from the *Sample solution*, at an R_f value corresponding to the principal spot from the *Standard solution*, is not greater in size or intensity than the principal spot from the *Standard solution* (0.2%). ■ 2S (USP39)

Delete the following:

■ ● **Procedure 2**

Mobile phase, Standard solution, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Ciprofloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity

r_T = sum of the responses of all the peaks

Acceptance criteria

Individual impurities: NMT 0.2% for the ciprofloxacin ethylenediamine analog or any other individual impurity peak

Total impurities: NMT 0.5%

■ 2S (USP39)

Add the following:

■ ● **Organic Impurities**

Buffer: Dilute 3.4 mL of phosphoric acid with water to 2000 mL. Adjust with triethylamine to a pH of 3.0 ± 0.1 .

Solution A: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Solution A (%)
0	87	13
10	87	13
11	50	50
16	50	50
16.1	87	13
20	87	13

Diluent: *Solution A* and *Buffer* (13:87)

System suitability solution: 7.5 µg/mL each of USP Ciprofloxacin Ethylenediamine Analog RS and USP Ciprofloxacin Hydrochloride RS in *Diluent*

Standard stock solution: 0.1 mg/mL each of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin Hydrochloride RS prepared as follows. Add suitable amounts of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin Hydrochloride RS to a suitable volumetric flask. Add 0.1% of the flask volume of 6 M ammonium hydroxide and dilute with water to volume.

Standard solution: 0.7 µg/mL each of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin Hydrochloride RS from *Standard stock solution* in *Diluent*

Sample solution: 0.35 mg/mL of Ciprofloxacin Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 263 and 278 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 30 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6.0 between ciprofloxacin ethylenediamine analog and ciprofloxacin at 278 nm, *System suitability solution*

Tailing factor: NMT 2.0 for the ciprofloxacin peak at 278 nm, *Standard solution*

Relative standard deviation: NMT 5.0% for ciprofloxacin at 278 nm; NMT 5.0% for fluoroquinolonic acid at 263 nm, *Standard solution*

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of fluoroquinolonic acid in the portion of Ciprofloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluoroquinolonic acid at 263 nm from the *Sample solution*

r_S = peak response of fluoroquinolonic acid at 263 nm from the *Standard solution*

C_S = concentration of USP Fluoroquinolonic Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Ciprofloxacin Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of ciprofloxacin ethylenediamine analog and any individual unspecified impurity in the portion of Ciprofloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity at 278 nm from the *Sample solution*

r_S = peak response of ciprofloxacin at 278 nm from the *Standard solution*

C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Ciprofloxacin Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Wavelength (nm)	Acceptance Criteria, NMT (%)
Ciprofloxacin ethylenediamine analog	0.70	278	0.2
Ciprofloxacin	1.00	278	—
Fluoroquinolonic acid	1.89	263	0.2
Any individual unspecified impurity	—	278	0.2
Total impurities ^a	—	—	0.5
^a Total impurities does not include fluoroquinolonic acid impurity.			

■ 2S (USP39)

SPECIFIC TESTS

- **pH** (791)

Sample solution: 25-mg/mL solution in water

Acceptance criteria: 3.0–4.5

- **Water Determination** (921), *Method I*: 4.7%–6.7%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at 25°,

excursions permitted between 15° and 30°.

Change to read:

• **USP Reference Standards** (11)

USP Ciprofloxacin Ethylenediamine Analog RS

~~1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.~~

~~C₁₅H₁₆FN₃O₃·HCl 341.77~~

■ 7-(2-Aminoethylamino)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

C₁₅H₁₆FN₃O₃ 305.30 ■_{2S} (USP39)

USP Ciprofloxacin Hydrochloride RS

USP Fluoroquinolonic Acid RS

■ 7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

C₁₃H₉ClFNO₃ 281.67 ■_{2S} (USP39)

BRIEFING

Codeine Sulfate Tablets, *USP 38* page 2933. As part of the USP monograph modernization initiative, it is proposed to make the following changes:

1. Replace *Identification* test *A*, which is based on IR and uses a toxic solvent, chloroform, with the retention time agreement from the *Assay*.
2. Delete *Identification* test *B* for sulfate. The salt form is controlled in the drug substance monograph.
3. Add *Identification* test *B* for codeine, a second orthogonal procedure based on the UV (210–400 nm) spectrum of the codeine peak in the *Assay*.
4. In the *Assay*, it is proposed to delete the system suitability requirement for *Column efficiency* and *Signal-to-noise ratio*. *Tailing factor* and *Relative standard deviation* are sufficient for the system suitability requirements.
5. Update the molecular weights of codeine sulfate, trihydrate and anhydrous compounds, to be consistent with the *USAN* dictionary.
6. The *Sensitivity solution* and system suitability requirement for *Signal-to-noise ratio* was moved from the *Assay* to the *Organic Impurities* test because this requirement is applicable to the *Organic Impurities* test.
7. Delete the *Procedure for content uniformity* in the test for *Uniformity of Dosage Units* to allow flexibility.
8. Add the disregard limit to the *Organic Impurities* test based on the submission data.
9. Update the storage requirement in the *Packaging and Storage* section based on the information in the label insert for an approved drug product.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM2: P. Pabba.)

Correspondence Number—C153117

Comment deadline: November 30, 2015

Codeine Sulfate Tablets

DEFINITION

Codeine Sulfate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of codeine sulfate trihydrate $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O]$.

IDENTIFICATION**Delete the following:**

- ● **A. Infrared Absorption** ~~(197K)~~

Standard solution: 50 mg of USP Codeine Sulfate RS dissolved in 15 mL of water, then rendered alkaline with *ammonium hydroxide, 6 N* and extracted with several 10 mL portions of *chloroform*. Evaporate the combined *chloroform* extracts on a steam bath at 80° for 4 h to dryness.

Sample solution: Digest an equivalent to 50 mg of codeine sulfate, from finely powdered Tablets, with 15 mL of water and 5 mL of 2 N sulfuric acid for 1 h. Filter, if necessary, and wash any undissolved residue with a few mL of water. Render the filtrate alkaline with *ammonium hydroxide, 6 N*, extract with several small portions of *chloroform*. Evaporate the *chloroform* solution on a steam bath at 80° for 4 h to dryness.

Acceptance criteria: Meet the requirements ■ 2S (USP39)

Add the following:

- ● **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

Delete the following:

- ● **B. Identification Tests—General, Sulfate** ~~(191)~~ A filtered solution of Tablets meets the requirements. ■ 2S (USP39)

Add the following:

- ● **B.**

Standard solution and Sample solution: Proceed as directed in the *Assay*.

Analysis: Inject 2 µL each of the *Standard solution* and the *Sample solution* using the *Chromatographic system* in the *Assay*.

Acceptance criteria: The spectrum of the codeine peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the corresponding peaks of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

ASSAY**Change to read:**

- **Procedure**

Solution A: *Acetonitrile* and 0.1% ammonium hydroxide (1.0 mL of concentrated *ammonium hydroxide* and 1000 mL of water) (1:19)

Solution B: *Acetonitrile* and 0.1% ammonium hydroxide (9:11)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40	0	100
41	100	0
50	100	0

[Note—The *Standard solution* and *Sample solution*, for the degradation products, are stable for 4 days when stored at room temperature in amber vials.]

Diluent: 0.5% Phosphoric acid (5 mL of concentrated *phosphoric acid* and 1000 mL of water)

Standard solution: 1.2 mg/mL of USP Codeine Sulfate RS in *Diluent*

~~**Sensitivity solution:** 0.6 µg/mL of USP Codeine Sulfate RS from the *Standard solution* in *Diluent*~~

■ ■2S (USP39)

Sample solution: Nominally 1.2 mg/mL of codeine sulfate trihydrate in *Diluent*. [Note—~~Dissolve 20 Tablets in 80% of the flask volume of *Diluent*, and sonicate for 15–30 min with occasional swirling before diluting with *Diluent* to volume.~~]

■ Dissolve 20 Tablets in 80% of the flask volume of *Diluent* and sonicate for 15–30 min with occasional swirling before diluting with *Diluent* to volume. ■2S (USP39)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 282 nm. For *Identification test B*, use a diode-array detector in the range of 210–400 nm.

Column: 4.6-mm × 15-cm; 3-µm packing *L1*

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 40 µL

System suitability

~~**Sample:** *Standard solution* and *Sensitivity solution*~~

■ ■2S (USP39)

Suitability requirements

~~**Tailing factor:** NMT 2.0, *Standard solution*~~

■ ■2S (USP39)

~~**Relative standard deviation:** NMT 2.0%, *Standard solution*~~

~~**Column efficiency:** NLT 10,000 theoretical plates, *Standard solution*~~

~~**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*~~

■ ■2S (USP39)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of codeine sulfate trihydrate $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O]$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of codeine sulfate from the *Sample solution*

r_S = peak area of codeine sulfate from the *Standard solution*

C_S = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of codeine sulfate trihydrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of codeine sulfate trihydrate, 750.87

■ 750.85 ■ 2S (USP39)

M_{r2} = molecular weight of codeine sulfate, anhydrous, 696.82

■ 696.81 ■ 2S (USP39)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• Dissolution (711)

Medium: Water; 500 mL

Apparatus 2: 25 rpm

Time: 45 min

Detector: UV maxima at about 284 nm

Cell: 1 cm

Blank: *Medium*

Standard solution: USP Codeine Sulfate RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.8- μ m pore size.

Tolerances: NLT 75% (Q) of the labeled amount of codeine sulfate trihydrate $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O]$ is dissolved.

Change to read:

• Uniformity of Dosage Units (905):

■ Meet the requirements ■ 2S (USP39)

~~Procedure for content uniformity:~~

~~**Standard solution:** 110 μ g/mL of USP Codeine Sulfate RS in 0.2 N sulfuric acid~~

~~**Sample solution:** Transfer 1 Tablet to a 50 mL volumetric flask. Add 20 mL of 0.5 N sulfuric acid and 10 mL of water. Shake until the Tablet is disintegrated, and allow to stand for 16 h. Dilute with water to volume, and filter, discarding the first few mL of the filtrate. Dilute the resulting filtrate with 0.2 N sulfuric acid to obtain a solution containing nominally 120 μ g/mL of codeine sulfate trihydrate.~~

~~Spectrometric conditions~~

(See *Spectrophotometry and Light Scattering* (851).)

Mode: UV-Vis

Analytical wavelength: About 284 nm

Cell: 1 cm

Blank: 0.2 N sulfuric acid

Analysis

Samples: *Standard solution, Sample solution, and Blank*

Calculate the percentage of codeine sulfate trihydrate ($C_{18}H_{21}NO_3$)₂·H₂SO₄·3H₂O in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Codeine Sulfate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of codeine sulfate trihydrate in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of codeine sulfate trihydrate, 750.87

M_{r2} = molecular weight of anhydrous codeine sulfate, 696.82

Acceptance criteria: Meet the requirements

■ 2S (USP39)

IMPURITIES

Change to read:

● Organic Impurities

Procedure

■ 2S (USP39)

Solution A, Solution B, Mobile phase, Diluent, Standard solution, Sensitivity solution,

■ 2S (USP39)

Sample solution, and Chromatographic system, and System suitability

■ 2S (USP39)

Proceed as directed in the Assay.

■ **Sensitivity solution:** 0.6 µg/mL of USP Codeine Sulfate RS from the *Standard solution* in *Diluent* ■ 2S (USP39)

System suitability

Samples: *Standard solution*

■ and *Sensitivity solution* ■ 2S (USP39)

Suitability requirements

▪ **Tailing factor:** NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution* ■ 2S (USP39)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of codeine sulfate from the *Standard solution*

C_S = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of codeine sulfate trihydrate in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

▪ Disregard any impurity peak less than 0.05%. ■ 2S (USP39)

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Codeine- <i>N</i> -oxide ^a	0.39	1.25	0.2
Codeine sulfate	1.00	—	—
Codeinone ^b	1.10	1.0	0.3
Individual unspecified degradant	—	— ▪ 1.0 ■ 2S (USP39)	0.2
Total impurities	—	—	0.5

^a 7,8-Didehydro-4,5a-epoxy-3-methoxy-17-methylmorphinan-6a-ol *N*-oxide.
^b 7,8-Didehydro-4,5a-epoxy-3-methoxy-17-methylmorphinan-6a-one.

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in well-closed containers.
 - Store at controlled room temperature. Protect from moisture and light. ■ 2S (USP39)
- **USP Reference Standards** <11>
 USP Codeine Sulfate RS

BRIEFING

Dolasetron Mesylate Injection, *USP 38* page 3192. It is proposed to omit this monograph for the following reasons. No drug products formulated as defined under Dolasetron Mesylate Injection are currently marketed in the United States. The drug product is currently not used in

veterinary medicine in the United States.

(CHM3: R.S. Prasad, E. Gonikberg.)

Correspondence Number—C162661

Comment deadline: November 30, 2015

Delete the following:

■ **Dolasetron Mesylate Injection**

» Dolasetron Mesylate Injection is a sterile solution, suitable for intravenous administration, containing Dolasetron Mesylate in a buffer solution. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dolasetron mesylate ($C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$).

Packaging and storage—Preserve in a single-dose container, protected from light. Store at controlled room temperature.

Labeling—Label it to indicate that it may be diluted with a suitable parenteral vehicle prior to intravenous infusion.

USP Reference standards (11)—

USP Dolasetron Mesylate RS

USP Endotoxin RS

Identification, Infrared Absorption (197K)—

Test specimen—Transfer a portion of Injection, equivalent to about 100 mg of dolasetron mesylate, to a 150-mL beaker. Add about 20 mL of water and 10 mL of a sodium hydroxide solution (1 in 10). Mix, and allow to stand at room temperature for 30 minutes. Pass through a filtering crucible with fritted disk having a medium porosity, using about 100 mL of water to aid in the transfer. Dry the precipitate in a vacuum oven at 105° for 4 hours. Prepare a 1.5% mixture of the dried powder with potassium bromide.

Bacterial endotoxins (85)—It contains not more than 2.7 USP Endotoxin Units per mg of dolasetron mesylate.

pH (791): between 3.2 and 3.8.

Particulate matter (788): meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections* (1).

Assay—

Mobile phase—Proceed as directed in the Assay under *Dolasetron Mesylate*.

System suitability preparation—Dissolve accurately weighed quantities of USP Dolasetron Mesylate RS and indole-3-carboxylic acid in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 0.1 mg per mL and 0.02 mg per mL, respectively.

Standard preparation—Dissolve an accurately weighed quantity of USP Dolasetron Mesylate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Using a “to contain” pipet, transfer 2.5 mL of Injection to a 50-mL

volumetric flask. Rinse the pipet with several portions of *Mobile phase*, and collect the rinses in the same flask. Dilute with *Mobile phase* to volume, and mix. Pipet 5.0 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))— Prepare as directed in the *Assay* under *Dolasetron Mesylate*. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the resolution, R , between indole-3-carboxylic acid and dolasetron mesylate is not less than 4; and the tailing factor for the dolasetron mesylate peak is not more than 1.8. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

Procedure— Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of dolasetron mesylate ($C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$) in each mL of Injection taken by the formula:

$$200C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dolasetron Mesylate RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. ■ 2S (USP39)

BRIEFING

Dolasetron Mesylate Tablets, USP 38 page 3193. It is proposed to omit this monograph for the following reasons. No drug products formulated as defined under Dolasetron Mesylate Tablets are currently marketed in the United States. The drug product is currently not used in veterinary medicine in the United States.

(CHM3: R.S. Prasad, E. Gonikberg.)

Correspondence Number—C162664

Comment deadline: November 30, 2015

Delete the following:

■ **Dolasetron Mesylate Tablets**

» Dolasetron Mesylate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dolasetron mesylate ($C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$).

Packaging and storage— Preserve in well-closed containers, protected from light.

USP Reference standards (11)—

USP Dolasetron Mesylate RS

Identification, Infrared Absorption (197K)—

Test specimen— Transfer a quantity of powdered Tablets, equivalent to about 200 mg of dolasetron mesylate, to a capped tube. Add 10 mL of acetonitrile, and shake for about 5 minutes. Allow to settle, filter, and collect the filtrate in a glass vial. Evaporate the solvent at 85°. Add an additional 5 mL of acetonitrile to the vial, and evaporate at 85°. Then evaporate

to dryness in a vacuum oven at 80°. Mix about 2 mg of the crystals obtained with about 300 mg of potassium bromide.

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure— Determine the amount of $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 284 nm on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a Standard solution having a known concentration of USP Dolasetron Mesylate RS in the same *Medium*.

Tolerances— Not less than 80% (*Q*) of the labeled amount of $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase— Proceed as directed in the *Assay* under *Dolasetron Mesylate*.

System suitability preparation— Dissolve accurately weighed quantities of USP Dolasetron Mesylate RS and indole-3-carboxylic acid in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 0.1 mg and 0.05 mg per mL, respectively.

Standard preparation— Dissolve an accurately weighed quantity of USP Dolasetron Mesylate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation— Transfer 10 Tablets to a 500 mL volumetric flask, add about 400 mL of *Mobile phase*, and stir vigorously with a magnetic stirrer for at least 40 minutes to dissolve. Dilute with *Mobile phase* to volume, mix, and allow the insoluble excipients to settle. Quantitatively dilute a portion of the supernatant with *Mobile phase* to obtain a solution having a concentration of about 0.1 mg of dolasetron mesylate per mL.

Chromatographic system (see *Chromatography* (621))— Prepare as directed in the *Assay* under *Dolasetron Mesylate*. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between indole-3-carboxylic acid and dolasetron mesylate is not less than 4; and the tailing factor for the dolasetron mesylate peak is not more than 1.8. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

Procedure— Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of dolasetron mesylate ($C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$) in each Tablet taken by the formula:

$$(TC/D)(r_U/r_S)$$

in which *T* is the labeled quantity, in mg, of dolasetron mesylate in the Tablet; *C* is the concentration, in mg per mL, of USP Dolasetron Mesylate RS in the *Standard preparation*; *D* is

~~the concentration, in mg per mL, of dolasetron mesylate in the Assay preparation, based on the labeled quantity per Tablet and the extent of dilution; and r_U and r_S are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.~~ ■ 2S (USP39)

BRIEFING

Doxycycline for Oral Suspension, USP 38 page 3221. As part of USP monograph modernization efforts, it is proposed to make the following revisions:

1. Replace the TLC *Identification* procedure, *Identification—Tetracyclines* (193), with a more specific FTIR procedure.
2. Add a second *Identification* test based on retention time using the HPLC procedure in the *Assay*.
3. A validated stability-indicating HPLC procedure for the *Organic Impurities* test is added. The proposed procedure uses the Acquity UPLC BEH C8 brand of L7 column manufactured by Waters where doxycycline elutes at about 3 min. A guard-column of the same brand of stationary phase was used in the validation.
4. Replace the *Assay* procedure with one that uses a new chromatographic method, which will also be used in the *Organic Impurities* test.
5. Acceptance limits for 4-epidoxycycline and any other unspecified impurity are proposed. Manufacturers with different limits are encouraged to submit their approved specifications to USP.
6. A *Dissolution* test is added. The analytical procedure uses the PRP-1 styrene divinyl benzene polymer brand of L21 column manufactured by Hamilton where doxycycline elutes at about 7.7 min.
7. Reference Standards are added to the *USP Reference Standards* section to support the revised procedures. USP Doxycycline Monohydrate RS is introduced as the Reference Standard for *Identification* test A. USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS are introduced in the *Assay* and the *Organic Impurities* test for system suitability evaluation and peak identification.
8. The test for *Water Determination* is deleted because the acceptance criterion for water is formulation specific.
9. The *Packaging and Storage* section is updated to include storage conditions consistent with the product label.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(CHM1: A. Potts.)

Correspondence Number—C115232; C115233; C157134

Comment deadline: November 30, 2015

Doxycycline for Oral Suspension

DEFINITION

Doxycycline for Oral Suspension contains the equivalent of NLT 90.0% and NMT 125.0% of the labeled amount of doxycycline (C₂₂H₂₄N₂O₈) when constituted as directed. It contains one or

more suitable buffers, colors, diluents, flavors, and preservatives.

IDENTIFICATION

Delete the following:

■ ● ~~A.~~

Test solution: Nominally 1 mg/mL of doxycycline in methanol from Doxycycline for Oral Suspension. Shake, allow to settle, and use the clear supernatant.

Analysis: Proceed as directed in *Identification—Tetracyclines* (193), Method II.

Acceptance criteria: Meets the requirements ■2S (USP39)

Add the following:

■ ● **A. Infrared Absorption** (197A)

Standard: Transfer about 25 mg of USP Doxycycline Monohydrate RS to a suitable flask. Add 25 mL of *acetonitrile* and mix for approximately 5 min with a magnetic stir bar. Pass through a suitable filter, evaporate the solvent, and dry under vacuum.

Sample: Place an amount of Doxycycline for Oral Suspension, equivalent to about 25 mg of doxycycline, in a suitable flask. Add 25 mL of *acetonitrile* and mix for approximately 5 min with a magnetic stir bar. Pass through a suitable filter and evaporate the acetonitrile under vacuum.

Analysis: Examine the spectra of the *Standard* and the *Sample* in the range between 4000 and 650 cm^{-1} .

Acceptance criteria: The *Sample* exhibits bands at about 1685, 1610, 1585, 1531, 1000, 958, 935, and 620 cm^{-1} , similar to the *Standard*. ■2S (USP39)

Add the following:

- ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■2S (USP39)

ASSAY

Change to read:

● **Procedure**

Throughout the following sections, protect the *Standard solution* and the *Sample solution* from light.

Mobile phase: Transfer 2.72 g of monobasic potassium phosphate, 0.74 g of sodium hydroxide, 0.50 g of tetrabutylammonium hydrogen sulfate, and 0.40 g of edetate disodium to a 1000 mL volumetric flask. Add 850 mL of water, and stir to dissolve. Add 60 g of tertiary butyl alcohol with the aid of water, dilute with water to volume, and adjust with 1 N sodium hydroxide to a pH of 8.0 ± 0.1 . Pass this solution through a filter of 0.5 μm or finer pore size, and degas before using. Decreasing the proportion of tertiary butyl alcohol results in a longer retention time of doxycycline and improved separation of doxycycline from the related compounds.

Diluent: 0.01 N hydrochloric acid

System suitability stock solution: 6 mg/mL of doxycycline from USP Doxycycline Hyclate

RS in Diluent

System suitability solution: Transfer 5 mL of *System suitability stock solution* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in 0.01 N hydrochloric acid, and dilute with *Diluent* to volume. Pass a portion of this solution through a filter of 0.5 μm or finer pore size, and use the filtrate. This solution contains a mixture of 4-epidoxycycline, 6-epidoxycycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.

Standard solution: 1.2 mg/mL of USP Doxycycline Hyclate *RS in Diluent*. Sonicate as needed to dissolve.

Sample solution: Nominally 1 mg/mL of doxycycline in *Diluent*, prepared as follows: Constitute Doxycycline for Oral Suspension as directed in the labeling. Transfer a suitable aliquot of the constituted suspension, freshly mixed and free from air bubbles, to a suitable volumetric flask, add 50% of the final volume of 0.1 N hydrochloric acid, and shake by mechanical means for 15 min. Dilute with *Diluent* to volume. Pass through filter paper, discarding the first 10 mL of the filtrate, then pass through a filter of 0.5 μm or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6 mm \times 25 cm; packing L21

Column temperature: 60 \pm 1 $^{\circ}$

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: 1.7 times the retention time of doxycycline

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for 4-epidoxycycline (the main degradation product), 6-epidoxycycline, and doxycycline are about 0.4, 0.7, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the 4-epidoxycycline peak and the doxycycline peak; *System suitability solution*

Tailing factor: NMT 2.0 for doxycycline, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$) in the portion of Doxycycline for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

f_U = peak response from the *Sample solution*

f_S = peak response from the *Standard solution*

C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of doxycycline from the *Sample solution* (mg/mL)

P = potency of doxycycline in USP Doxycycline Hyclate RS ($\mu\text{g}/\text{mg}$)

F = unit conversion factor, 0.001 mg/ μg

Acceptance criteria: 90.0%–125.0%

- Protect solutions containing doxycycline from light.

Solution A: Transfer 3.1 g of *monobasic potassium phosphate*, 0.5 g of *edetate disodium*, and 0.5 mL of *triethylamine* to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with 1 N *sodium hydroxide* to a pH of 8.5 \pm 0.2.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
2.0	90	10
4.0	60	40
6.0	90	10
9.0	90	10

Diluent: 0.01 N hydrochloric acid

System suitability stock solution 1: 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS in *Diluent*

System suitability stock solution 2: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*

System suitability solution: Transfer 5 mL of *System suitability stock solution 2* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in *Diluent*, add 0.5 mL of *System suitability stock solution 1*, and dilute with *Diluent* to volume. Pass the solution through a suitable filter and use the filtrate. This solution contains a mixture of 4-epidoxycycline, doxycycline related compound A, methacycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.

Standard solution: 0.3 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.

Sample solution: Nominally 0.25 mg/mL of doxycycline in *Diluent*, prepared as follows. Constitute Doxycycline for Oral Suspension as directed in the labeling. Transfer an accurately measured portion of the constituted suspension, freshly mixed and free from air bubbles, equivalent to about 25 mg of doxycycline, to a suitable volumetric flask. Add 50% of the final volume of *Diluent*, shake by mechanical means for about 15 min and dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of

0.2- μm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 350 nm

Column: 2.1-mm \times 5-cm; 1.7- μm packing L7. [Note—A 1.7- μm guard-column with packing L7 was used during method validation.]

Column temperature: 60°

Flow rate: 0.6 mL/min

Injection volume: 5 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 1.5 between doxycycline related compound A and doxycycline, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$) in the portion of Doxycycline for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

P = potency of doxycycline in USP Doxycycline Hyclate RS ($\mu\text{g}/\text{mg}$)

F = conversion factor, 0.001 mg/ μg

Acceptance criteria: 90.0%–125.0% \blacksquare 2S (*USP39*)

PERFORMANCE TESTS

- **Deliverable Volume** (698): Meets the requirements
- **Uniformity of Dosage Units** (905)

For single-unit containers

Acceptance criteria: Meets the requirements

Add the following:

■ ● **Dissolution** (711)

Protect solutions containing doxycycline from light.

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 25 rpm

Time: 10 min

Solution A: Transfer 1.36 g of *monobasic potassium phosphate*, 0.37 g of *sodium hydroxide*, 0.25 g of *tetrabutylammonium hydrogen sulfate*, and 0.2 g of *edetate disodium* to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Add 0.75 g of *tert-butyl alcohol* and dilute with water to volume. Adjust with 1 N *sodium hydroxide* to a pH of 8.0 ± 0.05 .

Solution B: *Acetonitrile* and water (80:20)

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
13.0	100	0
15.0	20	80
21.0	20	80

Return to original conditions and equilibrate the system.

Standard solution: 32 µg/mL of USP Doxycycline Hyclate RS in *Medium*

Sample solution: Reconstitute the suspension according to the label instructions for three separate containers. Transfer and combine the contents of the three containers into a separate suitable flask. Determine the density of the suspension. Using suitable syringes, measure portions of the reconstituted suspension containing nominally 25 mg of doxycycline. With the paddles lowered, gently empty the contents of each syringe into each vessel containing *Medium*. Start the paddle rotation. At the specified time, sample per *Dissolution* (711) and pass a portion through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 355 nm

Column: 4.6-mm × 250-cm; 5-µm packing *L21*

Temperatures

Column: 60°

Autosampler: 10°

Flow rate: 1.5 mL/min

Injection volume: 100 µL

Run time: 30 min

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage (*Q*) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times (d/W) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of doxycycline in the *Standard solution* (mg/mL)

L = label claim of Doxycycline for Oral Suspension (mg/5 mL)

D = dilution factor, necessary only if the *Sample solution* requires dilution (mL/mL)

d = density of the *Sample solution* (g/mL)

W = weight of Doxycycline for Oral Suspension taken (g)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

■ 2S (USP39)

IMPURITIES

Add the following:

■ ● **Organic Impurities**

Mobile phase, Diluent, System suitability solution, and Chromatographic system:

Proceed as directed in the *Assay*.

Standard solution: 1.5 µg/mL of USP Doxycycline Hyclate RS in *Diluent*

Sample solution: Constitute Doxycycline for Oral Suspension as directed in the labeling.

Nominally, a 0.25-mg/mL solution of doxycycline in *Diluent* is prepared as follows. Transfer an accurately measured portion of the constituted suspension, freshly mixed and free from air bubbles, equivalent to about 25 mg of doxycycline, to a 100-mL volumetric flask. Add 50 mL of *Diluent* and shake by mechanical means for about 15 min. Dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of 0.2-µm pore size.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 1.5 between doxycycline related compound A and doxycycline, *System suitability solution*

Relative standard deviation: NMT 5.0% for the doxycycline peak, *Standard solution*

Analysis**Samples:** *Standard solution and Sample solution*

Calculate the percentage of each impurity in the portion of Doxycycline for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of doxycycline from the *Standard solution*

C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

P = potency of doxycycline in USP Doxycycline Hyclate RS ($\mu\text{g}/\text{mg}$)

F = conversion factor, 0.001 mg/ μg

Acceptance criteria: See *Table 3*. Disregard any impurity peaks less than 0.2%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methacycline ^{a,b}	0.64	—
4-Epidoxycycline ^c	0.79	1.5
Doxycycline related compound A (6-epidoxycycline) ^{a,d}	0.88	—
Doxycycline	1.0	—
Any individual unspecified impurity	—	0.5
Total impurities	—	2.5

^a Process impurities are controlled in the drug substance and are not to be reported here. They are not included in total impurities.

^b (4*S*,4*aR*,5*S*,5*aR*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide.

^c (4*R*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

^d (4*S*,4*aR*,5*S*,5*aR*,6*S*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

■ 2*S* (USP39)

SPECIFIC TESTS

- pH (791)

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: 5.0–6.5

Delete the following:

- ● ~~Water Determination, Method 1 (921): NMT 3.0%~~ ■ 2*S* (USP39)

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
 - Store dry powder at controlled room temperature. ■ 2S (USP39)

Change to read:

- **USP Reference Standards** (11)
 - USP Doxycycline Hyclate RS
 - USP Doxycycline Monohydrate RS
 - USP Doxycycline Related Compound A RS
 - (4S,4aR,5S,5aR,6S,12aS)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.
 - C₂₂H₂₄N₂O₈ 444.43
 - USP Methacycline Hydrochloride RS ■ 2S (USP39)

BRIEFING

Epoetin. Because there is no existing *USP* monograph for this drug substance, a new monograph based on validated methods is being proposed. The liquid chromatographic procedure in the test for *Peptide Mapping* is based on analyses performed with the Agilent Zorbax SB-C8 brand of L7 column. The liquid chromatographic procedure in the test for *Limit of High Molecular Weight Proteins* is based on analyses performed with the Tosoh TSKgel G3000SW xl brand of L20 column. The liquid chromatographic procedure in the test for *N-Glycan Profiling* is based on analyses performed with the Dionex CarboPac PA1 brand of L46 guard column and the Dionex CarboPac PA1 brand of L46 column. The Nomenclature, Safety and Labeling Expert Committee in consultation with Biologics and Biotechnology Expert Committee 1 propose the title "Epoetin" because the monograph tests can apply to human recombinant erythropoietin with the same amino acid sequence and expressed in mammalian cell lines. Based on extensive discussion, the Biologics and Biotechnology Expert Committee 1 proposes the test for *N-Glycan Profiling* be included under *Specific Tests*.

(BIO2: K. Carrick.)

Correspondence Number—C108498

Comment deadline: November 30, 2015

Add the following:■ **Epoetin**

```

APPRLICDSR VLERYLLEAK EAENITTTGCA EHCSLNENIT VPDTKVNIFYA
WKRMEVGQQA VEVWQGLALL SEAVLRGQAL LVNSSQPWEP LQLHVDKAVS
GLRSLTTLLR ALGAQKEAIS PPDAASAAPL RTITADTFRK LFRVYSNFLR
GKLLKLYTGEA CRTGD
  
```

C₈₀₉H₁₃₀₁N₂₂₉O₂₄₀S₅ 18,236.06 Da (amino acid sequence)

DEFINITION

Epoetin is the recombinant form of human erythropoietin. It is a 165 amino acid glycoprotein manufactured using recombinant DNA technology. The presence of the impurities, host cell DNA, and host cell protein in Epoetin is process-specific, and is controlled through the purification process. The impurity levels are determined by validated methods and limits approved by the competent regulatory authority. It has a potency of NLT 140,000 and NMT 200,000 International Units (IU) per absorbance unit (AU) at 280 nm.

IDENTIFICATION

- **A. Erythropoietin Bioassays** (124): Meets the requirements
- **B. Peptide Mapping**

Solution A: Trifluoroacetic acid and water (1.5: 1000)

Solution B: Acetonitrile, trifluoroacetic acid, and water (900: 1.2: 100)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
30.0	78	22
110.0	58	42
130.0	35	65
130.1	10	90
140.0	10	90
140.1	90	10

Phosphate buffer saline: 137 mM sodium chloride, 2.7 mM potassium chloride, 8.1 mM disodium hydrogen phosphate, and 1.47 mM potassium dihydrogen phosphate

Buffer A: 20 mM sodium citrate and 100 mM sodium chloride, pH 6.9, prepared as follows. Dissolve 5.80 g of sodium citrate dihydrate, 0.057 g of citric acid, and 5.84 g of sodium chloride in 800 mL of water, and mix. Adjust with hydrochloric acid to a pH of 6.9 and dilute with water to 1 L. Pass through a nylon filter of 0.2- μ m pore size.

Digest stop solution: 8 M guanidine hydrochloride

Digest buffer stock: 1.0 M *tris*(hydroxymethyl) aminomethane solution. Adjust with hydrochloric acid to a pH of 7.3.

Lys-C stock solution: Dissolve Lysyl Endopeptidase (Lys-C), containing approximately 4.5 AU/mg, in *Digest buffer stock* to a final concentration of 2 mg/mL. Expiry of reconstituted Lys-C is 2 years at $-70 \pm 10^\circ$, and 1 day at $5 \pm 3^\circ$.

Lys-C digest solution: Add 20 μ L of *Lys-C stock solution* to 180 μ L of *Digest buffer stock*. Expiry is 1 day stored on ice or at $5 \pm 3^\circ$.

Standard solution: 100 μ g of USP Erythropoietin RS in 50 μ L of water. Buffer exchange by a suitable method into *Buffer A*. Add 5 μ L of *Lys-C digest solution* to 50 μ L of buffer exchanged USP Erythropoietin RS. Incubate at 37° for 30 min. Add 50 μ L of *Digest stop solution* and mix.

Sample solution: 100 μ g of Epoetin in 50 μ L of *Phosphate buffer saline*. Add 5 μ L of *Lys-C*

digest solution. Incubate at 37° for 30 min. Add 50 µL of *Digest stop solution* and mix.

Blank solution: 50 µL of *Phosphate buffered saline*. Add 5 µL of *Lys-C digest solution*. Incubate at 37° for 30 min. Add 50 µL of *Digest stop solution* and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 3.0-mm × 25-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 0.2 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements: 10 major peaks must be present as illustrated in the typical chromatogram provided with the USP certificate for USP Erythropoietin RS. The absolute difference between the retention times of peaks 5, 8, and 9 must be ±1.2 min for three replicate *Standard solution* injections. The ratio of relative heights % (RRH%) for each peak 8 and peak 9 in the *Standard solution* injections must be within 94%–106%.

Signal-to-noise ratio: NLT 3 for peak 6

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank solution*

Follow each injection of *Standard solution* with a *Blank solution* injection. Inject *Standard solution* before and after all *Sample solution* injections. Record the retention time and response (peak height) of each of the 10 major peaks for both the *Standard solution* and *Sample solution*.

Calculate the relative peak height (RH%) of peak 8 and peak 9 as compared to peak 5 for both the *Standard solution* and *Sample solution* taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak height of peak 8 or 9

r_S = peak height of peak 5

Calculate the ratio of relative peak height (RRH%) for peak 8 and peak 9:

$$\text{Result} = (RH_x/RH_S) \times 100$$

RH_x = RH% of peak 8 or 9 for the *Standard solution*

RH_S = RH% of peak 8 or 9 for the *Sample solution*

Acceptance criteria: The chromatographic profile of the *Sample solution* is similar to that of the *Standard solution*. The absolute difference between the retention times of peaks 5, 8, and 9 in the *Sample solution* must be within 1.2 min when compared to the initial *Standard solution*. The RRH% for each peak 8 and peak 9 in the *Sample solution* must be

within 94%–106% when compared to the initial *Standard solution*. There must be no new peaks > peak 6 in height in the *Sample solution* that are not present in the *Standard solution*.

ASSAY

• Erythropoietin Bioassays (124)

Acceptance criteria: Meets the requirements. It has a potency of NLT 140,000 and NMT 200,000 IU per absorbance unit at 280 nm.

IMPURITIES

• Limit of High Molecular Weight Proteins

Mobile phase: 20 mM sodium citrate and 100 mM sodium chloride, pH 6.9, prepared as follows. Dissolve 5.80 g of sodium citrate dihydrate, 0.057 g of citric acid, and 5.84 g of sodium chloride in 800 mL of water and mix. Adjust with hydrochloric acid to a pH of 6.9 and dilute with water to 1 L. Pass through a nylon filter of 0.2- μ m pore size.

System suitability solution: 2 mg/mL of USP Erythropoietin RS. Heat at 80° for 30 min, and use immediately.

Sample solution: 2 mg/mL of Epoetin

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 7.8-mm \times 30-cm; packing L20

Temperatures

Autosampler: 2–8°

Column: 25°

Flow rate: 1.0 mL/min

Injection volume: 40 μ L

Run time: 30 min

System suitability

Sample: *System suitability solution*

[Note—The relative retention time for the aggregate is approximately 0.7.]

Suitability requirements: The monomer retention time must be within 8–10 min for *System suitability solution* injections. The relative retention times for the dimer and monomer are about 0.9 and 1.0, respectively.

Relative standard deviation: The precision of the monomer peak area must be <2%.

Analysis

Sample: *Sample solution*

[Note—Condition the *Chromatographic system* with the *Standard solution*. Inject the *Standard solution* initially in duplicate before, and in a single bracket after, all *Sample solutions* injected.]

Record the chromatogram, and measure the areas of the main peak and of the peaks eluting near the main peak, excluding the solvent peaks.

Acceptance criteria: The total impurities (aggregates and dimer) should be NMT 0.1%.

SPECIFIC TESTS

• N-Glycan Profiling

Solution A: Water. Degas before use.

Solution B: Dissolve 41.0 g of anhydrous sodium acetate in 900 mL of water. Transfer the solution to a 1-L volumetric flask, and dilute with water to volume. Pass the solution through a nylon membrane filter of NMT 0.45- μ m pore size, and degas before use.

Solution C: To 900 mL of water add 26 mL of 50% (w/w) sodium hydroxide solution. Dilute the solution with water to a final volume of 1000 mL. Pass the solution through an alkaline-resistant nylon membrane filter of NMT 0.45- μ m pore size, and degas before use.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	80	10	10
15	80	10	10
70	60	30	10
94	0	90	10
99	0	90	10
105	0	10	90
110	0	10	90
111	80	10	10
130	80	10	10

2-AB labeling solution: Add 150 μ L of glacial acetic acid to 350 μ L of dimethyl sulfoxide (DMSO) and mix. Add 110 μ L of the acetic acid–DMSO mixture to a tube containing 5 mg of 2-aminobenzamide acid (2-AB) labeling dye and mix. Transfer the complete acetic-acid/DMSO/2-AB mixture to a tube containing 6 mg of sodium cyanoborohydride and mix. Use immediately and protect from light.

Buffer A: 20 mM sodium citrate, 100 mM sodium chloride, pH 6.9. Dissolve 5.80 g of sodium citrate dihydrate, 0.057 g of citric acid, and 5.84 g of sodium chloride in 800 mL of water and mix. Adjust with hydrochloric acid to a pH of 6.9 and dilute with water to 1 L. Pass through a nylon filter of 0.2- μ m pore size.

0.5 M DTT: Add 77 mg of dithiothreitol to 1 mL of water and mix. Use immediately.

Enzyme reaction buffer: 0.5 M sodium phosphate, pH 7.5

Standard solution: Reconstitute 100 μ g of USP Erythropoietin RS in 50 μ L of water. If necessary, buffer exchange by a suitable method into *Buffer A*. Transfer the entire contents to a 1.5-mL polypropylene tube (or equivalent). Add 12.0 μ L of *Enzyme reaction buffer*, 2.5 μ L of 0.5 M DTT, and 38.5 IUB milliunits of Peptide-N-Glycosidase F (PNGase F). Dilute with water to a final volume of 100 μ L, mix, and briefly centrifuge. Incubate at 37° for 30 min. Briefly centrifuge the sample, then freeze on dry ice for at least 5 min.

After the sample is frozen, uncap the tube and place it in the centrifugal evaporator and operate under vacuum without heat for approximately 70–80 min or until completely dry. Add 5.0 μL of 2-AB labeling solution, vortex, briefly centrifuge, and incubate at 60° for 3 h. Following incubation, add 120 μL of water, vortex, and centrifuge the sample. Remove excess 2-AB using a microcentrifuge column filled with an appropriate amount of G-10 gel filtration stationary phase for desalting a 75–150- μL sample. To prepare the resin, add 0.5 mL of water to the microcentrifuge column and swell for 15 min. Centrifuge at maximum speed for 5–10 s. Repeat wash and remove residual water. Apply labeled glycans, place the column in the microcentrifuge and spin at 200 $\times g$ for 1 min. Apply the flow through to a second column and repeat centrifugation. Transfer the repeat flow through to an HPLC vial for analysis.

Sample solution: Proceed as directed in the *Standard solution* using 100 μg of Epoetin.

Blank: Water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Fluorescence (330- and 420-nm emission wavelength)

Columns

Guard: 4.0-mm \times 5-cm; 10- μm packing L46

Analytical: 4.0-mm \times 25-cm; 10- μm packing L46

Temperatures

Column: 25°

Autosampler: 4°

Flow rate: 0.5 mL/min

Injection volume: 25 μL

System suitability

Samples: *Standard solution* and *Blank*

Suitability requirements: The % interference of the *Blank* injection must be <1% of the total peak area of the initial *Standard solution*. The *Standard solution* chromatogram must be consistent with the typical chromatogram provided with the USP certificate for USP Erythropoietin RS, including the presence of peaks 1–7. [Note—Peak 6 may exhibit splitting or shouldering but must be consistent for all *Standard solution* injections within a run.]

Relative standard deviation: Precision of total integrated peak area must be NMT 1.5%, *Standard solution*.

Analysis

Sample: *Sample solution*

Calculate the peak area response of each of the three peak groups, bi-sialylated (2 N), tri-sialylated (3 N), and tetra-sialylated (4 N), using the following formula: [Note—Refer to the typical chromatogram provided with the USP certificate for USP Erythropoietin RS.]

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = total peak response for each of the three peak groups, bi-sialylated (2 N), tri-sialylated (3 N), and tetra-sialylated (4 N)

r_T = sum of the total peak responses for the three peak groups, bi-sialylated (2 N), tri-sialylated (3 N), and tetra-sialylated (4 N)

Acceptance criteria: The *N*-glycan percentages must be within the following ranges.

2 N: 4.5%–6.0%

3 N: 18%–25%

4 N: 69%–77%

● **Isoform Distribution**

Cathode solution: Measure 0.31 g of L-histidine and dilute with water to a final volume of 10 mL.

Anode solution: 0.2 N sulfuric acid

Initiator: Measure 0.072 g of potassium persulfate and dilute with water to a final volume of 10 mL.

Gel: Prepare a single horizontal gel using plate dimensions of 265-mm × 128-mm × 3-mm with a gel of 0.5-mm thickness prepared as follows. Combine 9.0 g of urea, 5.9 mL of acrylamide (30%), 2.4 mL of bis-acrylamide (2%), 450 μL of 3-10 ampholyte, and 1.1 mL of 3-5 ampholyte. Dilute with water to 28 mL, mix thoroughly, and pass the solution through a filter of 0.45-μm pore size. Add 2 mL of *Initiator* and mix by inversion. Transfer the solution to the gel cassette assembly.

Fixing solution: Prepare a solution containing 200 mL of 60% (w/v) of a mixture containing trichloroacetic acid and 17.5% 5-sulfosalicylic acid, 100 mL of glacial acetic acid, 400 mL of absolute methanol, and 300 mL of water.

Gel wash solution: Prepare a solution containing 400 mL of absolute methanol and 100 mL of glacial acetic acid. Dilute with water to 1 L.

Stain solution: Dissolve 1.25 g of Coomassie Brilliant Blue R-250 in 1 L of *Gel wash solution*. Pass through a filter of 0.45-μm pore size.

Destain solution: Prepare a solution containing 75 mL of absolute methanol and 100 mL of glacial acetic acid. Dilute with water to 1 L.

Standard stock solution: 2 μg/μL of USP Erythropoietin RS in water

Standard solution A: 0.13 μg/mL of USP Erythropoietin RS, prepared by diluting *Standard stock solution* in water

Standard solution B: 1.3 μg/mL of USP Erythropoietin RS, prepared by diluting *Standard stock solution* in water

Sample solution: 20 μg of Epoetin in 15 μL of water

Electrophoretic system

Mode: Horizontal electrophoretic system with integrated cooling apparatus capable of maintaining 2°–8°

Temperature: Cool to 2°–8° before applying voltage and maintain the temperature during electrophoresis.

Prefocusing: Prefocus the gel for 20–40 min at a constant 10 watts (maximum of 3,000

V and 50 mAmps).

Loading profile: Load 15 µL of *Standard solution A*, *Standard solution B*, and *Sample solution* onto separate lanes of the gel at the cathode site.

Focusing: Focus the gel for 2.5 h at a constant 10 watts (maximum of 3,000 V and 50 mAmps).

Staining: Incubate the gel in *Fixing solution* two times for 15 min each. Decant and incubate the gel in *Gel wash solution* for at least 30 min. Decant and incubate the gel in *Stain solution* for 15–60 min. Decant and rinse the gel with *Destain*. Decant and incubate the gel in *Destain* until the background is clear and *Standard solution A* is still visible.

System suitability

Samples: *Standard solution A* and *Standard solution B*

There is no artifact of staining that obscures visualization of protein lanes and the location and intensity of bands are appropriate in *Standard solution A* and *Standard solution B*. Isoforms 10, 11, 12, and 13 are present in *Standard solution A* and *Standard solution B*. No bands are present below Isoform 9 in *Standard solution B*. Refer to the typical image provided with the USP certificate for USP Erythropoietin RS.

Analysis

Sample: *Sample solution*

Scan the gel with a densitometer.

Using data from the densitometric scan, calculate the relative percentage of each individual isoform in the portion of the *Sample solution* taken:

$$\text{Result} = (I/T) \times 100$$

I = individual band intensity

T = total band intensity in the *Sample solution*

Acceptance criteria: Isoforms 10–13 must be present at the following percentages.

Isoform 13: NLT 13%

Isoforms 12 + 13: 46%–72%

Isoforms 10 + 11: NLT 52%

Isoforms 10–13: NLT 91%

Isoforms 9 and 14 may be present in the *Sample solution*. No isoform below isoform 9 is observed and any minor band present in the *Sample solution* must also be present at the same location in *Standard solution B*. Refer to the typical image provided with the USP certificate for USP Erythropoietin RS.

● Protein Content

Sample solution: Epoetin, undiluted

Blank: Use an appropriate buffer solution consistent with the Epoetin under test.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 280 nm

Cell: Quartz cuvette

Analysis

Samples: *Sample solution* and *Blank*

Calculate the protein concentration, in mg/mL, of the sample taken:

$$\text{Result} = (A/e) \times l$$

A = absorbance at 280 nm, corrected with the *Blank*

e = extinction coefficient, 0.74 mL/mg·cm⁻¹

l = path length (cm)

- **Sterility Tests** (71): Meets the requirements
- **Bacterial Endotoxins Test** (85): It contains NMT 2.5 USP Endotoxin Units/mL.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in fluorinated ethylene propylene (FEP) or equivalent containers between 2° and 8°. Protect from light.
 - **Labeling:** Label it to indicate that the material is of recombinant DNA origin.
 - **USP Reference Standards** (11)
 - USP Endotoxin RS
 - USP Erythropoietin RS
- 2S (USP39)

BRIEFING

Exenatide. Because there is no existing *USP* monograph for this drug substance, a new monograph based on validated methods of analyses is being proposed. The liquid chromatographic procedure in the *Assay* is based on analyses performed with the XBridge 130 C18 brand of L1 column. The liquid chromatographic procedures in the test for *Product-Related Substances and Impurities, Procedures 1* and *2* are based on analyses performed with the XBridge 130 C18 brand of L1 column and YMC ODS AQ brand of L1 column. The typical retention time for exenatide in the *Assay* and the test for *Product-Related Substances and Impurities* are 14–20 min and 24–27 min, respectively. The liquid chromatographic procedure in *Process-Related Impurities, Limit of Phosphate* is based on analyses performed with the Dionex IonPac AS17-C brand of L83 column.

(BIO1: A. Szajek.)

Correspondence Number—C145255

Comment deadline: November 30, 2015

Add the following:

■ Exenatide

HGEGTFTSDL SKQMEEEAVR LFIEWLKNGG PSSGAPPPS —NH₂

C₁₈₄H₂₈₂N₅₀O₆₀S 4186.57

L-Histidyl-glycyl-L-glutamyl-glycyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-aspartyl-L-leucyl-L-seryl-L-lysyl-L-glutamyl-L-methionyl-L-glutamyl-L-glutamyl-L-alanyl-L-valyl-L-arginyl-L-leucyl-L-phenylalanyl-L-isoleucyl-L-glutamyl-L-tryptophyl-L-leucyl-L-lysyl-L-asparaginyl-glycyl-glycyl-L-prolyl-L-seryl-L-seryl-glycyl-L-alanyl-L-prolyl-L-prolyl-L-prolyl-L-serinamide [141758-74-9].

DEFINITION

Exenatide is a 39 amino acid synthetic peptide agonist for glucagon-like peptide-1 (GLP-1) receptor. Exenatide contains NLT 95% and NMT 105% of exenatide ($C_{184}H_{282}N_{50}O_{60}S$), calculated on the anhydrous, acetic acid-free basis. Exenatide is a white to off-white powder. [Note—Exenatide is very hygroscopic. Protect from exposure to moisture.]

IDENTIFICATION

- **A. HPLC:** *Solution A, Solution B, Mobile phase, System suitability solution, Standard solutions, Sample solutions, Chromatographic system, and System suitability.* Proceed as directed in the Assay.

Identity sample solution: Mix equal volumes of the *Standard solution* and the *Sample solution*.

Analysis

Samples: *Standard solution, Sample solution, and Identity sample solution*

Examine the chromatograms of the *Standard solution, Sample solution, and Identity sample solution*.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, and the major peak from the *Identity sample solution* elutes as a single peak.

- **B. Amino Acid Analysis**

For further discussion of the theory and applications, see *Biotechnology-Derived Articles—Amino Acid Analysis* (1052), which may be a helpful, but not mandatory, resource. Standardize the instrument with a mixture containing equal molar per volume amounts (except for L-cystine which is half the molar amount) of glycine and the L-form of the following amino acids: alanine, arginine, aspartic acid, cystine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, norleucine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan, valine, and tryptophan.

Hydrolysis solution: 6 N hydrochloric acid containing 4% of phenol

Sample solution: Accurately weigh out between 0.4 and 1.0 mg of Exenatide Acetate in glass ampuls. Add a minimum of 1.0 mL of *Hydrolysis solution*, freeze the sample ampul, and flame seal under vacuum. Hydrolyze at 110° for about 22 h. After hydrolysis, dry the test sample under vacuum to remove any residual acid. To the ampul add 2 mL of a buffer solution that is suitable for the amino acid analyzer, and pass through a filter of 0.45- μ m pore size.

Procedure: Prepare a co-injection of the *Standard solution* and the test sample. Inject a suitable volume into the amino acid analyzer, and record and measure the responses for each amino acid peak in the *Standard solution*. Express the content of each amino acid in

nmoles.

Calculate the mean nmol of the amino acids:

Result = (nmol found in the *Analysis* for Ala, Arg, Asx, Glx, Gly, His, Ile, Leu, Lys, Phe, Pro, Val)/30

Divide the nmol of each amino acid by the *Result* to determine the amino acid ratios that must meet the *Acceptance criteria*.

Acceptance criteria: See *Table 1*.

Table 1

Name	Acceptance Criteria (amino acid ratio)
Asparagine, threonine, phenylalanine, lysine, alanine	1.5–2.5
Serine	4.2–5.5
Glutamine	5.2–6.8
Proline	3.5–4.5
Glycine	4.5–5.5
Valine	0.5–1.5
Methionine, isoleucine, histidine, arginine	0.5–1.5
Leucine	2.5–3.5
Tryptophan	0.5–1.5

- **C.** The average mass by *Mass Spectrometry* (736) is 4186.6 ± 1.0 mass units.

ASSAY

- **Procedure**

Solution A: 10 mM ammonium hydrogen carbonate, pH 9.5

Solution B: Acetonitrile and *Solution A* (90:10)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	74	26
0.5	74	26
33	63	37
35	10	90
36	10	90
36.1	74	26
42	74	26

System suitability solution: 1.0 mg/mL of USP Exenatide RS and 0.005 mg/mL each of USP [Glu¹³]-Exenatide RS and USP [Met(O)¹⁴]-Exenatide RS

Standard solutions: 1.0 mg/mL of USP Exenatide RS in water, prepared in duplicate

Sample solutions: 1.0 mg/mL of Exenatide in water, prepared in duplicate

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

Temperatures

Column: 60°

Autosampler: 10°

Flow rate: 0.8 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solutions*

Suitability requirements

Mean peak area: Maximum difference between the mean peak area of the two *Standard solutions* is ± 2%.

Resolution: NLT 1.0 between the [Glu¹³]-exenatide and [Met(O)¹⁴]-exenatide peaks, *System suitability solution*

Retention time: 14–20 min for the exenatide peak, *Standard solutions*

Relative standard deviation: NMT 2.0% for the exenatide peak area and retention time from three replicate injections of the two *Standard solutions*

Analysis

Sample: *Sample solutions*

Calculate the percentage of exenatide (C₁₈₄H₂₈₂N₅₀O₆₀S) in the portion of Exenatide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of exenatide from the *Sample solutions*

r_S = peak response of exenatide from the *Standard solutions*

C_S = concentration of USP Exenatide RS in the *Standard solutions* (mg/mL)

C_U = concentration of Exenatide in the *Sample solutions* (mg/mL), calculated on the anhydrous, acetic acid-free basis

Acceptance criteria: 95%–105% of exenatide on the anhydrous, acetic acid-free basis

PRODUCT RELATED SUBSTANCES IMPURITIES

• Procedure 1: Exenatide Related Substances and Impurities

[Note—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]

Mobile phase, System suitability solution, Standard solutions, Sample solutions, and Chromatographic system: Proceed as directed in the *Assay*.

Acceptance criteria

Individual impurities: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
[Glu ¹³]exenatide	0.65–0.68	0.50
Sum of [Asp ²⁸]-exenatide and [Met(O) ¹⁴]-exenatide	0.68–0.76	0.50
Exenatide	1.00	—
Impurity A	1.19–1.20	0.50
Unspecified impurities	—	0.50

Total impurities: NMT 3.0% from *Procedure 1*

● **Procedure 2: N-Acetyl His¹-Exenatide**

[Note—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]

Solution A: 0.1 M sodium perchlorate, pH 2.7

Solution B: Acetonitrile

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	63	37
40	59	41
60	39	61

System suitability solution: 1.0 mg/mL of USP Exenatide RS and 0.005 mg/mL of USP [N-Acetyl-His¹]-Exenatide RS

Standard solution: 1.0 mg/mL of USP Exenatide RS in water

Sample solution: 1.0 mg/mL of Exenatide in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.0-mm × 15-cm; 3-μm packing L1

Temperatures

Column: 55°

Autosampler: 10°

Flow rate: 0.6 mL/min

Injection volume: 15 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between the main exenatide peak and the [*N*-acetyl-His¹]-exenatide impurity peak, *System suitability solution*

Retention time variability: NMT $\pm 5\%$ of mean for the exenatide peak, *Standard solution*

Relative standard deviation: NMT 2.0% for the exenatide peak from three replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Exenatide Acetate taken, disregarding any peak with an area less than 0.05% of the main peak:

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = peak area of each individual impurity from the *Sample solution*, other than the solvent peak and the main exenatide acetate peak

r_T = sum of the areas of all the peaks from the *Sample solution*, excluding that of the solvent peak

Acceptance criteria

Individual impurities: See *Table 5*.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Exenatide	1.00	—
[<i>N</i> -Acetyl-His ¹]-exenatide	1.10–1.12	1.0
Unspecified impurities	—	0.50

Total impurities: NMT 3.0% from *Procedure 2*

Procedure 3: Limit of d-His¹ Exenatide

Standard solution: 100 mg of l-amino acids and 0.5 mg of corresponding d-enantiomers are dissolved in 500 mL water. An aliquot of 700 μ L is dried in a SpeedVac and derivatized as described in the *Sample solution*.

Sample solution: Hydrolyze 1 mg of the sample in 350 μ L of 6 N deuterium chloride (DCI) in heavy water (D₂O) at 110° for 8 h. After removal of excess reagent by a stream of nitrogen, the sample is esterified with 250 μ L of 4 M deuteriochloric acid-ethyl alcohol at 110° for 20 min. After cooling to about 40°, open the vial and evaporate the reagent with a gentle stream of nitrogen at moderate temperature. Dissolve the residue in 250 μ L of trifluoroacetic anhydride in trifluoroacetic acid ethyl ester (1:2). Tightly close the vials

and heat to 130° for 10 min. After cooling to room temperature, remove the excess reagent by a stream of nitrogen. Add 50 µL of isobutyl chloroformate to the sample and heat the closed vial to 110° for 10 min. After removal of the excess reagent under a nitrogen stream, dissolve the residue in 250 µL of dichloromethane.

System suitability solution: About 100 µg of each d, l-amino acid is derivatized as described in *Sample solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC-MS (see *Mass Spectrometry* (736))

Detector: Mass

Column: 20-m × 0.28-mm Chirasil Val, 0.28 µm; liquid phase Chirasil Val or equivalent chiral capillary column¹

Temperatures

Oven: 155°, 3 min isotherm, 4°/min to 190°, 5 min

Injection port: 220°

Carrier gas: Hydrogen

Flow rate: 26 mL/min

Injection volume: 1.0 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 1.0 for enantiomers of the analyte and for any additional peak next to the analyte, *System suitability solution*

Tailing factor: NMT 2.0 for the representative enantiomer, *System suitability solution*

Relative standard deviation: NMT ±0.1% from the calculated content of d-Ala, d-Pro, d-Asp, d-Glu, d-Lys, and d-Arg, *Standard solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of d-His-exenatide impurity in the portion of Exenatide taken:

$$\text{Result} = [A_D / (A_D + A_L)] \times 100$$

A_D = peak response of d-His from the *Sample solution*

A_L = peak response of l-His from the *Sample solution*

Acceptance criteria: NMT 1.0%

PROCESS RELATED IMPURITIES

● **Procedure 4: Limit of Phosphate**

[Note—Limit of phosphate should be performed if phosphate is used in manufacture.]

Solution A: 50 mM potassium hydroxide in water

Solution B: Water

Mobile phase: See Table 6.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	2	98
3	2	98
20	50	50
21	100	0

Diluent: 1 mM sodium hydroxide in water

Standard solution: 2 µg/mL of phosphate in *Diluent*

Sample solution: 2 mg/mL of Exenatide in *Diluent*

System suitability solution: *Standard solution* and *Sample solution* (1:1)

Chromatographic system

(See *Ion Chromatography* (1065).)

Mode: IC

Detector: Conductivity

Column: 2-mm × 25-cm; 10.5-µm packing L83

Temperatures

Column: 30°

Sample: 15°

Suppressor: Anion self-regenerating suppressor

Suppressant: Autosuppression

Flow rate: 0.25 mL/min

Injection volume: 25 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Tailing factor: NMT 3%, *Standard solution*

Relative standard deviation: NMT 10%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

The peak area responses for the phosphate ion peak in the chromatograms obtained with the *Standard solution* shows a peak corresponding to phosphate ion at a retention time of 15–21 min. The identity of the phosphate peak in the exenatide sample is confirmed by the *System suitability solution* showing one single peak. The phosphate content in exenatide is >0.1%, if the mean peak area of the *Sample solution* is higher than the mean peak area of the *Standard solution*. The phosphate content in exenatide is ≤0.1%, if the mean peak area of the *Sample solution* is equal to or smaller than the mean peak area for

the *Standard solution*.

Acceptance criteria: NMT 0.1%

- **Procedure 5, Trifluoroacetic Acid (TFA) in Peptides** <503.1>: The exenatide shall contain NMT 0.25% trifluoroacetic acid.

OTHER COMPONENTS

- **Acetic Acid In Peptides** <503>: NMT 5.0%

SPECIFIC TESTS

- **Bacterial Endotoxins Test** <85>: It contains NMT 10 USP Endotoxin Units/mg of exenatide.
- **Microbial Enumeration Tests** <61> and **Tests for Specified Microorganisms** <62>: The total aerobic microbial count is less than 200 cfu/g and the total combined yeasts and molds count does not exceed 200 cfu/g.
- **Water Determination** <921>, *Method Ic*: NMT 7.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers at $-20 \pm 5^\circ$
- **USP Reference Standards** <11>
 - USP [*N*-Acetyl-His¹]-Exenatide RS
 - USP Endotoxin RS
 - USP Exenatide RS
 - USP [Glu¹³]-Exenatide RS
 - USP [Met(O)¹⁴]-Exenatide RS

■ 2S (*USP39*)

¹ AA suitable capillary is available through C.A.T. GmbH & Co. K.G.

BRIEFING

Frovatriptan Tablets. Because there is no existing monograph for this drug product, a new monograph based on validated methods of analysis is proposed.

The liquid chromatographic system used in the *Assay* and the *Dissolution* test was validated using the Inertsil C8-3 brand of *L7* column. The typical retention time for frovatriptan is about 5.5 min. The liquid chromatographic system used in the proposed test for *Organic Impurities* was validated using the Inertsil C8-3 brand of *L7* column. The typical retention time for frovatriptan is 24 min.

This monograph is contingent on FDA approval of a product that meets the proposed monograph.

(CHM4: H. Joyce.)

Correspondence Number—C136127

Comment deadline: November 30, 2015

Add the following:

► **Frovatriptan Tablets**

DEFINITION

Frovatriptan Tablets contain frovatriptan succinate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of frovatriptan (C₁₄H₁₇N₃O).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the frovatriptan peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as the frovatriptan peak of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• Procedure**

Solution A: Add 2.0 mL of *phosphoric acid* per L of water.

Buffer: 3.9 g/L of *ammonium acetate* in water. Add 2.0 mL of *triethylamine* per L and adjust with *glacial acetic acid* to a pH of 6.8.

Mobile phase: *Chromatographic methanol* and *Buffer* (20:80)

Diluent: *Chromatographic acetonitrile* and *chromatographic methanol* (50:50)

Standard stock solution: 0.3 mg/mL of USP Frovatriptan Succinate RS prepared as follows. Transfer a suitable quantity of USP Frovatriptan Succinate RS to an appropriate volumetric flask. Add 50% of the total flask volume of *Solution A*. Sonicate to promote dissolution. Dilute with *Diluent* to volume.

Standard solution: 0.06 mg/mL of USP Frovatriptan Succinate RS (equivalent to 0.04 mg/mL of frovatriptan) from *Standard stock solution* in *Mobile phase*

Sample stock solution: Nominally 0.2 mg/mL of frovatriptan from Tablets prepared as follows. Transfer NLT 20 Tablets to an appropriate volumetric flask. Add 50% of the final flask volume of *Solution A* and sonicate for 15 min with intermittent shaking. Add 30% of the final flask volume of *Diluent*, and sonicate for 15 min. Allow to cool to room temperature and dilute with *Diluent* to volume.

Sample solution: Nominally 0.04 mg/mL of frovatriptan from *Sample stock solution* in *Mobile phase* passed through a suitable filter. Use the filtrate.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 245 nm. For *Identification* test B, use a diode array detector in the range of 210–300 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection volume: 10 µL

Run time: NLT 1.5 times the retention time of frovatriptan

System suitability**Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of frovatriptan ($C_{14}H_{17}N_3O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Frovatriptan Succinate RS in the *Standard solution* (mg/mL) C_U = nominal concentration of frovatriptan in the *Sample solution* (mg/mL) M_{r1} = molecular weight of frovatriptan, 243.30 M_{r2} = molecular weight of anhydrous frovatriptan succinate, 361.40**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS****• Dissolution** (711)**Medium:** pH 5.5 phosphate buffer (6.8 g/L of *monobasic potassium phosphate* adjusted with sodium hydroxide solution to a pH of 5.5); 900 mL**Apparatus 2:** 50 rpm**Time:** 20 min**Buffer:** 3.9 g/L of *ammonium acetate* in water. Add 2.0 mL of *triethylamine* per 1 L and adjust with *glacial acetic acid* to a pH of 6.8.**Mobile phase:** *Chromatographic methanol* and *Buffer* (20:80)**Standard stock solution:** 0.4 mg/mL of USP Frovatriptan Succinate RS prepared as follows. Transfer a suitable amount of USP Frovatriptan Succinate RS to an appropriate volumetric flask. Add 20% of the final flask volume of *chromatographic methanol*. Sonicate to promote dissolution and then dilute with *Medium* to volume.**Standard solution:** ($L/900$) mg/mL of USP Frovatriptan Succinate RS from *Standard stock solution* in *Medium*, where L is the label claim of frovatriptan in mg/Tablet**Sample solution:** Pass a portion of solution under test through a suitable filter and use the filtrate.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 245 nm

Column: 4.6-mm × 25.0-cm; 5-μm packing L7

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection volume: 50 μL

Run time: NLT 1.5 times the retention time of frovatriptan

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of frovatriptan (C₁₄H₁₇N₃O) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

r_U = peak response of frovatriptan from the *Sample solution*

r_S = peak response of frovatriptan from the *Standard solution*

C_S = concentration of USP Frovatriptan Succinate RS in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of frovatriptan, 243.30

M_{r2} = molecular weight of anhydrous frovatriptan succinate, 361.40

V = volume of *Medium*, 900 mL

L = label claim of frovatriptan (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of frovatriptan (C₁₄H₁₇N₃O)

- **Uniformity of Dosage Units** (905): Meets the requirements

IMPURITIES

- **Organic Impurities**

Solution A: 3.9 g/L of *ammonium acetate* in water

Solution B: *Chromatographic methanol*

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	92	8
15	92	8
30	88	12
50	80	20
60	80	20
65	92	8
70	92	8

Diluent: *Chromatographic methanol* and *Solution A* (10:90)

System suitability solution: 0.1 mg/mL of USP Frovatriptan Succinate RS and 0.1 µg/mL of USP Frovatriptan Related Compound A RS in *Diluent*. Sonication may be used to aid in dissolution.

Standard solution: 0.8 µg/mL of USP Frovatriptan Succinate RS (equivalent to 0.5 µg/mL of frovatriptan) in *Diluent*. Sonication may be used to aid in dissolution.

Sensitivity solution: 0.08 µg/mL of USP Frovatriptan Succinate RS (equivalent to 0.05 µg/mL of frovatriptan) from *Standard solution* in *Diluent*

Sample solution: Nominally 100 µg/mL of frovatriptan from Tablets prepared as follows. Transfer NLT 10 Tablets into an appropriate volumetric flask. Add 40% of the final flask volume of *Diluent* and sonicate for 20 min. Allow to cool to room temperature and then dilute with *Diluent* to volume. Pass the resulting solution through a suitable filter. Use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 4.6-mm × 25-cm; 5-µm packing *L7*

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.0 between frovatriptan related compound A and frovatriptan, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of frovatriptan from *Standard solution*

C_S = concentration of USP Frovatriptan Succinate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of frovatriptan in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of frovatriptan, 243.30

M_{r2} = molecular weight of anhydrous frovatriptan succinate, 361.40

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Frovatriptan related compound A ^a	0.86	—
Frovatriptan	1.0	—
Any individual, unspecified degradation product	—	0.26
Total degradation products	—	1.0

^a This is a process impurity that is included in the table for identification purposes only. It is controlled in the drug substance and is not to be reported or included in the total degradation products for the drug product.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.
- **USP Reference Standards** <11>
 - USP Frovatriptan Succinate RS
 - USP Frovatriptan Related Compound A RS
 - (R)-3-Amino-2,3,4,9-tetrahydro-1H-carbazole-6-carboxamide.
 - C₁₃H₁₅N₃O 229.28

◀(TBD)

BRIEFING

Frovatriptan Succinate. Because there is no existing monograph for this drug substance, a new monograph based upon the Authorized Pending Monograph (posted December 27, 2013) is being proposed. On the basis of comments received, the chemical formula for frovatriptan succinate in the *Definition* and the calculation description in the *Assay* are for the anhydrous form of frovatriptan succinate.

The isocratic liquid chromatographic procedure in the *Assay* was validated with the Inertsil C8-3 brand of *L7* column. The typical retention time for the frovatriptan peak is about 6.7 min. The gradient liquid chromatographic procedure in the test for *Organic Impurities* was validated with the Inertsil C8-3 brand of *L7* column. The typical retention time for the frovatriptan peak is about 11.7 min. The liquid chromatographic procedure in the test for the *Limit of d-Camphor Sulfonic Acid* was validated with the Zorbax SB C8 brand of *L7* column. The typical retention time for the d-camphor sulfonic acid peak is about 6.7 min. The liquid chromatographic

procedure in the test for *Enantiomeric Purity* was validated with the Chiralpak AS-H brand of L90 column. The typical retention time for the frovatriptan peak is about 7.9 min.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style. This monograph is contingent on FDA approval of a product that meets the proposed monograph.

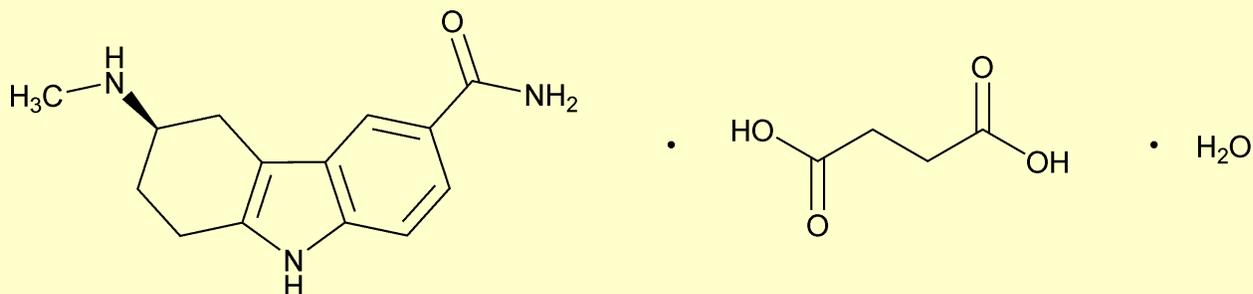
(CHM4: H. Joyce.)

Correspondence Number—C154837

Comment deadline: November 30, 2015

Add the following:

►Frovatriptan Succinate



$C_{14}H_{17}N_3O \cdot C_4H_6O_4 \cdot H_2O$ 379.41

(+)-(R)-2,3,4,9-Tetrahydro-3-(methylamino)-1H-carbazole-6-carboxamide butanedioate (1:1), monohydrate;

(+)-(R)-5,6,7,8-Tetrahydro-6-(methylamino)carbazole-3-carboxamide succinate (1:1), monohydrate [158930-17-7].

DEFINITION

Frovatriptan Succinate contains NLT 98.0% and NMT 102.0% of frovatriptan succinate ($C_{14}H_{17}N_3O \cdot C_4H_6O_4$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• Procedure

Solution A: 3.9 g/L of *ammonium acetate* in water

Solution B: *Chromatographic acetonitrile* and *chromatographic methanol* (50:50)

Mobile phase: *Solution A* and *Solution B* (85:15)

Diluent: *Chromatographic acetonitrile*, *chromatographic methanol*, and water (5:5:90)

Standard solution: 50 µg/mL of USP Frovatriptan Succinate RS in *Diluent*. Sonication may

be used to aid in dissolution.

Sample solution: 50 µg/mL of Frovatriptan Succinate in *Diluent*. Sonication may be used to aid in dissolution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 4.6-mm × 25.0-cm; 5-µm packing *L7*

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 2.2 times the retention time of frovatriptan

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of frovatriptan succinate ($C_{14}H_{17}N_3O \cdot C_4H_6O_4$) in the portion of Frovatriptan Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Frovatriptan Succinate RS in the *Standard solution* (µg/mL)

C_U = concentration of Frovatriptan Succinate in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.20%
- **Limit of Palladium**

Diluent: *Nitric acid* and water (20:80)

Standard solutions: Prepare five solutions having known concentrations ranging from 1.0 to 6.25 µg/mL of palladium, from a commercially available palladium atomic absorption standard in *Diluent*.

Sample solution: Transfer 2.5 g of Frovatriptan Succinate into a crucible that was previously ignited at $800 \pm 50^\circ$ for 30 min, cooled, and tared. Add 1 mL of *sulfuric acid*, and heat gently to thoroughly char the Frovatriptan Succinate. After cooling, moisten the residue with a small amount of sulfuric acid. Heat gently until white fumes are no longer evolved. Ignite at $800 \pm 50^\circ$ until the residue is completely incinerated. Ensure that fumes

are not produced during the ignition. Cool in a desiccator over silica gel. Dissolve the residue in 1 mL of *nitric acid*, and dilute with water to 5 mL.

Blank: *Diluent*

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrometry

Analytical wavelength: 247.6 nm

Lamp: Hollow cathode

Flame: Air-acetylene

System suitability

Samples: *Standard solutions* and *Blank*

Construct a calibration curve using the corrected absorbance values of the *Standard solutions*.

Suitability requirements

Correlation coefficient: NLT 0.99

Analysis

Samples: *Sample solution* and *Blank*

Using linear regression, calculate the slope (*S*) and the intercept (*I*) from the calibration curve.

Calculate the amount of palladium in the portion of Frovatriptan Succinate taken to prepare the *Sample solution*:

$$\text{Result} = [(A_U - I) \times (1/S) \times V] / W$$

A_U = corrected absorbance of palladium from the *Sample solution* (μg/mL)

I = intercept from the linear regression

S = slope from the linear regression

V = volume of the *Sample solution* (mL)

W = initial weight of Frovatriptan Succinate in the *Sample solution* (g)

Acceptance criteria: NMT 5 ppm

Limit of d-Camphor Sulfonic Acid

Solution A: To each L of a 2.5-g/L solution of *octanesulfonic acid sodium salt* add 2 mL of *triethylamine*, and adjust with *perchloric acid* to a pH of 2.0.

Solution B: *Chromatographic acetonitrile*

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
8	90	10
25	75	25
30	75	25
31	90	10
40	90	10

[Note—The gradient was established on an HPLC system with a dwell volume of approximately 0.9 mL.]

Standard solution: 20 µg/mL of USP d-Camphor Sulfonic Acid RS in water. Sonication may be used to aid in dissolution.

Sensitivity solution: 4 µg/mL of USP d-Camphor Sulfonic Acid RS from the *Standard solution* in water

Sample solution: 4 mg/mL of Frovatriptan Succinate in water. Sonication may be used to aid in dissolution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 195 nm

Column: 4.6-mm × 25.0-cm; 5-µm packing L7

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of d-camphor sulfonic acid in the portion of Frovatriptan Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of d-camphor sulfonic acid from the *Sample solution*

r_S = peak response of d-camphor sulfonic acid from the *Standard solution*

C_S = concentration of USP d-Camphor Sulfonic Acid RS in the *Standard solution* (µg/mL)

C_U = concentration of Frovatriptan Succinate in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.10%

- **Organic Impurities**

Solution A: 3.9 g/L of ammonium acetate in water

Solution B: *Chromatographic acetonitrile* and *Chromatographic methanol* (50:50)

Diluent: *Chromatographic acetonitrile*, *Chromatographic methanol*, and water (5:5:90)

System suitability stock solution A: 0.1 mg/mL of USP Frovatriptan Related Compound A RS, prepared as follows. Dissolve the Reference Standard first in *Solution B* using 10% of the final flask volume, then dilute with *Diluent* to volume.

System suitability stock solution B: 0.015 mg/mL of USP Frovatriptan Related Compound A RS from *System suitability stock solution A* in *Diluent*

System suitability solution: 0.5 mg/mL of USP Frovatriptan Succinate RS and 0.75 µg/mL of USP Frovatriptan Related Compound A RS from *System suitability stock solution B*, prepared as follows. Transfer a suitable amount of USP Frovatriptan Succinate RS to an appropriate volumetric flask. Add 5% of the final flask volume of *System suitability stock solution B* and 50% of the final flask volume of *Diluent*. Sonication may be used to aid in dissolution. Dilute with *Diluent* to the final flask volume.

Standard solution: 0.5 µg/mL of USP Frovatriptan Succinate RS in *Diluent*. Sonication may be used to aid in dissolution.

Sample solution: 0.5 mg/mL of Frovatriptan Succinate in *Diluent*. Sonication may be used to aid in dissolution.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
30	90	10
45	70	30
55	70	30
57	90	10
65	90	10

[Note—The gradient was established on an HPLC system with a dwell volume of approximately 0.9 mL.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 4.6-mm × 25.0-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 3* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.5 between frovatriptan related compound A and frovatriptan succinate, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Frovatriptan Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of frovatriptan succinate from the *Standard solution*

C_S = concentration of USP Frovatriptan Succinate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of Frovatriptan Succinate in the *Sample solution* ($\mu\text{g/mL}$)

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any peaks less than 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Frovatriptan related compound A racemate ^a	0.87	1.6	0.10 ^b
Frovatriptan succinate	1.0	—	—
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

^a (RS)-3-Amino-2,3,4,9-tetrahydro-1H-carbazole-6-carboxamide.

^b This procedure is not intended to resolve the two enantiomers. The acceptance criteria applies to the sum of both enantiomers.

- Enantiomeric Purity**

Mobile phase: *Chromatographic solvent hexane, dehydrated alcohol, and diethylamine* (65: 35: 0.1)

System suitability stock solution: 0.3 mg/mL of USP Frovatriptan Racemate RS in a solution prepared as follows. Dissolve the Reference Standard first in *absolute alcohol* using 20% of the final flask volume. Then dilute with *Mobile phase* to volume.

System suitability solution: 30 $\mu\text{g/mL}$ of USP Frovatriptan Racemate RS from the *System suitability stock solution* in *Mobile phase*

Standard solution: 0.002 mg/mL of USP Frovatriptan Succinate RS in *Mobile phase*. Sonication may be used to aid in dissolution.

Sample solution: 0.2 mg/mL of Frovatriptan Succinate in *Mobile phase*. Sonication may be used to aid in dissolution.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 4.6-mm × 25.0-cm; 5-μm packing *L90*

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: 2.5 times the retention time of frovatriptan

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for frovatriptan enantiomer and frovatriptan succinate are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between frovatriptan enantiomer and frovatriptan, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of frovatriptan enantiomer in the portion of Frovatriptan Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of frovatriptan enantiomer from the *Sample solution*

r_S = peak response of frovatriptan from the *Standard solution*

C_S = concentration of USP Frovatriptan Succinate RS in the *Standard solution* (mg/mL)

C_U = concentration of Frovatriptan Succinate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.15% of frovatriptan enantiomer

SPECIFIC TESTS

- **Water Determination** <921>, *Method I*: 4.3%–5.7%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at room temperature.
- **USP Reference Standards** <11>
 - USP d-Camphor Sulfonic Acid RS
 - [(1*S*,4*R*)-7,7-Dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl]methanesulfonic acid.
 - C₁₀H₁₆O₄S 232.30
 - USP Frovatriptan Racemate RS
 - (*RS*)-3-(Methylamino)-2,3,4,9-tetrahydro-1*H*-carbazole-6-carboxamide.
 - C₁₄H₁₇N₃O 243.30

USP Frovatriptan Related Compound A RS

(R)-3-Amino-2,3,4,9-tetrahydro-1*H*-carbazole-6-carboxamide.C₁₃H₁₅N₃O 229.28

USP Frovatriptan Succinate RS

◀(TBD)

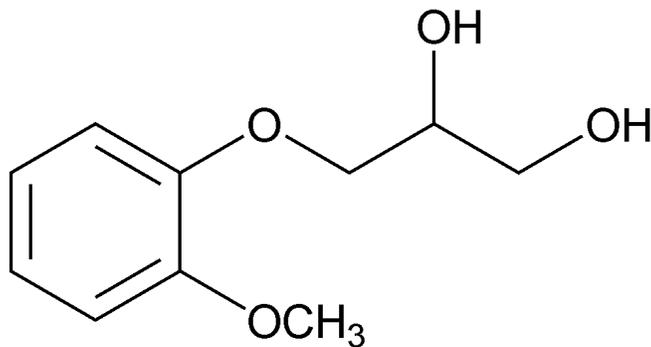
BRIEFING**Guaifenesin**, *USP 38* page 3725. The following revisions are being proposed:

1. Revise *Identification* test A to allow for the use of either *Infrared Absorption* (197K) or (197A).
2. Delete *Identification* test C, which is a reaction of the pseudoalkaloid, guaifenesin, with a Marquis reagent. The test is nonspecific and is non-value added. Conformance with both of the remaining tests by IR absorption and UV absorption is adequate for identification.
3. Delete the *Melting Range or Temperature* test. The remaining tests adequately ensure the purity of the material.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM6: A. Potts.)

Correspondence Number—C162208

Comment deadline: November 30, 2015**Guaifenesin**C₁₀H₁₄O₄ 198.22

1,2-Propanediol, 3-(2-methoxyphenoxy)-(±)-;
(±)-3-(*o*-Methoxyphenoxy)-1,2-propanediol [93-14-1].

DEFINITION

Guaifenesin contains NLT 98.0% and NMT 102.0% of guaifenesin (C₁₀H₁₄O₄), calculated on the dried basis.

IDENTIFICATION***Change to read:***

- **A. Infrared Absorption** (197):

- [Note—Methods described in (197K) or (197A) may be used.] ■ 2S (USP39)

- **B. Ultraviolet Absorption** (197U)

Sample solution: 40 µg/mL in *methanol*

Acceptance criteria: Meets the requirements

Delete the following:

- • ~~€~~

~~**Analysis:** Mix about 5 mg with 1 drop of formaldehyde and a few drops of sulfuric acid.~~

~~**Acceptance criteria:** A deep cherry red to purple color is produced. ■ 2S (USP39)~~

ASSAY

- **Procedure**

Solution A: *Glacial acetic acid* and water (1:99)

Solution B: *Acetonitrile*

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
32	50	50
35	80	20

System suitability solution: 0.5 mg/mL of USP Guaifenesin RS and 0.02 mg/mL of USP Guaiacol RS in *Solution B*

Standard solution: 0.5 mg/mL of USP Guaifenesin RS in *Solution B*

Sample solution: 0.5 mg/mL of Guaifenesin in *Solution B*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 276 nm

Column: 4.6-mm × 25-cm; 5-µm packing *L1*

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 3 between guaifenesin and guaiacol, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of guaifenesin ($C_{10}H_{14}O_4$) in the portion of Guaifenesin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of guaifenesin from the *Sample solution*

r_S = peak response of guaifenesin from the *Standard solution*

C_S = concentration of USP Guaifenesin RS in the *Standard solution* (mg/mL)

C_U = concentration of Guaifenesin in the *Sample solution* (mg/mL)

To this value, add the percentage of guaifenesin β -isomer found in the test for *Organic Impurities*.

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES**Delete the following:**

• **Heavy Metals, Method I (231):** NMT 25 ppm (Official 1-Dec-2015)

• **Organic Impurities**

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Sample solution: 2 mg/mL of Guaifenesin in *Solution B*

Diluted sample solution: 0.02 mg/mL of Guaifenesin from the *Sample solution* in *Solution B*

Analysis

Samples: *Sample solution* and *Diluted sample solution*

[Note—See *Table 2* for relative retention times.]

[Note—All of the peaks are baseline resolved.]

Calculate the percentage of each individual impurity in the portion of Guaifenesin taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of guaifenesin from the *Diluted sample solution*

F = relative response factor

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Guaifenesin β -isomer ^a	0.9	1.0	1.5
Guaifenesin	1.0	—	—
Guaiacol	1.4	1.6	0.03
Any individual unspecified impurity	—	1.0	0.5
Total impurities ^b	—	—	1.0

^a 2-(2-Methoxyphenoxy)-1,3-propanediol.
^b Excluding guaifenesin β -isomer and guaiacol.

SPECIFIC TESTS

- **Loss on Drying** (731)

Analysis: Dry under vacuum (NLT 10 mm of mercury) at 60° to a constant weight.

Acceptance criteria: NMT 0.5%

Delete the following:

- ● **Melting Range or Temperature** (741): 78°–82°, but the range between beginning and end of melting does not exceed 3° ■ 2S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** (11)
 USP Guaiacol RS
 USP Guaifenesin RS

BRIEFING

Hydroxyprogesterone Caproate Injection, USP 38 page 3810. As part of USP monograph modernization efforts, it is proposed to make the following changes.

1. Replace *Identification* test B, which is based on TLC and uses a toxic solvent, chloroform, with a retention time agreement in the proposed *Assay*.
2. Replace the nonspecific UV procedure in the *Assay* with a stability-indicating HPLC method. This procedure was validated using the Waters Symmetry C18 brand of L1 column. The retention time for hydroxyprogesterone caproate in the analysis is about 15 min.
3. Add a test for *Organic Impurities* to the monograph based on the proposed HPLC procedure in the *Assay*. The procedure was validated using the same chromatographic parameters as were used in the *Assay*.
4. Add an additional storage requirement to the *Packaging and Storage* section based on the information for an approved drug product.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(CHM5: P. Pabba, D. Min.)

Correspondence Number—C112399

Comment deadline: November 30, 2015

Hydroxyprogesterone Caproate Injection

DEFINITION

Hydroxyprogesterone Caproate Injection is a sterile solution of Hydroxyprogesterone Caproate in a suitable vegetable oil. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydroxyprogesterone caproate ($C_{27}H_{40}O_4$).

IDENTIFICATION

• A.

Sample solution: Transfer a volume of Injection, equivalent to 125 mg of hydroxyprogesterone caproate, to a 60-mL separator containing 10 mL of solvent hexane, 8 mL of methanol, and 2 mL of water. Insert the stopper, shake for 2 min, and allow the phases to separate. The lower layer is the *Sample solution*.

Analysis: To 3 mL of the *Sample solution* add sulfuric acid dropwise until a color develops, then add 3 mL of methanol.

Acceptance criteria: A purple color develops and the solution, when viewed under long-wavelength UV light, exhibits a pale yellow fluorescence.

Delete the following:

■ • B. Thin-Layer Chromatography

Standard solution: 400 µg/mL of USP Hydroxyprogesterone Caproate RS in chloroform

Sample solution: Evaporate 4 mL of the *Sample solution* prepared in the *Assay* to dryness and dissolve the residue in 0.5 mL of chloroform.

Chromatographic system

(See *Chromatographic system* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Chloroform and ethyl acetate (3:1)

Spray reagent: Sulfuric acid and alcohol (1:3)

Analysis: Apply the *Sample solution* and the *Standard solution* 2 cm apart on a line 2.5 cm from the bottom edge of a thin-layer chromatographic plate. Place the plate in a chamber that contains and that has been equilibrated with the *Developing solvent system*. Develop the plate until the solvent front has moved to 10 cm above the points of application. Remove the plate, mark the solvent front, and dry. Spray the plate with the *Spray reagent*, and heat in an oven at 105° for 5 min.

Acceptance criteria: The R_f value of the principal yellowish green spot of the *Sample solution* corresponds to that of the *Standard solution*. ■ 2S (USP39)

Add the following:

- ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

ASSAY**Change to read:**● **Procedure**

Isoniazid reagent: Dissolve 375 mg of isoniazid and 0.47 mL of hydrochloric acid in 500 mL of methanol.

Standard solution: 50 µg/mL of USP Hydroxyprogesterone Caproate RS in methanol

Sample solution: Nominally 50 µg/mL of hydroxyprogesterone caproate from an appropriate volume of Injection diluted with methanol

Spectrometric conditions

Mode: UV-Vis

Analytical wavelength: 380 nm

Blank: Methanol and *Isoniazid reagent* (5:10)

Analysis: Pipet 5 mL of *Sample solution* into a glass stoppered, 50 mL conical flask. Pipet 5 mL of *Standard solution* into a similar flask. To each flask add 10.0 mL of *Isoniazid reagent*, mix, and allow to stand in a water bath at 30° for 45 min.

Determine the absorbances of the *Sample solution* and the *Standard solution*. Calculate the percentage of hydroxyprogesterone caproate ($C_{27}H_{40}O_4$) in the portion of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Hydroxyprogesterone Caproate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of hydroxyprogesterone caproate the *Sample solution* (µg/mL)

- **Mobile phase:** Methanol and water (80:20)

Standard solution: 0.05 mg/mL of USP Hydroxyprogesterone Caproate RS in methanol

Sample solution: Nominally 0.05 mg/mL of hydroxyprogesterone caproate from an appropriate volume of Injection in methanol

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 μL

Run time: Twice the retention time of hydroxyprogesterone caproate

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 4000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydroxyprogesterone caproate ($\text{C}_{27}\text{H}_{40}\text{O}_4$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Hydroxyprogesterone Caproate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydroxyprogesterone caproate in the *Sample solution* (mg/mL)

■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

■ ● Organic Impurities

Mobile phase and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 0.5 $\mu\text{g/mL}$ of USP Hydroxyprogesterone Caproate RS in methanol

Sample stock solution: Transfer 2.0 mL of Injection, equivalent to 500 mg of hydroxyprogesterone caproate, to a 100-mL volumetric flask and dilute with methanol to volume.

Sample solution: Nominally 0.25 mg/mL of hydroxyprogesterone caproate prepared as follows. Transfer an appropriate amount of *Sample stock solution* to a suitable volumetric flask and dilute with methanol to volume.

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Signal-to-noise ratio: NLT 10

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of hydroxyprogesterone or any individual unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each corresponding degradation product from the *Sample solution*

r_S = peak response of hydroxyprogesterone caproate from the *Standard solution*

C_S = concentration of USP Hydroxyprogesterone Caproate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of hydroxyprogesterone caproate in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Hydroxyprogesterone ^a	0.4	1.3	1.0
Hydroxyprogesterone caproate	1.0	—	—
Individual unspecified degradation product	—	1.0	0.2
Total impurities	—	—	2.0

^a 17-Hydroxypregn-4-ene-3,20-dione.

■ 2S (USP39)

SPECIFIC TESTS

- **Water Determination** (921), *Method I*: NMT 0.2%
- **Other Requirements:** Meets the requirements in *Injections* (1)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type III glass.
 - Store at controlled room temperature. ■ 2S (USP39)
- **USP Reference Standards** (11)
USP Hydroxyprogesterone Caproate RS

BRIEFING

Imipramine Pamoate Capsules. The previous proposal of the new monograph, which appeared as an *In-Process Revision* in PF 39(6) [Nov.–Dec. 2013], is canceled and is now being re-proposed with changes based on comments received.

1. *Identification* test *B* based on spectral agreement using the test for *Organic Impurities*

has been added.

2. A statement has been added to the *Sample stock solution* preparation within the *Assay* to indicate that the solution must be allowed to stand for 5 min before use.
3. A two-tiered approach has been added to *Dissolution Test 1* to address possible cross-linking.
4. *Dissolution Test 2* is added to accommodate the drug product, which was approved with different dissolution conditions and tolerances.
5. The requirement for using low-actinic glassware is replaced with a more flexible light protection statement in the test for *Organic Impurities*.
6. The composition of *Solution A* has been modified slightly to reflect the sponsor's solution description within the liquid chromatographic test for *Organic Impurities* and to reflect additional validation data.
7. A *Labeling* section was added to support the inclusion of *Dissolution Test 2*.

The liquid chromatographic test procedure in the *Assay* is based on analyses performed with the XTerra RP18 brand of L1 column manufactured by Waters Corporation. The typical retention time for imipramine in the *Assay* procedure is about 27 min.

The liquid chromatographic test procedure in *Dissolution Test 1* is based on analyses performed with the XTerra RP18 brand of L1 column manufactured by Waters Corporation. The typical retention time for imipramine in *Dissolution Test 1* is about 9.3 min.

The liquid chromatographic test procedure in the *Organic Impurities* is based on analyses performed with the XTerra RP18 brand of L1 column manufactured by Waters Corporation. The typical retention times for imipramine are 24–25 min.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM4: H. Joyce.)

Correspondence Number—C118410; C142769; C143061

Comment deadline: November 30, 2015

Add the following:

■ Imipramine Pamoate Capsules

DEFINITION

Imipramine Pamoate Capsules contain imipramine pamoate $[(C_{19}H_{24}N_2)_2 \cdot C_{23}H_{16}O_6]$ equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of imipramine hydrochloride $(C_{19}H_{24}N_2 \cdot HCl)$.

IDENTIFICATION

- **A.** The retention time of the imipramine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the imipramine peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Organic Impurities*.

ASSAY

- Procedure**

Buffer: 5.2 g/L of *dibasic potassium phosphate* in water

Solution A: *Chromatographic acetonitrile* and *Buffer* (15:85). Adjust with *phosphoric acid* to a pH of 8.0.

Solution B: *Chromatographic acetonitrile* and *Buffer* (38:62). Adjust with *phosphoric acid* to a pH of 8.0.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	70	30
20	35	65
30	35	65
31	90	10
35	90	10

Diluent: *Chromatographic acetonitrile* and water (75:25)

Standard stock solution: 0.75 mg/mL of USP *Imipramine Pamoate RS* in *Diluent*

Standard solution: 0.23 mg/mL of USP *Imipramine Pamoate RS* (equivalent to 0.15 mg/mL of *imipramine hydrochloride*) from the *Standard stock solution* in *Solution A*. Pass a portion through a suitable filter of 0.45- μ m pore size. Use the filtrate.

Sample stock solution: Transfer the contents of *Capsules (NLT 5)* into a suitable volumetric flask, and add the corresponding capsule shells. Add 10% of the final flask volume of *acetonitrile*, and sonicate for 10 min with intermittent shaking. Add 80% of the final flask volume of *Diluent*, and sonicate for 15 min with intermittent shaking. Allow to cool to room temperature, and dilute with *Diluent* to volume. Allow to stand for 5 min.

Sample solution: Nominally 0.23 mg/mL of *imipramine pamoate* (equivalent to 0.15 mg/mL of *imipramine hydrochloride*) from the *Sample stock solution* in *Solution A*. Pass a portion of the resulting solution through a suitable filter of 0.45- μ m pore size. Use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 269 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Autosampler temperature: 10°

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times for *pamoic acid* and *imipramine* are 0.3 and 1.0,

respectively.]

Suitability requirements

Resolution: NLT 2.0 between pamoic acid and imipramine

Tailing factor: NMT 2.0 for imipramine

Relative standard deviation: NMT 2.0% for imipramine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) in the portion of Imipramine Pamoate Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M \times (M_{r1}/M_{r2})] \times 100$$

r_U = peak area of imipramine from the *Sample solution*

r_S = peak area of imipramine from the *Standard solution*

C_S = concentration of USP Imipramine Pamoate RS in the *Standard solution* (mg/mL)

C_U = equivalent concentration of imipramine hydrochloride in the *Sample solution* (mg/mL)

M = number of moles of imipramine hydrochloride equivalent to each mole of imipramine pamoate, 2

M_{r1} = molecular weight of imipramine hydrochloride, 316.87

M_{r2} = molecular weight of imipramine pamoate, 949.18

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution (711)

Test 1

Tier 1

Medium 1: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 30 and 90 min

Tier 2

Medium 2: 0.1 N hydrochloric acid with 0.3% purified pepsin; 900 mL

Apparatus 1: 100 rpm

Time: 30 and 90 min

Buffer: 4.4 g/L of *dibasic potassium phosphate* in water

Mobile phase: *Acetonitrile*, *triethylamine*, and *Buffer* (400:5:600). Adjust with *phosphoric acid* to a pH of 8.0.

Diluent A: *Acetonitrile* and water (75:25)

Diluent B: 20.4 g/L of *monobasic potassium phosphate* and 3 g/L of *sodium hydroxide*. Adjust with 1 N *sodium hydroxide* or 1 N *phosphoric acid* to a pH of 7.4.

Standard stock solution: 0.63 mg/mL of USP Imipramine Pamoate RS in *Diluent A*

Standard solution: 0.038 mg/mL of USP Imipramine Pamoate RS from the *Standard stock solution* prepared as follows. Transfer a suitable volume of the *Standard stock solution* to an appropriate flask that already contains 60% of the final flask volume of *Diluent B* and 30% of the final flask volume of *Medium*. Dilute with *Diluent B* to volume.

Sample stock solution: Centrifuge a portion of the solution under test. Use the supernatant. Replace the portion of solution removed from the vessel with the same volume of fresh *Medium 1* or *Medium 2* at 37°. [Note—The use of a centrifuge speed of 5000 rpm for 10 min may be suitable.]

Sample solution: Nominally equivalent to about 0.025 mg/mL of imipramine hydrochloride prepared from the *Sample stock solution* in *Diluent B* in a suitable volumetric flask

Dissolution procedure: Perform the test using the conditions under *Tier 1*. In the presence of cross-linking, repeat the test with a new set of Capsules using the conditions under *Tier 2*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Temperatures

Autosampler: 10°

Column: 30°

Flow rate: 1.2 mL/min

Injection volume: 50 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_i = (r_i/r_S) \times C_S \times [M \times (M_{r1}/M_{r2})] \times V \times (1/L) \times 100$$

r_i = peak area of imipramine from the *Sample solution* at each time point

r_S = peak area of imipramine from the *Standard solution*

C_S = concentration of USP Imipramine Pamoate RS in the *Standard solution* (mg/mL)

M = number of moles of imipramine hydrochloride equivalent to each mole of imipramine pamoate, 2

M_{r1} = molecular weight of imipramine hydrochloride, 316.87

M_{r2} = molecular weight of imipramine pamoate, 949.18

V = volume of *Medium 1* or *Medium 2*, 900 mL

L = label claim (mg/Capsule)

Tolerances: See *Table 2*.

Table 2

Time Point (<i>i</i>)	Time (min)	Amount Dissolved NLT (%)
1	30	40
2	90	75

The percentage of imipramine pamoate dissolved equivalent to the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) dissolved at the times specified conforms to *Dissolution* (711), *Acceptance Table 1*.

Test 2: If the product complies with this test, the labeling indicates that the product meets *USP Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Times: 30 and 150 min

Standard solution: A solution containing USP Imipramine Pamoate RS at the concentrations listed in *Table 3* prepared as follows. Transfer a suitable quantity of USP Imipramine Pamoate RS to an appropriate volumetric flask. Add 5% of the final flask volume of *methanol* and sonicate for 5 min. Add 75% of the final flask volume of *Medium* that has been heated to NLT 60° and stir for 30 min. Allow to cool to room temperature. Dilute with *Medium* to volume and mix. Pass through a suitable filter and use the filtrate.

Table 3

Labeled Amount of Imipramine Hydrochloride (mg/Capsule)	Concentration of USP Imipramine Pamoate RS (mg/mL)	Equivalent Concentration of Imipramine Hydrochloride (mg/mL)
75	0.12	0.08
100	0.17	0.11
125	0.21	0.14
150	0.26	0.17

Sample solution: Pass a portion of the solution under test through a suitable filter. Use the filtrate.

Instrumental conditions

Mode: UV-Vis

Wavelength: 251 nm

Cell: 0.2 cm

Analysis**Samples:** *Standard solution* and *Sample solutions*

Calculate the percentage of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_i = (A_i/A_S) \times C_S \times [M \times (M_{r1}/M_{r2})] \times V \times (1/L) \times 100$$

A_i = absorbance of imipramine from the *Sample solution* at each time point

A_S = absorbance of imipramine from the *Standard solution*

C_S = concentration of USP Imipramine Pamoate RS in the *Standard solution* (mg/mL)

M = number of moles of imipramine hydrochloride equivalent to each mole of imipramine pamoate, 2

M_{r1} = molecular weight of imipramine hydrochloride, 316.87

M_{r2} = molecular weight of imipramine pamoate, 949.18

V = volume of *Medium*, 900 mL

L = label claim (mg/Capsule)

Tolerances: See *Table 4*.**Table 4**

Time Point (i)	Time (min)	Amount Dissolved NLT (%)
1	30	25
2	150	80

The percentage of imipramine pamoate dissolved equivalent to the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) dissolved at the times specified conforms to *Dissolution (711), Acceptance Table 1*.

- **Uniformity of Dosage Units (905):** Meet the requirements

IMPURITIES

- **Organic Impurities**

Protect solutions containing imipramine from light.

Buffer: 5.2 g/L of *dibasic potassium phosphate* in water

Solution A: *Chromatographic acetonitrile* and *Buffer* (3:100). Adjust with *phosphoric acid* to a pH of 7.2.

Solution B: *Methanol* and *chromatographic acetonitrile* (70:30)

Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	62	38
12	62	38
25	50	50
65	20	80
70	20	80
75	62	38
95	62	38

System suitability solution: 1.5 mg/mL of USP Imipramine Pamoate RS (equivalent to 1 mg/mL of imipramine hydrochloride), and 0.001 mg/mL each of USP Desipramine Hydrochloride RS and USP Depramine RS in *Solution B*. Pass a portion through a suitable membrane filter of 0.2- μ m pore size, and use the filtrate.

Standard solution: 0.015 mg/mL of USP Imipramine Pamoate RS (equivalent to 0.010 mg/mL of imipramine hydrochloride) in *Solution B*. Pass a portion through a suitable membrane filter of 0.2- μ m pore size, and use the filtrate.

Sample solution: Nominally 1.5 mg/mL of imipramine pamoate (equivalent to 1.0 mg/mL of imipramine hydrochloride) from NLT 20 Capsules prepared as follows. Transfer a portion of the contents of the Capsules equivalent to 50 mg of imipramine hydrochloride to a 50-mL volumetric flask. Add 30 mL of *Solution B*, and sonicate for 10 min in a cool water bath with intermittent shaking. Dilute with *Solution B* to volume. Pass a portion through a suitable membrane filter of 0.2- μ m pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm. For *Identification* test B, a diode-array detector may be used in the wavelength range of 200–300 nm.

Column: 4.6-mm \times 15-cm; 3.5- μ m packing L1

Temperatures

Autosampler: 10°

Column: 45°

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 6* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between the desipramine and depramine peaks; NLT 2.0 between the depramine and imipramine peaks, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis**Samples:** *Standard solution and Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M \times (M_{r1}/M_{r2})] \times (1/F) \times 100$$

 r_U = peak response of each imipramine impurity from the *Sample solution* r_S = peak response of imipramine from the *Standard solution* C_S = concentration of the USP Imipramine Pamoate RS in the *Standard solution* (mg/mL) C_U = equivalent concentration of imipramine hydrochloride in the *Sample solution* (mg/mL) M = number of moles of imipramine hydrochloride equivalent to each mole of imipramine pamoate, 2 M_{r1} = molecular weight of imipramine hydrochloride, 316.87 M_{r2} = molecular weight of imipramine pamoate, 949.18 F = relative response factor (see *Table 6*)**Acceptance criteria:** See *Table 6*. Disregard any degradation product peaks less than 0.02%.**Table 6**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (% w/w)
Pamoic acid ^a	0.1	—	—
Desipramine	0.40	1.0	0.2
Depramine	0.66	0.87	0.10
Imipramine	1.0	—	—
Iminodibenzyl ^b	1.3	1.5	0.2
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	0.75

^a Included for identification only. This peak is due to the pamoate counterion; hence it is not an impurity.

^b 10,11-Dihydro-5H-dibenzo[*b,f*]azepine.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **Labeling:** The labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP Reference Standards** <11>
USP Depramine RS
3-(5H-Dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine.

$C_{19}H_{22}N_2$ 278.39

USP Desipramine Hydrochloride RS

USP Imipramine Pamoate RS

■ 2S (USP39)

BRIEFING

Insulin Injection, *USP 38* page 3871. On the basis of comments received, it is proposed to revise the monograph as follows:

1. In the *Definition* and *Assay*, *Acceptance criteria*, change “USP Insulin Units” to “USP Insulin Units/mL” to align with the potency specification in the *Assay* calculation. Clarify the components contributing to the potency in the *Definition*.
2. Replace the test for *Limit of High Molecular Weight Proteins* with the test for *Physicochemical Analytical Procedures for Insulins* (121.1), *Limit of High Molecular Weight Proteins*, but retain the specific instructions for the *Sample solution* and *Acceptance criteria*.
3. In the *USP Reference Standards* section, remove USP Insulin RS because the reference material is no longer available. The *System suitability solution* and concentration of the *Standard solution* in the formula of the *Assay* have been modified to specify use of USP Insulin Beef RS and USP Insulin Pork RS in lieu of USP Insulin RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BIO1: M. Crivellone.)

Correspondence Number—C155178

Comment deadline: November 30, 2015

Insulin Injection

DEFINITION

Change to read:

Insulin Injection is an isotonic, sterile solution of Insulin. Its potency

■, based on the sum of the insulin and desamido insulin components, ■ 2S (USP39) is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in ~~USP Insulin Units~~

■ USP Insulin Units/mL. ■ 2S (USP39)

IDENTIFICATION

- **A.** The retention time of the insulin peak of *Sample solution A* or *Sample solution B* corresponds to that of the appropriate species of the *Identification solution*, as obtained in the *Assay*. [Note—It may be necessary to inject a mixture of *Sample solution* and *Identification solution*.]

ASSAY

Change to read:

- **Procedure**

Solution A: Dissolve 28.4 g of *anhydrous sodium sulfate* in 1000 mL of water. Pipet 2.7 mL of *phosphoric acid* into the solution, and adjust with *ethanolamine* to a pH of 2.3, if necessary.

Mobile phase: *Acetonitrile* and *Solution A* (26:74). [Note—The *acetonitrile* is warmed to NLT 20° to avoid precipitation.]

System suitability solution: 1.5 mg/mL of insulin

- of the appropriate species, either insulin beef or insulin pork, ■_{2S} (USP39) in 0.01 N *hydrochloric acid*.

- For insulin of mixed species, prepare a solution containing 1.3 mg/mL of insulin beef and 0.25 mg/mL of insulin pork in 0.01 N *hydrochloric acid*. ■_{2S} (USP39)

Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.

Identification solution: 0.6 mg/mL of USP Insulin Beef RS and 0.6 mg/mL of USP Insulin Pork RS in 0.01 N *hydrochloric acid*

Standard solution: 1.5 mg/mL of USP Insulin RS of the appropriate species, either USP Insulin Beef RS or USP Insulin Pork RS, in 0.01 N *hydrochloric acid*. For insulin of mixed species, prepare a solution containing 1.3 mg/mL of USP Insulin Beef RS and 0.25 mg/mL of USP Insulin Pork RS in 0.01 N *hydrochloric acid*.

Sample solution A (for Insulin Injection labeled as containing 40 USP Insulin Units/mL):

Add 2.5 µL of 9.6 N *hydrochloric acid* for each mL of an accurately measured volume of Injection. Allow the suspension, if present, to clarify, and mix.

Sample solution B (for Insulin Injection labeled as containing 100 USP Insulin Units/mL):

Add 2.5 µL of 9.6 N *hydrochloric acid* for each mL of an accurately measured volume of Injection. Allow the suspension, if present, to clarify, and mix. [Note—Pooling several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N *hydrochloric acid* to volume, and mix.

[Note—The *Identification solution*, *Standard solution*, and *Sample solutions* may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; packing *L1*

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between insulin and A-21 desamido insulin, *System suitability solution*

Tailing factor: NMT 1.8 for the insulin peak, *System suitability solution*

Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis

Samples: *Identification solution, Standard solution, and either Sample solution A or Sample solution B*

Measure the peak responses for insulin and A-21 desamido insulin using the chromatogram of the *Identification solution* to identify the insulin peaks.

For Insulin Injection prepared from a single species, calculate the potency, in USP Insulin Units/mL, of the portion of Injection taken:

$$\text{Result} = (\Sigma r_U / \Sigma r_S) \times C_S \times D$$

Σr_U = sum of the peak responses of insulin and A-21 desamido insulin from the *Sample solution*

Σr_S = sum of the peak responses of insulin and A-21 desamido insulin from the *Standard solution*

C_S = concentration of USP Insulin RS

▪ of the appropriate species, either USP Insulin Beef RS or USP Insulin Pork RS, ■ 2S (USP39) in the *Standard solution* (USP Insulin Units/mL)

D = dilution factor used to prepare the *Sample solution*

For Insulin Injection prepared from a mixture of insulin beef and insulin pork, calculate the total potency as the sum of the potencies of insulin beef and insulin pork, determined separately, as directed above.

Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin

Units/mL ■ 2S (USP39)

OTHER COMPONENTS

- **Zinc Determination (591):** 10–40 µg for every 100 USP Insulin Units of the appropriate species

PRODUCT RELATED SUBSTANCES IMPURITIES

Delete the following:

● **Limit of High Molecular Weight Proteins**

Solution A: 1 mg/mL of L-arginine in water

Mobile phase: ~~Solution A, acetonitrile, and glacial acetic acid (65:20:15)~~

Resolution solution: 4 mg/mL of insulin containing NLT 0.4% of high molecular weight proteins in 0.01N hydrochloric acid. Store in a refrigerator, and use within 7 days.

[Note—Insulin containing NLT 0.4% of high molecular weight proteins may be prepared by allowing insulin to stand at room temperature for about 5 days.]

Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each mL of an accurately measured volume of Injection, and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV-276 nm

Column: 7.8 mm × 30 cm; packing L20

Flow rate: 0.5 mL/min

Injection volume: 100 µL

System suitability

Sample: *Resolution solution*

Suitability requirements

Retention times: 13–17 min for the polymeric insulin complexes, about 17.5 min for the covalent insulin dimer, and 18–22 min for the insulin monomer, with salts eluting after the insulin monomer

Peak-to-valley ratio: The ratio of the height of the covalent insulin dimer peak to the height of the valley between the covalent insulin dimer peak and the insulin monomer peak is NLT 2.0

Analysis

Sample: *Sample solution*

Disregard any peaks having retention times greater than that of the insulin monomer. Calculate the percentage of high molecular weight proteins in the portion of insulin taken:

$$\text{Result} = 100 \times \sum r_H / (\sum r_H + r_M)$$

$\sum r_H$ = sum of the responses of all peaks having retention times less than that of the insulin monomer

r_M = peak response of the insulin monomer

Acceptance criteria: NMT 2.0% ■ 2S (USP39)

Add the following:

- ● **Physicochemical Analytical Procedures for Insulins** (121.1), *Limit of High Molecular Weight Proteins*

Proceed as directed in *Limit of High Molecular Weight Proteins*, except prepare the following *Sample solution*. It meets the requirements.

Sample solution: Quantitatively add 4 µL of 6 N *hydrochloric acid* to each mL of an accurately measured volume of Injection, and mix.

Acceptance criteria: NMT 2.0% ■ 2S (USP39)

SPECIFIC TESTS

- **pH** (791): 7.0–7.8
- **Particulate Matter in Injections** (788): Meets the requirements for small-volume injections

- **Bacterial Endotoxins Test** (85): NMT 80 USP Endotoxin Units/100 USP Insulin Units
- **Sterility Tests** (71), *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **Injections** (1): Meets the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage**: Preserve in the unopened, multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.
- **Labeling**: Label it to indicate the one or more animal species to which it is related, as porcine, bovine, or a mixture of porcine and bovine. If the Insulin Injection is made from insulin that is purified, label it as such. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Units/mL.

Change to read:

- **USP Reference Standards** (11)

USP Endotoxin RS

USP Insulin RS

■ 2S (USP39)

USP Insulin Beef RS

USP Insulin Pork RS

BRIEFING

Isophane Insulin Suspension, *USP 38* page 3881. On the basis of comments received, it is proposed to revise the monograph as follows:

1. Replace the test for *Limit of High Molecular Weight Proteins* with the test for *Physicochemical Analytical Procedures for Insulins* (121.1), *Limit of High Molecular Weight Proteins*, but retain the specific instructions for the *Sample solution* and *Acceptance criteria*.
2. In the test for *Physicochemical Analytical Procedures for Insulins*, correct the *Acceptance criteria* of "NMT 3.0% for the insulin monomer" to "NMT 3.0%."
3. Include directions on how to use the *Identification solution* in the *Analysis* section of the *Assay*.
4. The *Assay* in the currently official monograph instructs the user to "Proceed as directed in the *Assay* under *Insulin Injection*." The *Insulin Injection* monograph indicates, "Note —The *Identification solution*, *Standard solution*, and *Sample solutions* may be stored at room temperature for up to 12 hours or in a refrigerator for up to 48 hours." Stability of the *Standard solution* and *Sample solutions* is specific to the *Insulin Injection* monograph, and as such is not included in the *Isophane Insulin Suspension* monograph.
5. In the *Standard solution* of the *Assay* replace "USP Insulin Human RS" with "USP Insulin RS of the appropriate species, either USP Insulin Beef RS or USP Insulin Pork RS" and specify the use of the two Reference Standards for insulin of mixed species. In the

Analysis section of the *Assay* replace "USP Insulin Human RS" with "USP Insulin RS of the appropriate species, either USP Insulin Beef RS or USP Insulin Pork RS."

6. Modify the *System suitability solution* of the *Assay* to specify use of USP Insulin Beef RS and USP Insulin Pork RS for added clarity.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BIO1: M. Crivellone.)

Correspondence Number—C157912

Comment deadline: November 30, 2015

Isophane Insulin Suspension

DEFINITION

Isophane Insulin Suspension is a sterile suspension of zinc–insulin crystals and Protamine Sulfate in buffered Water for Injection, combined in a manner such that the solid phase of the suspension consists of crystals composed of insulin, protamine, and zinc. The Protamine Sulfate is prepared from the sperm or from the mature testes of fish belonging to the genus *Oncorhynchus* Suckley, or *Salmo* Linné (Fam. Salmonidae). Its potency, based on the sum of its insulin and desamido insulin components, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Units/mL.

IDENTIFICATION

- **A.** The retention time of the insulin peak of *Sample solution A* or *Sample solution B* corresponds to that of the appropriate species of the *Identification solution*, as obtained in the *Assay*. [Note—It may be necessary to inject a mixture of *Sample solution* and *Identification solution*.]

ASSAY

Change to read:

- **Procedure**

Solution A: Dissolve 28.4 g of *anhydrous sodium sulfate* in 1000 mL of water. Pipet 2.7 mL of *phosphoric acid* into the solution, and adjust with *ethanolamine* to a pH of 2.3, if necessary.

Mobile phase: *Acetonitrile* and *Solution A* (26:74). [Note—The *acetonitrile* is warmed to NLT 20° to avoid precipitation.]

System suitability solution: 1.5 mg/mL of insulin

- of the appropriate species, either insulin beef or insulin pork, ■2S (USP39)

in 0.01 N *hydrochloric acid*.

- For insulin of mixed species, prepare a solution containing 1.3 mg/mL of insulin beef and 0.25 mg/mL of insulin pork in 0.01 N *hydrochloric acid*. ■2S (USP39)

Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.

Identification solution: 0.6 mg/mL of USP Insulin Beef RS and 0.6 mg/mL of USP Insulin Pork RS in 0.01 N *hydrochloric acid*. [~~Note—The Identification solution, Standard solution, and Sample solutions~~

■ **2S (USP39)**

may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Standard solution: 1.5 mg/mL of ~~USP Insulin Human RS~~

■ USP Insulin RS of the appropriate species, either USP Insulin Beef RS or USP Insulin Pork RS, **2S (USP39)**

in 0.01 N *hydrochloric acid*.

■ For insulin of mixed species, prepare a solution containing 1.3 mg/mL of USP Insulin Beef RS and 0.25 mg/mL of USP Insulin Pork RS in 0.01 N *hydrochloric acid*. **2S (USP39)**

Sample solution A (for Isophane Insulin Suspension labeled as containing 40 USP Insulin Units/mL): Add 2.5 μ L of 9.6 N *hydrochloric acid* for each mL of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix.

Sample solution B (for Isophane Insulin Suspension labeled as containing 100 USP Insulin Units/mL): Add 2.5 μ L of 9.6 N *hydrochloric acid* for each mL of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix. [Note—Pooling several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N *hydrochloric acid* to volume, and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm \times 15-cm; packing *L1*

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between insulin and A-21 desamido insulin, *System suitability solution*

Tailing factor: NMT 1.8 for the insulin peak, *System suitability solution*

Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis

Samples:

■ *Identification solution*, **2S (USP39)**

Standard solution, and either *Sample solution A* or *Sample solution B*

■ Measure the peak responses for insulin and A-21 desamido insulin of the appropriate species, using the chromatogram of the *Identification solution* to identify the insulin peaks.

■ 2S (USP39)

For Isophane Insulin Suspension prepared from a single species, calculate the potency, in USP Insulin Units/mL, in the portion of Suspension taken:

$$\text{Result} = (\Sigma r_U / \Sigma r_S) \times C_S \times D$$

Σr_U = sum of the peak responses of insulin and A-21 desamido insulin from the *Sample solution*

Σr_S = sum of the peak responses of insulin and A-21 desamido insulin from the *Standard solution*

C_S = concentration of USP Insulin Human RS

■ USP Insulin RS of the appropriate species, either USP Insulin Beef RS or USP Insulin Pork RS, ■ 2S (USP39) in the *Standard solution* (USP Insulin Units/mL)

D = dilution factor used to prepare the *Sample solution*

For Isophane Insulin Suspension prepared from a mixture of insulin beef and insulin pork, calculate the total potency as the sum of the potencies of insulin beef and insulin pork, determined separately, as directed above.

Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Units/mL

OTHER COMPONENTS

- **Zinc Determination** (591): 10–40 µg for every 100 USP Insulin Units

PRODUCT RELATED SUBSTANCES IMPURITIES

Delete the following:

■ ● **Limit of High Molecular Weight Proteins**

Solution A: 1 mg/mL of L-arginine in water

Mobile phase: *Solution A*, acetonitrile, and glacial acetic acid (65:20:15)

Resolution solution: 4 mg/mL of insulin containing NLT 0.4% of high molecular weight proteins in 0.01 N hydrochloric acid. Store in a refrigerator, and use within 7 days.

[Note—Insulin containing NLT 0.4% of high molecular weight proteins may be prepared by allowing insulin to stand at room temperature for about 5 days.]

Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each mL of an accurately measured volume of Suspension, and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 276 nm

Column: 7.8 mm × 30 cm; packing L20

Flow rate: 0.5 mL/min

Injection volume: 100 µL

System suitability**Sample:** *Resolution solution***Suitability requirements****Retention times:** 13–17 min for the polymeric insulin complexes, about 17.5 min for the covalent insulin dimer, and 18–22 min for the insulin monomer, with salts eluting after the insulin monomer**Peak-to-valley ratio:** The ratio of the height of the covalent insulin dimer peak to the height of the valley between the covalent insulin dimer peak and the insulin monomer peak is NLT 2.0**Analysis****Sample:** *Sample solution*

Disregard any peaks having retention times greater than that of the insulin monomer. Calculate the percentage of high molecular weight proteins in the portion of Suspension taken:

$$\text{Result} = 100 \times \Sigma f_H / (\Sigma f_H + f_M)$$

Σf_H = sum of the responses of all peaks having retention times less than that of the insulin monomer

f_M = peak response of the insulin monomer

Acceptance criteria: NMT 3.0% for the monomer ■ 2S (USP39)**Add the following:**

- **Physicochemical Analytical Procedures for Insulins** (121.1), *Limit of High Molecular Weight Proteins*

Proceed as directed in *Limit of High Molecular Weight Proteins*, except prepare the following *Sample solution*. It meets the requirements.

Sample solution: Quantitatively add 4 µL of 6 N *hydrochloric acid* to each mL of an accurately measured volume of Suspension, and mix.**Acceptance criteria:** NMT 3.0% ■ 2S (USP39)**SPECIFIC TESTS**• **Insulin in the Supernatant****Sample solution:** Centrifuge 10 mL of the Suspension at 1500 ×g for 10 min. Use the supernatant.**Analysis:** Determine the insulin content of the *Sample solution* by a suitable method.**Acceptance criteria:** NMT 1.0 USP Insulin Unit/mL

- **pH** (791): 7.0–7.8
- **Bacterial Endotoxins Test** (85): NMT 80 USP Endotoxin Units/100 USP Insulin Units
- **Sterility Tests** (71), *Test for Sterility of the Product to Be Examined, Membrane Filtration:* Meets the requirements when tested as directed, and the Suspension being filtered immediately after it has been put into solution using a validated suitable solvent

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in the unopened, multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.
- **Labeling:** Label it to indicate the one or more animal species to which it is related, as porcine, bovine, or a mixture of porcine and bovine. If the Isophane Insulin Suspension is made from insulin that is purified, label it as such. The Suspension container label states that the Suspension is to be shaken carefully before use. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Units/mL.
- **USP Reference Standards** (11)
 - USP Endotoxin RS
 - USP Insulin Beef RS
 - USP Insulin Pork RS

BRIEFING

Insulin Zinc Suspension, *USP 38* page 3885. On the basis of comments received, it is proposed to revise the monograph as follows:

1. Replace the test for *Limit of High Molecular Weight Proteins* with the test for *Physicochemical Analytical Procedures for Insulins* (121.1), *Limit of High Molecular Weight Proteins*, but retain the specific instructions for the *Sample solution* and *Acceptance criteria*.
2. The *Assay* in the official monograph instructs the user to "Proceed as directed in the *Assay* under *Insulin Injection*." The *Insulin Injection* monograph indicates, "Note—The *Identification solution*, *Standard solution*, and *Sample solutions* may be stored at room temperature for up to 12 hours or in a refrigerator for up to 48 hours." Stability of the *Standard solution* and *Sample solutions* is specific to the *Insulin Injection* monograph, and as such is not included in the *Insulin Zinc Suspension* monograph.
3. Modify the *System suitability solution* and the concentration of the *Standard solution* in the formula of the *Assay* to specify the use of USP Insulin Beef RS and USP Insulin Pork RS for added clarity.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BIO1: : M. Crivellone.)

Correspondence Number—C157913

Comment deadline: November 30, 2015

Insulin Zinc Suspension

Insulin zinc [8049-62-5].

DEFINITION

Insulin Zinc Suspension is a sterile suspension of Insulin in buffered Water for Injection,

modified by the addition of a suitable zinc salt in a manner such that the solid phase of the suspension consists of a mixture of crystalline and amorphous insulin in a ratio of approximately 7 parts of crystals to 3 parts of amorphous material. Its potency, based on the sum of its insulin and desamido insulin components, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Units/mL.

IDENTIFICATION

- **A.** The retention time of the insulin peak of *Sample solution A* or *Sample solution B* corresponds to that of the appropriate species of the *Identification solution*, as obtained in the *Assay*. [Note—It may be necessary to inject a mixture of *Sample solution* and *Identification solution*.]

ASSAY

Change to read:

- **Procedure**

Solution A: Dissolve 28.4 g of *anhydrous sodium sulfate* in 1000 mL of water. Pipet 2.7 mL of *phosphoric acid* into the solution, and adjust with *ethanolamine* to a pH of 2.3, if necessary.

Mobile phase: *Acetonitrile* and *Solution A* (26:74). [Note—The *acetonitrile* is warmed to NLT 20° to avoid precipitation.]

System suitability solution: 1.5 mg/mL of insulin

- of the appropriate species, either insulin beef or insulin pork, ■ 2S (USP39) in 0.01 N *hydrochloric acid*.

- For insulin of mixed species, prepare a solution containing 1.3 mg/mL of insulin beef and 0.25 mg/mL of insulin pork in 0.01 N *hydrochloric acid*. ■ 2S (USP39)

Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.

Identification solution: 0.6 mg/mL of USP Insulin Beef RS and 0.6 mg/mL of USP Insulin Pork RS in 0.01 N *hydrochloric acid*. [Note—The *Identification solution*, ~~Standard solution~~, and ~~Sample solution~~

- ■ 2S (USP39)

may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Standard solution: 1.5 mg/mL of USP Insulin RS of the appropriate species, either USP Insulin Beef RS or USP Insulin Pork RS, in 0.01 N *hydrochloric acid*. For insulin of mixed species, prepare a solution containing 1.3 mg/mL of USP Insulin Beef RS and 0.25 mg/mL of USP Insulin Pork RS in 0.01 N *hydrochloric acid*.

Sample solution A (for Insulin Zinc Suspension labeled as containing 40 USP Insulin Units/mL): Add 2.5 µL of 9.6 N *hydrochloric acid* for each mL of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix.

Sample solution B (for Insulin Zinc Suspension labeled as containing 100 USP Insulin Units/mL): Add 2.5 µL of 9.6 N *hydrochloric acid* for each mL of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix. [Note—Pooling several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N *hydrochloric acid* to volume, and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; packing *L1*

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between insulin and A-21 desamido insulin, *System suitability solution*

Tailing factor: NMT 1.8 for the insulin peak, *System suitability solution*

Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis

Samples: *Identification solution*, *Standard solution*, and either *Sample solution A* or *Sample solution B*

Measure the peak responses for insulin and A-21 desamido insulin using the chromatogram of the *Identification solution* to identify the insulin peaks.

For Insulin Zinc Suspension prepared from a single species, calculate the potency, in USP Insulin Units/mL, in the portion of Suspension taken:

$$\text{Result} = (\Sigma r_U / \Sigma r_S) \times C_S \times D$$

Σr_U = sum of the peak responses of insulin and A-21 desamido insulin from the *Sample solution*

Σr_S = sum of the peak responses of insulin and A-21 desamido insulin from the *Standard solution*

C_S = concentration of USP Insulin RS

■ of the appropriate species, either USP Insulin Beef RS or USP Insulin Pork RS, ■2S (USP39) in the *Standard solution* (USP Insulin Units/mL)

D = dilution factor used to prepare the *Sample solution*

For Insulin Zinc Suspension prepared from a mixture of insulin beef and insulin pork, calculate the total potency as the sum of the potencies of insulin beef and insulin pork, determined separately, as directed above.

Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Units/mL

OTHER COMPONENTS

- **Zinc Determination** (591): 0.12–0.25 mg for every 100 USP Insulin Units
- **Zinc in the Supernatant**

Analysis: Centrifuge a portion of Suspension sufficient for the test and determine the zinc content in the clear supernatant as directed in *Zinc Determination* (591).

Acceptance criteria: Concentration of zinc (mg/mL) is 20%–65% of the zinc concentration of the Suspension.

PRODUCT RELATED SUBSTANCES IMPURITIES

Delete the following:

• Limit of High Molecular Weight Proteins

Solution A: 1 mg/mL of L-arginine in water

Mobile Phase: *Solution A*, acetonitrile, and glacial acetic acid (65:20:15).

Resolution solution: 4 mg/mL of insulin containing NLT 0.4% of high molecular weight proteins in 0.01 N hydrochloric acid. Store in a refrigerator, and use within 7 days.

[Note—Insulin containing NLT 0.4% of high molecular weight proteins may be prepared by allowing insulin to stand at room temperature for about 5 days.]

Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each mL of an accurately measured volume of Suspension, and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 276 nm

Column: 7.8 mm × 30 cm; packing L20

Flow rate: 0.5 mL/min

Injection volume: 100 µL

System suitability

Sample: *Resolution solution*

Suitability requirements

Retention times: 13–17 min for the polymeric insulin complexes, about 17.5 min for the covalent insulin dimer, and 18–22 min for the insulin monomer, with salts eluting after the insulin monomer

Peak-to-valley ratio The ratio of the height of the covalent insulin dimer peak to the height of the valley between the covalent insulin dimer peak and the insulin monomer peak is NLT 2.0

Analysis

Sample: *Sample solution*

Disregard any peaks having retention times greater than that of the insulin monomer. Calculate the percentage of high molecular weight proteins in the portion of insulin taken:

$$\text{Result} = 100 \times \Sigma r_{H} / (\Sigma r_{H} + r_{M})$$

Σr_{H} = sum of the responses of all peaks having retention times less than that of the insulin monomer

r_{M} = peak response of the insulin monomer

Acceptance criteria: NMT 1.5% ■ 2S (USP39)

Add the following:

■ ● **Physicochemical Analytical Procedures for Insulins** (121.1), *Limit of High Molecular Weight Proteins*

Proceed as directed in *Limit of High Molecular Weight Proteins*, except prepare the following *Sample solution*. It meets the requirements.

Sample solution: Quantitatively add 4 μL of 6 N *hydrochloric acid* to each mL of an accurately measured volume of Suspension, and mix.

Acceptance criteria: NMT 1.5% ■ 2S (USP39)

SPECIFIC TESTS

● **Insulin Not Extracted by Buffered Acetone Solution**

Sample solution: Centrifuge a quantity of Suspension representing 1000 USP Insulin Units, and discard the supernatant. Suspend the residue in 8.4 mL of water, quickly add 16.6 mL of *buffered acetone TS*, shake or stir vigorously, and centrifuge within 3 min after the addition of the *buffered acetone TS*. Discard the supernatant, repeat the treatment with water and *buffered acetone TS*, centrifuge, and discard the supernatant. Dissolve the crystalline residue in 5 mL of dilute *hydrochloric acid* (1 in 100), transfer to a 25-mL flask, and dilute with water to volume.

Analysis: Use an appropriate method to determine the insulin concentration.

Acceptance criteria: Insulin concentration is 63%–77% of the insulin content of an equal amount of the Suspension.

● **Insulin in the Supernatant**

Sample solution: Centrifuge 10 mL of the Suspension at 1500 $\times g$ for 10 min. Use the supernatant.

Analysis: Determine the insulin content of the *Sample solution* by a suitable method.

Acceptance criteria: NMT 1.0 USP Insulin Unit/mL

● **pH** (791): 7.0–7.8

● **Bacterial Endotoxins Test** (85): NMT 80 USP Endotoxin Units/100 USP Insulin Units

● **Sterility Tests** (71), *Test for Sterility of the Product to Be Examined, Membrane Filtration:* Meets the requirements when tested as directed, and the Suspension being filtered immediately after it has been put into solution using a validated suitable solvent

ADDITIONAL REQUIREMENTS

● **Packaging and Storage:** Preserve in the unopened, multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid

freezing.

- **Labeling:** Label it to indicate the one or more animal species to which it is related, as porcine, bovine, or a mixture of porcine and bovine. If the Insulin Zinc Suspension is made from insulin that is purified, label it as such. The Suspension container label states that the Suspension is to be shaken carefully before use. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Units/mL.
- **USP Reference Standards** <11>
 - USP Endotoxin RS
 - USP Insulin Beef RS
 - USP Insulin Pork RS

BRIEFING

Extended Insulin Zinc Suspension, *USP 38* page 3886. On the basis of comments received, it is proposed to revise the monograph as follows:

1. Replace the test for *Limit of High Molecular Proteins* with the test for *Physicochemical Analytical Procedures for Insulins* <121.1>, *Limit of High Molecular Weight Proteins*, but retain the specific instructions for the *Sample solution* and *Acceptance criteria*.
2. The *Assay* in the currently official monograph instructs the user to "Proceed as directed in the *Assay* under *Insulin Injection*." The *Insulin Injection* monograph indicates, "Note —The *Identification solution*, *Standard solution*, and *Sample solutions* may be stored at room temperature for up to 12 hours or in a refrigerator for up to 48 hours." Stability of the *Standard solution* and *Sample solutions* is specific to the *Insulin Injection* monograph, and as such is not included in the *Extended Insulin Zinc Suspension* monograph.
3. Modify the *System suitability solution* and the concentration of the *Standard solution* in the formula of the *Assay* to specify the use of USP Insulin Beef RS and USP Insulin Pork RS for added clarity.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BIO1: M. Crivellone.)

Correspondence Number—C157914

Comment deadline: November 30, 2015

Extended Insulin Zinc Suspension

DEFINITION

Extended Insulin Zinc Suspension is a sterile suspension of Insulin in buffered Water for Injection, modified by the addition of a suitable zinc salt in a manner such that the solid phase of the suspension is predominantly crystalline. Its potency, based on the sum of its insulin and desamido insulin components, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Units/mL.

IDENTIFICATION

- **A.** The retention time of the insulin peak of *Sample solution A* or *Sample solution B* corresponds to that of the appropriate species of the *Identification solution*, as obtained in the *Assay*. [Note—It may be necessary to inject a mixture of *Sample solution* and *Identification solution*.]

ASSAY**Change to read:****Procedure**

Solution A: Dissolve 28.4 g of *anhydrous sodium sulfate* in 1000 mL of water. Pipet 2.7 mL of *phosphoric acid* into the solution, and adjust with *ethanolamine* to a pH of 2.3, if necessary.

Mobile phase: *Acetonitrile* and *Solution A* (26:74). [Note—The *acetonitrile* is warmed to NLT 20° to avoid precipitation.]

System suitability solution: 1.5 mg/mL of insulin

- of the appropriate species, either insulin beef or insulin pork, ■ 2S (USP39) in 0.01 N *hydrochloric acid*.

- For insulin of mixed species, prepare a solution containing 1.3 mg/mL of insulin beef and 0.25 mg/mL of insulin pork in 0.01 N *hydrochloric acid*. ■ 2S (USP39)

Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.

Identification solution: 0.6 mg/mL of USP Insulin Beef RS and 0.6 mg/mL of USP Insulin Pork RS in 0.01 N *hydrochloric acid*. [Note—The *Identification solution*, *Standard solution*, and *Sample solution*

- ■ 2S (USP39)

may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Standard solution: 1.5 mg/mL of USP Insulin RS of the appropriate species, either USP Insulin Beef RS or USP Insulin Pork RS, in 0.01 N *hydrochloric acid*. For insulin of mixed species, prepare a solution containing 1.3 mg/mL of USP Insulin Beef RS and 0.25 mg/mL of USP Insulin Pork RS in 0.01 N *hydrochloric acid*.

Sample solution A (for Extended Insulin Zinc Suspension labeled as containing 40 USP Insulin Units/mL): Add 2.5 µL of 9.6 N *hydrochloric acid* for each mL of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix.

Sample solution B (for Extended Insulin Zinc Suspension labeled as containing 100 USP Insulin Units/mL): Add 2.5 µL of 9.6 N *hydrochloric acid* for each mL of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix. [Note—Pooling several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N *hydrochloric acid* to volume, and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between insulin and A-21 desamido insulin, *System suitability solution*

Tailing factor: NMT 1.8 for the insulin peak, *System suitability solution*

Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis

Samples: *Identification solution*, *Standard solution*, and either *Sample solution A* or *Sample solution B*

Measure the peak responses for insulin and A-21 desamido insulin using the chromatogram of the *Identification solution* to identify the insulin peaks.

For Extended Insulin Zinc Suspension prepared from a single species, calculate the potency, in USP Insulin Units/mL, of the portion of Suspension taken:

$$\text{Result} = (\Sigma r_U / \Sigma r_S) \times C_S \times D$$

Σr_U = sum of the peak responses of insulin and A-21 desamido insulin from the *Sample solution*

Σr_S = sum of the peak responses of insulin and A-21 desamido insulin from the *Standard solution*

C_S = concentration of USP Insulin RS

▪ of the appropriate species, either USP Insulin Beef RS or USP Insulin Pork RS, ■ 2S (USP39)

in the *Standard solution* (USP Insulin Units/mL)

D = dilution factor used to prepare the *Sample solution*

For Extended Insulin Zinc Suspension prepared from a mixture of insulin beef and insulin pork, calculate the total potency as the sum of the potencies of insulin beef and insulin pork, determined separately, as directed above.

Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Units/mL

OTHER COMPONENTS

- **Zinc Determination** (591): 0.12–0.25 mg for every 100 USP Insulin Units
- **Zinc in the Supernatant**

Analysis: Centrifuge a portion of Suspension sufficient for the test and determine the zinc content of the clear supernatant as directed in *Zinc Determination* (591).

Acceptance criteria: Concentration of zinc (mg/mL) is 20%–65% of the zinc concentration of the Suspension.

PRODUCT RELATED SUBSTANCES IMPURITIES**Delete the following:****Limit of High Molecular Weight Proteins****Solution A:** 1 mg/mL of L-arginine in water**Mobile phase:** *Solution A*, acetonitrile, and glacial acetic acid (65:20:15)**Resolution solution:** 4 mg/mL of insulin containing NLT 0.4% of high molecular weight proteins in 0.01 N hydrochloric acid. Store in a refrigerator, and use within 7 days.

[Note—Insulin containing NLT 0.4% of high molecular weight proteins may be prepared by allowing insulin to stand at room temperature for about 5 days.]

Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each mL of an accurately measured volume of Suspension, and mix.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV-276 nm**Column:** 7.8 mm × 30 cm; packing L20**Flow rate:** 0.5 mL/min**Injection volume:** 100 µL**System suitability****Sample:** *Resolution solution***Suitability requirements****Retention times:** 13–17 min for the polymeric insulin complexes, about 17.5 min for the covalent insulin dimer, and 18–22 min for the insulin monomer, with salts eluting after the insulin monomer**Peak-to-valley ratio:** The ratio of the height of the covalent insulin dimer peak to the height of the valley between the covalent insulin dimer peak and the insulin monomer peak is NLT 2.0**Analysis****Sample:** *Sample solution*

Disregard any peaks having retention times greater than that of the insulin monomer. Calculate the percentage of high molecular weight proteins in the portion of insulin taken:

$$\text{Result} = 100 \times \Sigma r_H / (\Sigma r_H + r_M)$$

 Σr_H = sum of the responses of all peaks having retention times less than that of the insulin monomer r_M = peak response of the insulin monomer**Acceptance criteria:** NMT 1.5% ■ 2S (USP39)**Add the following:**

- ● **Physicochemical Analytical Procedures for Insulins** (121.1), *Limit of High Molecular Weight Proteins*
Proceed as directed in *Limit of High Molecular Weight Proteins*, except prepare the following *Sample solution*. It meets the requirements.
Sample solution: Quantitatively add 4 μL of 6 N *hydrochloric acid* to each mL of an accurately measured volume of Suspension, and mix.
Acceptance criteria: NMT 1.5% ■_{2S} (USP39)

SPECIFIC TESTS

● **Insulin Not Extracted by Buffered Acetone Solution**

Sample solution: Centrifuge a quantity of Suspension representing 1000 USP Insulin Units, and discard the supernatant. Suspend the residue in 8.4 mL of water, quickly add 16.6 mL of *buffered acetone TS*, shake or stir vigorously, and centrifuge within 3 min after the addition of the *buffered acetone TS*. Discard the supernatant, repeat the treatment with water and *buffered acetone TS*, centrifuge, and discard the supernatant. Dissolve the crystalline residue in 5 mL of dilute *hydrochloric acid* (1 in 100), transfer to a 25-mL flask, and dilute with water to volume.

Analysis: Use an appropriate method to determine the insulin concentration.

Acceptance criteria: The insulin concentration is NLT 90% of the insulin content of an equal amount of the Suspension.

● **Insulin in the Supernatant**

Sample solution: Centrifuge 10 mL of the Suspension at 1500 $\times g$ for 10 min. Use the supernatant.

Analysis: Determine the insulin content of the *Sample solution* by a suitable method.

Acceptance criteria: NMT 1.0 USP Insulin Unit/mL

● **pH** (791): 7.0–7.8

● **Bacterial Endotoxins Test** (85): NMT 80 USP Endotoxin Units/100 USP Insulin Units

● **Sterility Tests** (71), *Test for Sterility of the Product to Be Examined, Membrane Filtration:* Meets the requirements when tested as directed, and the Suspension being filtered immediately after it has been put into solution using a validated suitable solvent

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in the unopened, multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.
- **Labeling:** Label it to indicate the one or more animal species to which it is related, as porcine, bovine, or a mixture of porcine and bovine. If the Extended Insulin Zinc Suspension is made from insulin that is purified, label it as such. The container label states that the Suspension is to be shaken carefully before use. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Units/mL.

- **USP Reference Standards** (11)

USP Endotoxin RS

USP Insulin Beef RS

USP Insulin Pork RS

BRIEFING

Prompt Insulin Zinc Suspension, *USP 38* page 3886. On the basis of comments received, it is proposed to revise the monograph as follows:

1. Replace the test for *Limit of High Molecular Weight Proteins* with the test for *Physicochemical Analytical Procedures for Insulins* (121.1), *Limit of High Molecular Weight Proteins*, but retain the specific instructions for the *Sample solution* and *Acceptance criteria*.
2. The *Assay* in the official monograph instructs the user to "Proceed as directed in the *Assay* under *Insulin Injection*." The *Insulin Injection* monograph indicates, "Note—The *Identification solution*, *Standard solution*, and *Sample solutions* may be stored at room temperature for up to 12 hours or in a refrigerator for up to 48 hours." Stability of the *Standard solutions* and *Sample solutions* is specific to the *Insulin Injection* monograph, and as such is not included in the *Prompt Insulin Zinc Suspension* monograph."
3. Modify the *System suitability solution* and concentration of the *Standard solution* in the formula of the *Assay* to specify use of USP Insulin Beef RS and USP Insulin Pork RS for added clarity.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BIO1: M. Crivellone.)

Correspondence Number—C157915

Comment deadline: November 30, 2015

Prompt Insulin Zinc Suspension

DEFINITION

Prompt Insulin Zinc Suspension is a sterile suspension of Insulin in buffered Water for Injection, modified by the addition of a suitable zinc salt in a manner such that the solid phase of the suspension is amorphous. Its potency, based on the sum of its insulin and desamido insulin components, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Units/mL.

IDENTIFICATION

- **A.** The retention time of the insulin peak of *Sample solution A* or *Sample solution B* corresponds to that of the appropriate species of the *Identification solution*, as obtained in the *Assay*. [Note—It may be necessary to inject a mixture of *Sample solution* and *Identification solution*.]

ASSAY**Change to read:**● **Procedure**

Solution A: Dissolve 28.4 g of *anhydrous sodium sulfate* in 1000 mL of water. Pipet 2.7 mL of *phosphoric acid* into the solution, and adjust with *ethanolamine* to a pH of 2.3, if necessary.

Mobile phase: *Acetonitrile* and *Solution A* (26:74). [Note—The *acetonitrile* is warmed to NLT 20° to avoid precipitation.]

System suitability solution: 1.5 mg/mL of insulin of the appropriate species,

■ either insulin beef or insulin pork, ■2S (USP39)

in 0.01 N *hydrochloric acid*.

■ For insulin of mixed species, prepare a solution containing 1.3 mg/mL of insulin beef and 0.25 mg/mL of insulin pork in 0.01 N *hydrochloric acid*. ■2S (USP39)

Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.

Identification solution: 0.6 mg/mL of USP Insulin Beef RS and 0.6 mg/mL of USP Insulin Pork RS in 0.01 N *hydrochloric acid*. [Note—The *Identification solution*, ~~*Standard solution*~~, and ~~*Sample solution*~~

■ ■2S (USP39)

may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Standard solution: 1.5 mg/mL of USP Insulin RS of the appropriate species, either USP Insulin Beef RS or USP Insulin Pork RS, in 0.01 N *hydrochloric acid*. For insulin of mixed species, prepare a solution containing 1.3 mg/mL of USP Insulin Beef RS and 0.25 mg/mL of USP Insulin Pork RS in 0.01 N *hydrochloric acid*.

Sample solution A (for Prompt Insulin Zinc Suspension labeled as containing 40 USP Insulin Units/mL): Add 2.5 µL of 9.6 N *hydrochloric acid* for each mL of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix.

Sample solution B (for Prompt Insulin Zinc Suspension labeled as containing 100 USP Insulin Units/mL): Add 2.5 µL of 9.6 N *hydrochloric acid* for each mL of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix. [Note—Pooling several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N *hydrochloric acid* to volume, and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between insulin and A-21 desamido insulin, *System suitability solution*

Tailing factor: NMT 1.8 for the insulin peak, *System suitability solution*

Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis

Samples: *Identification solution*, *Standard solution*, and either *Sample solution A* or *Sample solution B*

Measure the peak responses for insulin and A-21 desamido insulin using the chromatogram of the *Identification solution* to identify the insulin peaks.

For Prompt Insulin Zinc Suspension prepared from a single species, calculate the potency, in USP Insulin Units/mL, of the portion of Suspension taken:

$$\text{Result} = (\Sigma r_U / \Sigma r_S) \times C_S \times D$$

Σr_U = sum of the peak responses of insulin and A-21 desamido insulin from the *Sample solution*

Σr_S = sum of the peak responses of insulin and A-21 desamido insulin from the *Standard solution*

C_S = concentration of USP Insulin RS

▪ of the appropriate species, either USP Insulin Beef RS or USP Insulin Pork RS, ■ 2S (USP39) in the *Standard solution* (USP Insulin Units/mL)

D = dilution factor used to prepare the *Sample solution*

For Prompt Insulin Zinc Suspension prepared from a mixture of insulin beef and insulin pork, calculate the total potency as the sum of the potencies of insulin beef and insulin pork, determined separately, as directed above.

Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Units/mL

OTHER COMPONENTS

- **Zinc Determination** (591): 0.12–0.25 mg for every 100 USP Insulin Units
- **Zinc in the Supernatant**

Analysis: Centrifuge a portion of Suspension sufficient for the test and determine the zinc content of the clear supernatant as directed in *Zinc Determination* (591).

Acceptance criteria: Concentration of zinc (mg/mL) is 20%–65% of the zinc concentration of the Suspension.

PRODUCT RELATED SUBSTANCES IMPURITIES**Delete the following:**

- • **Limit of High Molecular Weight Proteins**

Solution A: ± mg/mL of L-arginine

Mobile phase: *Solution A*, acetonitrile, and glacial acetic acid (65:20:15)

Resolution solution: 4 mg/mL of insulin containing NLT 0.4% of high molecular weight proteins in 0.01 N hydrochloric acid. Store in a refrigerator, and use within 7 days. [Note—Insulin containing NLT 0.4% of high molecular weight proteins may be prepared by allowing insulin to stand at room temperature for about 5 days.]

Sample solution: Quantitatively add 4 μL of 6 N hydrochloric acid to each mL of an accurately measured volume of Suspension, and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV-276 nm

Column: 7.8 mm \times 30 cm; packing L20

Flow rate: 0.5 mL/min

Injection volume: 100 μL

System suitability

Sample: *Resolution solution*

Suitability requirements

Retention times: 13–17 min for the polymeric insulin complexes, about 17.5 min for the covalent insulin dimer, and 18–22 min for the insulin monomer, with salts eluting after the insulin monomer

Peak-to-valley ratio: The ratio of the height of the covalent insulin dimer peak to the height of the valley between the covalent insulin dimer peak and the insulin monomer peak is NLT 2.0

Analysis

Sample: *Sample solution*

Disregard any peaks having retention times greater than that of the insulin monomer. Calculate the percentage of high molecular weight proteins in the portion of insulin taken:

$$\text{Result} = 100 \times \Sigma r_{\text{H}} / (\Sigma r_{\text{H}} + r_{\text{M}})$$

Σr_{H} = sum of the responses of all peaks having retention times less than that of the insulin monomer

r_{M} = peak response of the insulin monomer

Acceptance criteria: NMT 1.5% \pm 2S (USP39)

Add the following:

- **Physicochemical Analytical Procedures for Insulins** (121.1), *Limit of High Molecular Weight Proteins*

Proceed as directed in *Limit of High Molecular Weight Proteins*, except prepare the following *Sample solution*. It meets the requirements.

Sample solution: Quantitatively add 4 μL of 6 N *hydrochloric acid* to each mL of an accurately measured volume of Suspension, and mix.

Acceptance criteria: NMT 1.5% \blacksquare _{2S} (USP39)

SPECIFIC TESTS

- **Insulin Not Extracted by Buffered Acetone Solution**

Sample solution: Centrifuge 15 mL (40-Unit), 8 mL (80-Unit), or 6 mL (100-Unit) of Suspension, and discard the supernatant. Suspend the residue in 8.4 mL of water, quickly add 16.6 mL of *buffered acetone TS*, shake or stir vigorously, and centrifuge within 3 min after the addition of the *buffered acetone TS*. Discard the supernatant, repeat the treatment with water and *buffered acetone TS*, centrifuge, and discard the supernatant.

Acceptance criteria: No crystalline residue remains.

- **Insulin in the Supernatant**

Sample solution: Centrifuge 10 mL of the Suspension at $1500 \times g$ for 10 min. Use the supernatant.

Analysis: Determine the insulin content of the *Sample solution* by a suitable method.

Acceptance criteria: NMT 1.0 USP Insulin Unit/mL

- **pH** (791): 7.0–7.8

- **Bacterial Endotoxins Test** (85): NMT 80 USP Endotoxin Units/100 USP Insulin Units

- **Sterility Tests** (71), *Test for Sterility of the Product to Be Examined, Membrane Filtration:* Meets the requirements when tested as directed, and the Suspension being filtered immediately after it has been put into solution using a validated suitable solvent

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in the unopened, multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.
- **Labeling:** Label it to indicate the one or more animal species to which it is related, as porcine, bovine, or a mixture of porcine and bovine. If the Prompt Insulin Zinc Suspension has been made from insulin that is purified, label it as such. The container label states that the Suspension is to be shaken carefully before use. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Units/mL.
- **USP Reference Standards** (11)
 - USP Endotoxin RS
 - USP Insulin Beef RS
 - USP Insulin Pork RS

BRIEFING

Levonorgestrel, USP 38 page 4086. As part of the USP monograph modernization effort, the following changes are proposed:

1. The nonspecific UV-Vis procedure for the Assay is replaced with a specific liquid chromatographic procedure that is based on validated methods of analysis performed

with the Purospher STAR RP-18e brand of L1 column. The typical retention time for levonorgestrel is about 13 min.

2. *Identification* test C, based on a retention time match in the *Assay*, is added to strengthen the monograph.
3. A reference to *Titrimetry* (541) and a formula to calculate the amount of ethynyl group are added in the test for *Limit of Ethynyl Group*.
4. The TLC test for *Organic Impurities* is replaced with a stability-indicating liquid chromatographic procedure. This procedure was validated using the Ascentis Express C18 brand of L1 column. The typical retention time for levonorgestrel is about 12 min.

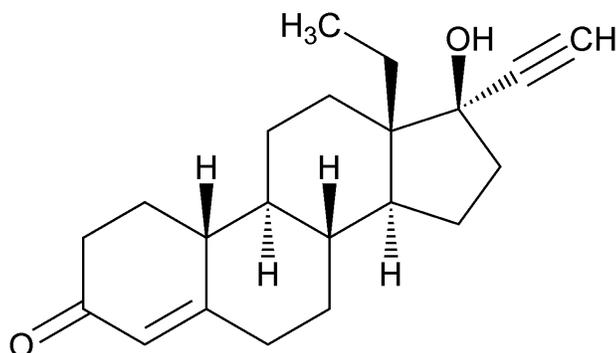
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM5: M. Koleck.)

Correspondence Number—C140857

Comment deadline: November 30, 2015

Levonorgestrel



$C_{21}H_{28}O_2$ 312.45

18,19-Dinorpregn-4-en-20-yn-3-one, 13-ethyl-17-hydroxy-, (17 α)-(–)-;
(–)-13-Ethyl-17-hydroxy-18,19-dinor-17 α -pregn-4-en-20-yn-3-one [797-63-7].

DEFINITION

Levonorgestrel contains NLT 98.0% and NMT 102.0% of levonorgestrel ($C_{21}H_{28}O_2$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** Meeting the requirements of the tests for *Optical Rotation* (781S), *Specific Rotation* and *Melting Range or Temperature* (741) provides identification distinguishing it from norgestrel.

Add the following:

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■2S (USP39)

ASSAY**Change to read:**● **Procedure**~~**Standard solution:** 10 µg/mL of USP Levonorgestrel RS in alcohol~~~~**Sample solution:** 10 µg/mL of Levonorgestrel in alcohol~~~~**Blank:** Alcohol~~~~**Instrumental conditions-**~~~~**Analytical wavelength:** Maximum absorbance at about 241 nm~~~~**Analysis-**~~~~**Samples:** *Standard solution* and *Sample solution*~~~~Calculate the percentage of levonorgestrel ($C_{21}H_{28}O_2$) in the portion of Levonorgestrel taken:~~

~~$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$~~

 ~~A_U = absorbance of the *Sample solution*~~ ~~A_S = absorbance of the *Standard solution*~~ ~~C_S = concentration of USP Levonorgestrel RS in the *Standard solution* (µg/mL)~~ ~~C_U = concentration of Levonorgestrel in the *Sample solution* (µg/mL)~~~~**Acceptance criteria:** 98.0%–102.0% on the dried basis~~~~■ **Diluent:** *Methanol* and water (50:50)~~~~**Mobile phase:** *Acetonitrile* and water (40:60)~~~~**Standard stock solution:** 1 mg/mL of USP Levonorgestrel RS in *methanol*~~~~**Standard solution:** 0.2 mg/mL of USP Levonorgestrel RS from the *Standard stock solution* in *Diluent*~~~~**Sample stock solution:** 1 mg/mL of Levonorgestrel in *methanol*~~~~**Sample solution:** 0.2 mg/mL of Levonorgestrel from the *Sample stock solution* in *Diluent*~~~~**Chromatographic system**~~~~(See *Chromatography* (621), *System Suitability*.)~~~~**Mode:** LC~~~~**Detector:** UV 240 nm~~~~**Column:** 4.6-mm × 15-cm; 5-µm packing *L1*~~~~**Autosampler temperature:** 10°~~~~**Flow rate:** 1.5 mL/min~~~~**Injection volume:** 10 µL~~~~**Run time:** NLT 2 times the retention time of levonorgestrel~~~~**System suitability**~~~~**Sample:** *Standard solution*~~

Suitability requirements**Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 1.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of levonorgestrel ($C_{21}H_{28}O_2$) in the portion of Levonorgestrel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Levonorgestrel RS in the *Standard solution* (mg/mL) C_U = concentration of Levonorgestrel in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the dried basis ■ 2S (USP39)**IMPURITIES**

- **Residue on Ignition** (281): NMT 0.3%

Change to read:

- **Limit of Ethynyl Group**

Sample solution: Dissolve 200 mg of Levonorgestrel in 40 mL of *tetrahydrofuran*.**Blank:** *Tetrahydrofuran***Titrimetric system**■ (See *Titrimetry* (541).)

■ 2S (USP39)

Mode: Direct titration**Titrant:** 0.1 N sodium hydroxide VS**Endpoint detection:** Potentiometric**Analysis****Samples:** *Sample solution* and *Blank*

Add 10 mL of silver nitrate solution (1 in 10) to the *Sample solution* and titrate with *Titrant*, using either a glass–calomel or a silver–silver chloride electrode system with potassium nitrate filling solution. Perform a blank determination and make any necessary correction.

- Calculate the percentage of ethynyl group in the portion of Levonorgestrel taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

 V_S = *Titrant* volume consumed by the *Sample solution* (mL) V_B = *Titrant* volume consumed by the *Blank* (mL)

N = normality of the *Titrant* (mEq/mL)
 F = equivalency factor, 25.03 mg/mEq
 W = *Sample weight* (mg) ■2S (USP39)

Acceptance criteria: 7.81%–8.18% of ethynyl group

Change to read:

• **Organic Impurities**

Standard solution A: 10 mg/mL of USP Levonorgestrel RS in chloroform

Standard solution B: 0.20 mg/mL of USP Levonorgestrel RS from *Standard solution A* in chloroform

Standard solution C: 0.10 mg/mL of USP Levonorgestrel RS from *Standard solution A* in chloroform

Standard solution D: 0.05 mg/mL of USP Levonorgestrel RS from *Standard solution A* in chloroform

Standard solution E: 0.02 mg/mL of USP Levonorgestrel RS from *Standard solution A* in chloroform

Standard solution F: 0.01 mg/mL of USP Levonorgestrel RS from *Standard solution A* in chloroform [Note—The *Standard solutions B–F* correspond to 2.0% (*Standard solution B*), 1.0% (*Standard solution C*), 0.5% (*Standard solution D*), 0.2% (*Standard solution E*), and 0.1% (*Standard solution F*), respectively, of the *Sample solution* concentration.]

Sample solution: 10.0 mg/mL of Levonorgestrel in chloroform

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Spray reagent: 0.1 g/mL of phosphomolybdic acid in alcohol. Stir the mixture for NLT 30 min and filter before use.

Application volume: 10 μ L

Developing solvent system: Chloroform and alcohol (96:4)

Analysis

Samples: *Standard solutions A–F* and *Sample solution*

[Note—Apply at equidistant points along a line 2.5 cm from one edge of a 20 × 20-cm, thin-layer chromatographic plate, previously activated by heating at 100° for 15 min.]

Place the plate in a suitable developing chamber that contains and has been equilibrated with *Developing solvent system*, seal the chamber, and allow the chromatogram to develop until the solvent front has moved 15 cm above the line of application. Remove the plate, allow the solvent to evaporate, then spray uniformly with *Spray reagent*, and heat it at 105° for 10–15 min. The lane of the *Sample solution* exhibits its principal spot at the same R_f as the principal spot of *Standard solution A*. If spots other than the principal spot are observed in the lane of the *Sample solution*, estimate the concentration of each by comparison with the *Standard solutions A–F*.

Acceptance criteria: ~~The sum of the impurities in the Sample solution does not exceed 2.0%, and no single impurity is greater than 0.5%.~~

▪ **Diluent:** Methanol and water (90:10)

Solution A: Acetonitrile and water (35:65)

Solution B: Acetonitrile and water (90:10)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
28	100	0
40	60	40
50	35	65
60	0	100
70	0	100
71	100	0
80	100	0

Sensitivity solution: 0.5 µg/mL of USP Levonorgestrel RS in Diluent

Standard solution: 5 µg/mL of USP Levonorgestrel RS in Diluent

Sample solution: 1 mg/mL of Levonorgestrel in Diluent

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 2.7-µm packing L1

Autosampler temperature: 10°

Flow rate: 1.2 mL/min

Injection volume: 25 µL

System suitability

Samples: Sensitivity solution and Standard solution

Suitability requirements

Tailing factor: NMT 1.5, Standard solution

Relative standard deviation: NMT 5.0%, Standard solution

Signal-to-noise ratio: NLT 10, Sensitivity solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of any individual unspecified impurity in the portion of Levonorgestrel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of levonorgestrel from the *Standard solution*

C_S = concentration of USP Levonorgestrel RS in the *Standard solution* (mg/mL)

C_U = concentration of Levonorgestrel in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks that are less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levonorgestrel	1.0	—	—
Levonorgestrel-5(10)-ene isomer ^a	2.05	0.18	0.15
3-Methoxy levonorgestrel 3,5-diene ^b	3.61	1.4	0.10
3-Methoxy levonorgestrel 2,5(10)-diene ^c	3.63	0.38	0.10
Deoxolevonorgestrel ^d	4.25	0.45	0.15
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.2

^a 13-Ethyl-17-hydroxy-18,19-dinor-17 α -pregn-5(10)-en-20-yn-3-one.
^b 13-Ethyl-3-methoxy-18,19-dinor-17 α -pregn-3,5-dien-20-yn-17-ol.
^c 13-Ethyl-3-methoxy-18,19-dinor-17 α -pregn-2,5(10)-dien-20-yn-17-ol.
^d 13-Ethyl-18,19-dinor-17 α -pregn-4-en-20-yn-17-ol.

■ 2S (USP39)

SPECIFIC TESTS

- **Melting Range or Temperature** (741): 232°–239°, but the range between beginning and end of melting does not exceed 4°
- **Optical Rotation** (781S), *Specific Rotation*
Sample solution: 20 mg/mL of Levonorgestrel in *chloroform*
Acceptance criteria: –30° to –35°
- **Loss on Drying** (731)
Analysis: Dry at 105° for 5 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
- **USP Reference Standards** (11)
 USP Levonorgestrel RS

Mannitol, page 7428 of the *First Supplement to USP 38*. On the basis of comments received, it is proposed to make the following changes to this **STAGE 6** harmonization document. See also *Notices of Stage 6 Harmonized Text, Mannitol*, posted on the USP website, with an official date of Aug. 1, 2015.

1. *Reducing Sugars*. Add the preparation procedure for *Ferric sulfate solution*.
2. *Melting Range or Temperature*. Clarify the requirement by adding "Melting point".

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

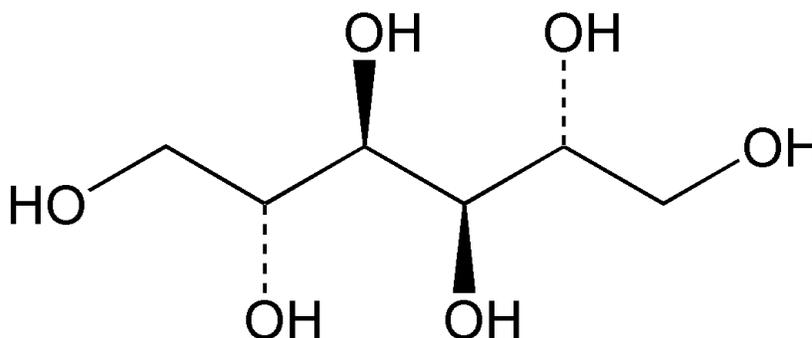
(EXC2: J. Liu.)

Correspondence Number—C160183

Comment deadline: November 30, 2015

Mannitol

Portions of the monograph text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (♦) to specify this fact.



$C_6H_{14}O_6$ 182.17

d-Mannitol [69-65-8].

DEFINITION

Change to read:

Mannitol contains NLT 97.0% and NMT 102.0% of mannitol ($C_6H_{14}O_6$), calculated on the **dried** basis.

IDENTIFICATION

• **A. Infrared Absorption** (197K)

If the spectra shows differences, proceed as directed.

Standard solution: Dissolve 25 mg of USP Mannitol RS in a glass vial with 0.25 mL of distilled water without heating. The solution is clear. Evaporate to dryness by one of the following methods. Heat in a microwave oven with a power range of 600–700 W for 20 min, or heat in an oven at 100° for 1 h, then gradually apply vacuum until a dry residue is

obtained. Non-sticky, white, or slightly yellowish powders are obtained.

Sample solution: Dissolve 25 mg of Mannitol in a glass vial with 0.25 mL of distilled water without heating. The solution is clear. Evaporate to dryness by one of the following methods. Heat in a microwave oven with a power range of 600–700 W for 20 min, or heat in an oven at 100° for 1 h, then gradually apply vacuum until a dry residue is obtained. Non-sticky, white, or slightly yellowish powders are obtained.

Analysis: Record new spectra using the residues from the *Standard solution* and the *Sample solution*.

ASSAY

Change to read:

• Procedure

Mobile phase: Degassed water

System suitability solution A: 25.0 mg/mL each of sorbitol and USP Mannitol RS

System suitability solution B: 1.0 mg/mL each of maltitol and isomalt

Standard solution A: 50.0 mg/mL of USP Mannitol RS

Standard solution B: Dilute 2.0 mL of the *Sample solution* with water to 100.0 mL.

Standard solution C: Dilute 0.5 mL of *Standard solution B* with water to 20.0 mL.

Sample solution: 50.0 mg/mL of Mannitol

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 7.8-mm × 30-cm; packing L19

Temperatures

Detector: 40° (maintain at a constant temperature)

Column: 85 ± 2°

Flow rate: 0.5 mL/min

Injection volume: 20 µL

Run time: NLT 1.5 times the retention time of the mannitol peak. [Note—The retention time for mannitol is about 20 min.]

System suitability

Samples: *System suitability solution A*, *System suitability solution B*, *Standard solution B*, and *Standard solution C*

Suitability requirements

Resolution: NLT 2.0 between sorbitol and mannitol, *System suitability solution*

■ *A*■_{2S} (USP39)

Analysis

Samples: *Standard solution A* and *Sample solution*

Calculate the percentage of mannitol ($C_6H_{14}O_6$) in the portion of Mannitol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from *Standard solution A*

C_S = concentration of USP Mannitol RS in *Standard solution A* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the ■ dried ■_{1S} (USP38) basis

IMPURITIES

Change to read:

- **Related Substances**

Mobile phase, System suitability solution A, System suitability solution B, Standard solution B, Standard solution C, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution B, Standard solution C, and Sample solution*

Acceptance criteria: See *Table 1* for the relative retention times.

Table 1

Name	Relative Retention Time
Isomalt (1st peak)	0.60
Maltitol	0.69
Isomalt (2nd peak)	0.73
Mannitol	1.0
Sorbitol	1.2

■ [Note—Impurity A—Sorbitol; Impurity B—Maltitol; Impurity C—Isomalt.]

■_{1S} (USP38)

[Note—Isomalt elutes in two peaks.]

[Note—Coelution of impurity B and the second peak of impurity C may be observed.]

Disregard limit: NMT 0.05%; any peak NMT the area of the principal peak obtained with *Standard solution C*

Sorbitol: NMT 2.0%; NMT the area of the principal peak obtained with *Standard solution B*

Sum of isomalt and maltitol: NMT 2.0%; NMT the area of the principal peak obtained with *Standard solution B*

Unspecified impurities: NMT 0.10% for each impurity; NMT twice the area of the principal peak obtained with *Standard solution C*

Total impurities: 2.0%; NMT the area of the principal peak obtained with *Standard solution B*

Change to read:● **Reducing Sugars**

■ **Ferric sulfate solution:** Dissolve 50 g of *ferric sulfate* in an excess of water, add 200 mL of *sulfuric acid*, and dilute with water to 1000 mL. ■_{2S} (USP39)

Copper sulfate solution: 69.2 mg/mL of *copper sulfate* ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) ■ in water ■_{1S} (USP38)

Sodium potassium tartrate solution: Dissolve 173 g of *sodium potassium tartrate* ($\text{C}_4\text{H}_4\text{KNaO}_6 \cdot 4\text{H}_2\text{O}$) and 50 g of *sodium hydroxide* in 400 mL of water. Heat to boiling, allow to cool, and dilute with carbon dioxide-free water to 500 mL. ■ ■_{1S} (USP38)

Cupri-tartaric solution: Mix equal volumes of *Copper sulfate solution* ■ and *Sodium potassium tartrate solution* ■_{1S} (USP38) immediately before use.

Sample: 7.0 g

Analysis: To the *Sample* add 13 mL of water. Boil gently with 40 mL of *Cupri-tartaric solution* for 3 min, and allow to stand for about 2 min. A precipitate is formed. Pass through a sintered-glass filter (10–16 μm) coated with diatomaceous earth or a sintered-glass filter (5–10 μm). Wash the precipitate with hot water (at about 50°–60°) until the washing is no longer alkaline, and pass the washings through the filter described above. Discard all the filtrate at this step. Immediately dissolve the precipitate in 20 mL of *ferric sulfate solution*

■ *Ferric sulfate solution*, ■_{2S} (USP39)

pass through the filter described above in a clean flask, and wash the filter with 15–20 mL of water. Combine the washings and the filtrate, heat to 80°, and titrate with 0.02 M *potassium permanganate VS*.

Acceptance criteria: NMT 0.1%, expressed as glucose; NMT 3.2 mL of 0.02 M *potassium permanganate VS* is required to change the color of the solution. The green color turns to pink, and the color persists at least 10 s.

Change to read:● **Nickel**

Sample solution: Suspend 10.0 g of Mannitol in 30 mL of dilute acetic acid [115–125 g/L of acetic acid ($\text{C}_2\text{H}_4\text{O}_2$)], add water, and shake to dissolve. Dilute with water to 100.0 mL. Add 2.0 mL of a *saturated solution of ammonium pyrrolidinedithiocarbamate* ($\text{C}_5\text{H}_{12}\text{N}_2\text{S}_2$) (about 10 g/L) and 10.0 mL of water-saturated *methyl isobutyl ketone* ($\text{C}_6\text{H}_{12}\text{O}$, 4-methyl-2-pentanone), and then shake for 30 s protected from bright light. Allow the layers to separate, and use the methyl isobutyl ketone layer.

Blank solution: Treat water-saturated *methyl isobutyl ketone* as described for preparation of the *Sample solution*, omitting the mannitol.

Standard solutions: Prepare three reference solutions in the same manner as the *Sample solution* but adding 0.5, 1.0, and 1.5 mL, respectively, of *nickel standard solution TS* [10 ppm nickel (Ni)] in addition to the 10.0 g of the substance to be examined.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 232.0 nm

Lamp: Nickel hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Sample solution* and *Standard solutions*

Set the zero of the instrument using the blank. Record the average of the steady readings for each of the *Standard solutions* and the *Sample solution*. Between each measurement rinse with water, and ascertain that the reading returns to zero with the blank. \blacksquare 1S (USP38) Plot the absorbances of the *Standard solutions* and the *Sample solution* versus the added quantity of nickel. Extrapolate the line joining the points on the graph until it meets the concentration axis. The distance between this point and the intersection of the axes represents the concentration of nickel in the *Sample solution*.

Acceptance criteria: NMT 1 $\mu\text{g/g}$

SPECIFIC TESTS

Change to read:

- **Melting Range or Temperature** (741), *Class I*

\blacksquare **Melting point:** 165°–170° \blacksquare 2S (USP39)

- **Appearance of Solution**

Hydrazine sulfate solution: 10.0 mg/mL of *hydrazine sulfate*. Allow to stand for 4–6 h.

Methenamine solution: 2.5 g of *methenamine* in 25 mL of water, in a ground-glass-stoppered flask

Primary opalescent suspension: To the *Methenamine solution*, add 25.0 mL of the *Hydrazine sulfate solution*. Mix, and allow to stand for 24 h. This suspension is stable for two months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Opalescence standard: Dilute 15.0 mL of the *Primary opalescent suspension* with water to 1000.0 mL. This suspension is freshly prepared and may be stored for up to 24 h.

Reference suspension: To 5.0 mL of *Opalescence standard* add 95.0 mL of water. Mix, and shake before use.

Standard solution: Pipet 3.0 mL of *ferric chloride CS*, 3.0 mL of *cobaltous chloride CS*, and 2.4 mL of *cupric sulfate CS* into a 1-L volumetric flask. Dilute with 1% (w/v) *hydrochloric acid* to volume.

Sample solution: 100.0 mg/mL of Mannitol

Analysis: Compare the color, clarity, and opalescence of equal volumes of the *Reference suspension*, *Standard solution*, and *Sample solution*.

Acceptance criteria: The *Sample solution* is clear and colorless; its clarity is the same as that of water, or its opalescence is not more pronounced than that of the *Reference suspension*, and it is not more intensely colored than the *Standard solution*.

- **Loss on Drying** (731)

Sample: 1.000 g

Analysis: Dry the *Sample* at 105° for 4 h.

Acceptance criteria: NMT 0.5%

- **Conductivity**

Sample: 20.0 g

Analysis: Dissolve the *Sample* in *carbon dioxide-free water* prepared from distilled water by heating to 40°–50°, and dilute with the same solvent to 100 mL. After cooling, measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Acceptance criteria: NMT 20 µS/cm at 25°

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): The total aerobic microbial count (TAMC) is NMT 10³ cfu/g, and the total combined molds and yeasts count is NMT 10² cfu/g. It meets the requirements of the test for absence of *Escherichia coli*.

If intended for use in the manufacture of parenteral dosage forms, the TAMC is NMT 10² cfu/g.

- **Bacterial Endotoxins Test** (85): If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins, less than 4 IU/g for parenteral dosage forms with a concentration of 100 g/L or less of mannitol, and less than 2.5 IU/g for parenteral dosage forms with a concentration of more than 100 g/L of mannitol

ADDITIONAL REQUIREMENTS

- **↑Packaging and Storage:** Preserve in well-closed containers.↓
- **Labeling**
The label states, where applicable, the maximum concentration of bacterial endotoxins.
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

Change to read:

- **USP Reference Standards** (11)

- USP Endotoxin RS ■ 1S (USP38)

USP Mannitol RS

BRIEFING

Methylergonovine Maleate, *USP 38* page 4343. On the basis of comments received, it is proposed to revise the monograph as follows:

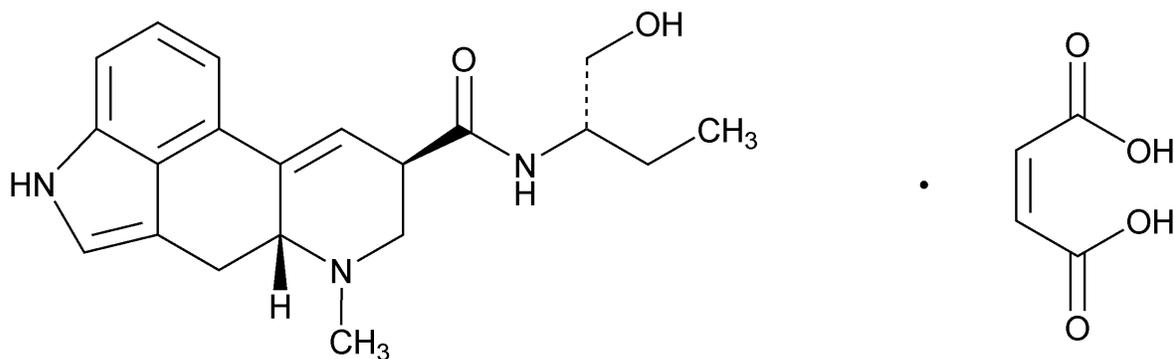
1. Define an emission wavelength for the analysis in the *Assay* and remove the reference to old detector technology.
2. Delete the *Column efficiency* requirement in the *Assay* because the remaining requirements are sufficient to evaluate the system suitability.
3. Update the CAS number to be consistent with the current CAS number for Methylergonovine Maleate.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM5: M. Koleck.)

Correspondence Number—C148447

Comment deadline: November 30, 2015

Methylergonovine Maleate**Change to read:**
 $C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$ 455.50

Ergoline-8-carboxamide, 9,10-didehydro-*N*-[1-(hydroxymethyl)propyl]-6-methyl-, [8 β (*S*)]-, (*Z*)-2-butenedioate (1:1) (salt);

9,10-Didehydro-*N*-[(*S*)-1-(hydroxymethyl)propyl]-6-methylergoline-[8 β -carboxamide maleate (1:1) (salt) [~~7054-07-1~~

■ 57432-61-8 ■ *2S* (USP39)

].

DEFINITION

Methylergonovine Maleate contains NLT 97.0% and NMT 103.0% of methylergonovine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The R_F values of the principal fluorescent spot and the principal blue spot of the *Sample solution* correspond to those of the *Standard stock solution*, as obtained in the test for *Related Alkaloids*.

ASSAY**Change to read:**• **Procedure**

Conduct this procedure with a minimum exposure to light.

Mobile phase: Acetonitrile and 2.0 g/L of *monobasic potassium phosphate* (1:4)

Diluent: 2.5 mg/mL of *tartaric acid* prepared as follows. Transfer a suitable amount of *tartaric acid* to a suitable volumetric flask, add 50% of the flask volume of water, and mix with shaking. Dilute with *methanol* to volume. Allow the mixture to cool before use.

Standard stock solution: 0.1 mg/mL of USP Methylergonovine Maleate RS in *Diluent*. Shake by mechanical means for 15 min.

Standard solution: 4 µg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Sample stock solution: 0.2 mg/mL of Methylergonovine Maleate in *Diluent*. Shake by mechanical means for 15 min or until completely dissolved.

Sample solution: 4 µg/mL of Methylergonovine Maleate from the *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Fluorescence with excitation at 315 nm; ~~the emission wavelength is set to zero, using a cutoff filter that passes light from 418 to 700 nm.~~

■ and emission at 423 nm ■ 2S (USP39)

Column: 4.6-mm × 25-cm; packing L7

Temperature: 30°

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

~~**Column efficiency:** NLT 1000 theoretical plates~~

■ ■ 2S (USP39)

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylergonovine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$) in the portion of Methylergonovine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methylergonovine Maleate RS in the *Standard solution* (µg/mL)

C_U = concentration of Methylergonovine Maleate in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

- **Related Alkaloids**

Conduct this test promptly, without exposure to daylight and with minimum exposure to artificial light. Solutions containing methylergonovine maleate should be prepared immediately before use.

Diluent: *Alcohol and ammonium hydroxide (9:1)*

Standard stock solution: 10 mg/mL of USP Methylergonovine Maleate RS in *Diluent*

Standard solution A: 0.20 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Standard solution B: 0.10 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Standard solution C: 0.05 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Sample solution: 10 mg/mL of Methylergonovine Maleate in *Diluent*

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

Developing solvent system: *Chloroform, methanol, and water (75:25:3)*, equilibrated for 30 min

Spray reagent: 10 mg/mL of *p*-dimethylaminobenzaldehyde in a cooled mixture of *alcohol* and *hydrochloric acid* (1:1)

Analysis

Samples: *Standard stock solution, Standard solution A, Standard solution B, Standard solution C, and Sample solution*

Proceed as directed in the chapter. Locate the spots on the plate by spraying thoroughly and evenly with *Spray reagent*. Immediately dry in a stream of nitrogen for 2 min.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard stock solution*. Estimate the concentration of any other spots observed from the *Sample solution* by comparison with *Standard solution A, Standard solution B, and Standard solution C*. The spots from *Standard solution A, Standard solution B, and Standard solution C* are equivalent to 2.0%, 1.0%, and 0.50% of impurities, respectively. The sum of the impurities is NMT 2.0%.

SPECIFIC TESTS

- **Optical Rotation** (781S), *Specific Rotation*

Sample solution: 5 mg/mL of Methylergonovine Maleate in water

Acceptance criteria: +44° to +50°

- **pH** (791)

Sample solution: 0.2 mg/mL of Methylergonovine Maleate in water

Acceptance criteria: 4.4–5.2

- **Loss on Drying** (731)

Analysis: Dry under vacuum at 80° to constant weight.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store in a cold place.

- **USP Reference Standards** (11)

USP Methylergonovine Maleate RS

BRIEFING

Methylnaltrexone Bromide. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods of analysis, is proposed. The liquid chromatographic procedure in the *Assay* and the test for *Organic Impurities* is based on analyses performed with the Acquity UPLC HSS T3 brand of L1 column. The typical retention time for methylnaltrexone is about 3.5 min.

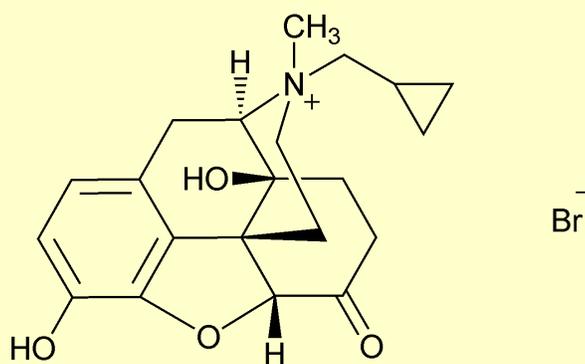
(CHM3: A. Wise, E. Gonikberg.)

Correspondence Number—C125865

Comment deadline: November 30, 2015

Add the following:

- **Methylnaltrexone Bromide**



C₂₁H₂₆BrNO₄ 436.34

Morphinanium, 17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxy-17-methyl-6-oxo-, bromide, (5a)-;

(17*RS*)-17-(Cyclopropylmethyl)-4,5*a*-epoxy-3,14-dihydroxy-17-methyl-6-oxomorphinanium bromide [73232-52-7].

DEFINITION

Methylnaltrexone Bromide contains NLT 98.0% and NMT 102.0% of methylnaltrexone bromide ($C_{21}H_{26}BrNO_4$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General** (191), *Bromide*: Meets the requirements

ASSAY

• Procedure

Protect solutions containing methylnaltrexone from light.

Solution A: 0.1% (v/v) of *trifluoroacetic acid* in water

Solution B: 0.1% (v/v) of *trifluoroacetic acid* in *methanol*

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
1	90	10
7	75	25
13.9	30	70
14	90	10

[Note—The gradient was established on an HPLC system with a dwell volume of approximately 120 μ L. For optimal results, condition the column with a 90:10 mixture of *Solution A* and *Solution B* for at least 30 min before the first injection.]

Diluent: *Solution A* and *Solution B* (90:10)

Standard solution: 1.4 mg/mL of USP Methylnaltrexone Bromide RS in *Diluent*

Sample solution: 1.4 mg/mL of Methylnaltrexone Bromide in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 2.1-mm \times 5-cm; 1.8- μ m packing *L1*

Temperatures

Column: 50°

Autosampler: 5°

Flow rate: 0.3 mL/min

Injection volume: 5 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylnaltrexone bromide ($C_{21}H_{26}BrNO_4$) in the portion of Methylnaltrexone Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methylnaltrexone Bromide RS in the *Standard solution* (mg/mL)

C_U = concentration of Methylnaltrexone Bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

- **Organic Impurities**

Protect solutions containing methylnaltrexone from light.

Solution A, Solution B, Mobile phase, Diluent, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 1.4 $\mu\text{g/mL}$ of USP Methylnaltrexone Bromide RS in *Diluent*

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5%

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Methylnaltrexone Bromide taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of methylnaltrexone from the *Sample solution*

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
S-Methylnaltrexone ^a	0.92	1.0	0.15
Methylnaltrexone	1.0	—	—
N-Butenyl oxymorphone ^b	1.26	1.0	0.15
Naltrexone ^c	1.31	1.0	0.10
2,2'-Bismethylnaltrexone ^d	1.63	1.6	0.15
O-Methyl methylnaltrexone ^e	1.82	1.0	0.15
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

^a (17S)-17-(Cyclopropylmethyl)-4,5a-epoxy-3,14-dihydroxy-17-methyl-6-oxomorphinanum bromide.

^b (17RS)-17-(But-3-en-1-yl)-4,5a-epoxy-3,14-dihydroxy-17-methyl-6-oxomorphinanum bromide.

^c 17-(Cyclopropylmethyl)-4,5a-epoxy-3,14-dihydroxymorphinan-6-one.

^d 2,2'-Bi[(17RS)-17-(Cyclopropylmethyl)-4,5a-epoxy-3,14-dihydroxy-17-methyl-6-oxomorphinanum] dibromide.

^e (17RS)-17-(Cyclopropylmethyl)-4,5a-epoxy-14-hydroxy-17-methyl-3-methoxy-6-oxomorphinanum bromide.

SPECIFIC TESTS

- **pH** (791)

Sample solution: 75 mg/mL in water; heat gently, if necessary, to dissolve. Allow the solution to cool to $25 \pm 2^\circ$ before measuring the pH.

Acceptance criteria: 4.3–5.3

- **Water Determination** (921), *Method Ic*: NMT 1.0%

- **Optical Rotation** (781S), *Specific Rotation*

Sample solution: 10 mg/mL in water

Acceptance criteria: -160° to -166° , measured at 20°

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers; store below 40° .

- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

- **USP Reference Standards** (11)

USP Methylnaltrexone Bromide RS

■ 2S (USP39)

BRIEFING

Metronidazole Extended-Release Tablets. Because there is no existing *USP* monograph for this dosage form, a new monograph, based on validated methods of analysis, is proposed. The liquid chromatographic procedures in the *Assay* and the test for *Organic Impurities* are based on analyses performed with the Inertsil ODS 3V brand of *L1* column. In the *Assay*, the typical retention time for metronidazole is about 6 min. In the test for *Organic Impurities*, the typical retention times for tinidazole related compound A and metronidazole are about 11 and 14 min, respectively.

(CHM1: S. Ramachandran, S. Shivaprasad.)
Correspondence Number—C151124

Comment deadline: November 30, 2015

Add the following:

■ **Metronidazole Extended-Release Tablets**

DEFINITION

Metronidazole Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$).

IDENTIFICATION

● **A. Ultraviolet Absorption** (197U)

Diluent: *Methanol* and *sulfuric acid* (350:1)

Standard stock solution: 15 mg/mL of USP Metronidazole RS in *dilute hydrochloric acid* (1 in 100). Sonicate to dissolve and pass through a suitable filter.

Standard solution: 18.8 μ g/mL of USP Metronidazole RS in *Diluent* from *Standard stock solution*

Sample stock solution: Nominally 15 mg/mL of metronidazole prepared as follows. Finely powder NLT 5 Tablets and transfer an amount equivalent to 300 mg of metronidazole into a 20-mL volumetric flask. Add about 15 mL of *dilute hydrochloric acid* (1 in 100) and shake mechanically for 30 min. Dilute with *dilute hydrochloric acid* (1 in 100) to volume and shake well. Pass through a suitable filter.

Sample solution: Nominally equivalent to 18.8 μ g/mL of metronidazole in *Diluent* from *Sample stock solution*.

Wavelength range: 200–400 nm

Acceptance criteria: Meet the requirement

● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

● **Procedure**

Buffer: 1.4 g/L of *monobasic potassium phosphate* in water

Mobile phase: *Methanol* and *Buffer* (30:70)

Standard solution: 0.1 mg/mL of USP Metronidazole RS in *Mobile phase*

Sample stock solution: Nominally 2.0 mg/mL of metronidazole from NLT 20 finely powdered Tablets in *Mobile phase*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 60% of the flask volume with *Mobile phase*, and shake by mechanical means for 30 min, dilute with *Mobile phase* to volume. Allow the solution to stand until the insoluble material settles.

Sample solution: Nominally 0.1 mg/mL of metronidazole in *Mobile phase* from the *Sample stock solution* supernatant. Pass the solution through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 315 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing *L1*

Temperatures

Column: 30°

Autosampler: 15°

Flow rate: 1 mL/min

Injection volume: 10 μ L

Run time: 15 min

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metronidazole from the *Sample solution*

r_S = peak response of metronidazole from the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Times: 2, 6, 10, and 16 h

Standard solution: 16.65 µg/mL of USP Metronidazole RS in *Medium*

Sample solution: At the times specified, withdraw 10 mL of the solution under test and pass through a suitable filter of 0.45-µm pore size. Replace the aliquots withdrawn for analysis with equal volumes of fresh portions of *Medium* maintained at 37°. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.

Blank: *Medium*

Instrumental conditions

Mode: UV

Analytical wavelength: 320 nm

Cell: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of metronidazole ($C_6H_9N_3O_3$) in the sample withdrawn from the vessel at each time point (i).

$$\text{Result} = (A_U/A_S) \times C_S \times D$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)

D = dilution factor, if needed

Calculate the percentage of the labeled amount (Q_i) of metronidazole ($C_6H_9N_3O_3$) dissolved at each time point (i).

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of metronidazole in the portion of sample withdrawn at the specified time point (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (i)	Time (h)	Amount Dissolved (%)
1	2	20–35
2	6	45–60
3	10	60–75
4	16	NLT 75

The percentages (Q) of the labeled amount of metronidazole ($C_6H_9N_3O_3$) released at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

- **Uniformity of Dosage Units** (905): Meet the requirements

IMPURITIES

- **Organic Impurities**

Buffer: Dissolve 1.5 g of *monobasic potassium phosphate* in 900 mL of water, adjust with *phosphoric acid* to a pH of 3.2, and dilute with water to 1000 mL.

Diluent: *Acetonitrile* and *Buffer* (45:55)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Buffer (%)	Acetonitrile (%)
0	95	5
5	95	5
25	50	50
30	95	5
35	95	5

System suitability solution: 0.5 mg/mL of USP Metronidazole RS and 2.5 μ g/mL of USP Tinidazole Related Compound A RS in *Diluent*. Sonicate, if necessary, to dissolve.

Standard solution: 0.75 μ g/mL of USP Metronidazole RS in *Diluent*

Sample solution: Nominally 0.5 mg/mL of metronidazole from NLT 20 finely powdered Tablets in *Diluent*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add *Diluent* equivalent to 70% of the flask volume, sonicate for 15 min with intermittent shaking, and dilute with *Diluent* to volume. Allow the solution to stand until the insoluble material settles, and pass the supernatant through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 315 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing *L1*

Autosampler temperature: 20°

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between tinidazole related compound A and metronidazole, *System suitability solution*

Relative standard deviation: NMT 5.0% for metronidazole, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution*

r_S = peak response of metronidazole from the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Disregard any impurity peaks less than 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.79	0.15
Metronidazole	1.0	—
Any individual unspecified degradation product	—	0.10
Total degradation products	—	0.50

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP Reference Standards** <11>
 - USP Metronidazole RS
 - USP Tinidazole Related Compound A RS
 - 2-Methyl-5-nitroimidazole.
 - $C_4H_5N_3O_2$ 127.10

■ 2S (USP39)

BRIEFING

Miconazole, *USP 38* page 4393. As part of the USP monograph modernization effort, it is proposed to make the following changes:

1. Revise the test method in *Identification* test A to allow the flexibility for the test.

2. Replace the UV-based *Identification* test *B* with a retention time agreement in the proposed *Assay*.
3. Replace the titration procedure for *Assay* with a stability-indicating liquid chromatographic procedure. The HPLC procedure is based on analyses performed with the Phenomenex Kinetex Phenyl-Hexyl brand of *L11* column. The typical retention time for the miconazole peak is about 24 min.
4. Replace the TLC procedure in the test for *Organic Impurities* with an HPLC procedure similar to that proposed in the *Assay*.
5. Add the new Reference Standards used in the *Assay* and *Organic Impurities* tests to the *USP Reference Standards* section.

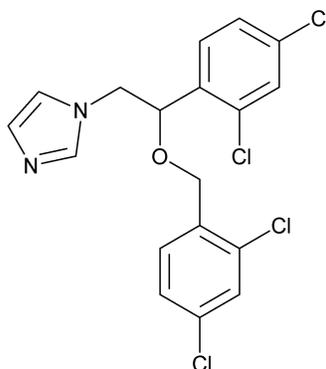
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM6: R.-H. Yeh.)

Correspondence Number—C132599

Comment deadline: November 30, 2015

Miconazole



$C_{18}H_{14}Cl_4N_2O$ 416.13

1*H*-Imidazole, 1-2-[(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]-, (±)-; (±)-1-[2,4-Dichloro-β-[(2,4-dichlorobenzyl)oxy]phenethyl]imidazole [22916-47-8].

DEFINITION

Miconazole contains NLT 98.0% and NMT 102.0% of miconazole ($C_{18}H_{14}Cl_4N_2O$), calculated on the dried basis.

IDENTIFICATION

Delete the following:

- • ~~A. Infrared Absorption~~ (197K) ■ 2S (USP39)

Add the following:

- • A. Infrared Absorption (197)

[Note—Methods described in *Infrared Absorption* (197K) or (197A) may be used.]

■ 2S (USP39)

Delete the following:

■ ● **B.**

Sample solution: Dissolve 40 mg in 50 mL of *isopropyl alcohol* in a 100-mL volumetric flask, and add 10 mL of 0.1 N hydrochloric acid. Dilute with *isopropyl alcohol* to volume.

Acceptance criteria: The UV-absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Miconazole RS, concomitantly measured. ■ 2S (USP39)

Add the following:

- ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (USP39)

ASSAY

Change to read:

● **Procedure**

Sample solution: 300 mg of Miconazole in 40 mL of *glacial acetic acid*. Add 4 drops of *p-naphtholbenzein TS*.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with *Titrant* to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 41.61 mg of miconazole ($C_{18}H_{14}Cl_4N_2O$).

■ **Solution A:** *Methanol*, water, and 1 M triethylammonium acetate (300:700:10)

Solution B: *Acetonitrile*, *methanol*, and 1 M triethylammonium acetate (250:750:2)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
5	70	30
10	44	56
27	44	56
30	25	75
35	25	75
36	70	30
40	70	30

Diluent: *Methanol* and water (70:30)

System suitability solution: 0.1 mg/mL of USP Miconazole RS and 6 µg/mL of USP Miconazole Related Compound F RS in *Diluent*. Sonication may be used to aid in dissolution.

Standard stock solution: 1.0 mg/mL of USP Miconazole RS prepared as follows. Weigh an appropriate amount of USP Miconazole RS into a suitable volumetric flask, and dissolve in 25% of the final flask volume of *methanol*, then dilute with *Diluent* to volume.

Standard solution: 0.1 mg/mL of USP Miconazole RS in *Diluent* from *Standard stock solution*

Sample stock solution: 1.0 mg/mL of Miconazole prepared as follows. Weigh an appropriate amount of Miconazole into a suitable volumetric flask, and dissolve in 25% of the final flask volume of *methanol*, then dilute with *Diluent* to volume.

Sample solution: 0.1 mg/mL of Miconazole in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 10-cm; 2.6-µm packing *L11*

Column temperature: 40°

Flow rate: 0.8 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for miconazole related compound F and miconazole are 0.96 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between miconazole related compound F and miconazole, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of miconazole (C₁₈H₁₄Cl₄N₂O) in the portion of Miconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of miconazole from the *Sample solution*

r_S = peak response of miconazole from the *Standard solution*

C_S = concentration of USP Miconazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Miconazole in the *Sample solution* (mg/mL)

■ 2S (USP39)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.2%

Change to read:

- **Organic Impurities**

~~**Standard solution A:** 10 mg/mL of USP Miconazole RS in chloroform~~

~~**Standard solution B:** 100 µg/mL of USP Miconazole RS from *Standard solution A* in chloroform~~

~~**Sample solution:** 10 mg/mL in chloroform~~

~~**Chromatographic system**~~

~~(See *Chromatography* (621), *Thin Layer Chromatography*.)~~

~~**Mode:** TLC~~

~~**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture~~

~~**Application volume:** 5 µL~~

~~**Developing solvent system:** *n*-Hexane, chloroform, methanol, and ammonium hydroxide (60:30:10:1)~~

~~**Analysis**~~

~~**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*~~

~~Apply the *Samples* separately to the starting line of the plate. Develop the chromatogram in a suitable chamber with freshly prepared *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow the solvent to evaporate. Expose the plate to iodine vapors in a closed chamber for 30 min, and locate the spots.~~

~~**Acceptance criteria:** The R_f value of the principal spot from the *Sample solution* corresponds to that from *Standard solution A*, and any other spot from the *Sample solution* does not exceed, in size or intensity, the principal spot from *Standard solution B* (1.0%).~~

- **Solution A, Solution B, Mobile phase, Diluent, Sample stock solution, and Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 0.5 mg/mL each of USP Miconazole RS, USP Econazole Nitrate RS, USP Miconazole Related Compound C RS, USP Miconazole Related Compound F RS, and USP Miconazole Related Compound I RS prepared as follows. Weigh an appropriate amount of each of the Reference Standards into a suitable volumetric flask, and dissolve in 25% of the final flask volume of *methanol*, then dilute with *Diluent* to volume.

Standard solution: 1.0 µg/mL each of USP Miconazole RS, USP Econazole Nitrate RS, USP Miconazole Related Compound C RS, USP Miconazole Related Compound F RS, and USP Miconazole Related Compound I RS in *Diluent* from *Standard stock solution*

Sample solution: 0.5 mg/mL of Miconazole in *Diluent* from *Sample stock solution*

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between miconazole related compound C and miconazole related compound I; NLT 1.5 between miconazole related compound I and econazole; NLT 1.5 between miconazole related compound F and miconazole

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of miconazole related compound C, miconazole related compound F, or miconazole related compound I in the portion of Miconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of miconazole related compound C, miconazole related compound F, or miconazole related compound I from the *Sample solution*

r_S = peak response of miconazole related compound C, miconazole related compound F, or miconazole related compound I from the *Standard solution*

C_S = concentration of USP Miconazole Related Compound C RS, USP Miconazole Related Compound F RS, or USP Miconazole Related Compound I RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of Miconazole in the *Sample solution* ($\mu\text{g/mL}$)

Calculate the percentage of econazole in the portion of Miconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of econazole from the *Sample solution*

r_S = peak response of econazole from the *Standard solution*

C_S = concentration of USP Econazole Nitrate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of Miconazole in the *Sample solution* ($\mu\text{g/mL}$)

M_{r1} = molecular weight of econazole free base, 381.68

M_{r2} = molecular weight of econazole nitrate, 444.70

Calculate the percentage of any other individual specified or unspecified impurity in the portion of Miconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual specified or unspecified impurity from the *Sample solution*

r_S = peak response of miconazole from the *Standard solution*

C_S = concentration of USP Miconazole RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of Miconazole in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 2*. Disregard limit: 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deschlorobenzyl econazole ^a	0.22	0.25
Miconazole quaternary salt ^b	0.57	0.25
Miconazole benzyl analog ^c	0.65	0.25
Miconazole related compound C	0.74	0.25
Miconazole related compound I	0.76	0.25
Econazole	0.78	0.25
Miconazole 2,6-isomer ^d	0.87	0.25
Miconazole 2,5-isomer ^e	0.94	0.25
Miconazole related compound F	0.96	0.25
Miconazole	1.0	—
Any individual unspecified impurity	—	0.25
Total impurities	—	0.5

^a 1-(2,4-Dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol.
^b 2-(3-{2-[(2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazol-3-ium-1-yl)-2-methylpropanoate.
^c 1-[2-(Benzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.
^d 1-{2-[(2,6-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.
^e 1-{2-[(2,5-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.

■ 2S (USP39)

SPECIFIC TESTS

- **Loss on Drying** (731)

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light. Store at 25°, excursions permitted between 15° and 30°.

Change to read:

- **USP Reference Standards** (11)

- USP Econazole Nitrate RS ■ 2S (USP39)

USP Miconazole RS

- USP Miconazole Related Compound C RS

2-[(2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethan-1-amine hydrochloride.

C₁₅H₁₃Cl₄NO·HCl 401.53

USP Miconazole Related Compound F RS

1-{2-[(3,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.

C₁₈H₁₄Cl₄N₂O 416.13

USP Miconazole Related Compound I RS 1-{2-[(2-Chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole mononitrate.C₁₈H₁₅Cl₃N₂O·HNO₃ 444.69

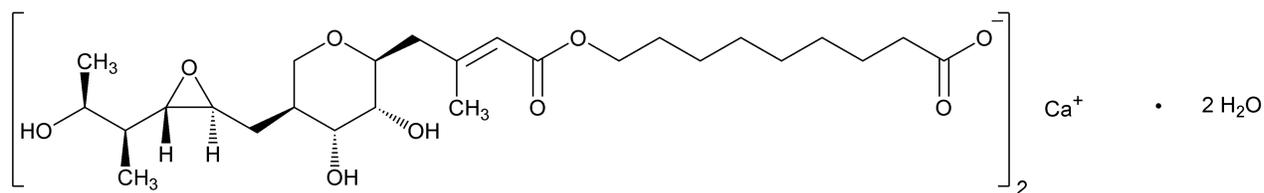
■ 2S (USP39)

BRIEFING

Mupirocin Calcium, USP 38 page 4460. In preparation for the omission of the flame tests from *Identification Tests—General* (191) proposed in PF 41(2) [Mar.–Apr. 2015], it is proposed to replace the flame test for calcium with the oxalate precipitation test in the chapter. This is similar to the test for calcium in the *Japanese Pharmacopoeia* monograph for *Mupirocin Calcium Hydrate*. *Identification test A* is revised to remove the redundant requirement to use an undried sample. Because the label for USP Mupirocin Calcium RS states “Do not dry”, *Spectrophotometric Identification Tests* (197) indicates that the sample should also be undried. Additionally, minor editorial changes have been made to update the monograph to current USP style.

(CHM1: A. Wise.)

Correspondence Number—C160925

Comment deadline: November 30, 2015**Mupirocin Calcium**C₅₂H₈₆CaO₁₈·2H₂O 1075.34

Nonanoic acid, 9-[[3-Methyl-1-oxo-4-[tetrahydro-3,4-dihydroxy-5-[[3-(2-hydroxy-1-methylpropyl)oxiranyl]methyl]-2*H*-pyran-2-yl]-2-butenyl]oxy-, calcium salt (2:1), dihydrate, [2*S*-[2*α*(*E*),3*β*,4*β*,5*α*[2*R**,3*R**(1*R**,2*R**)]]]-; (*αE*,2*S*,3*R*,4*R*,5*S*)-5-[(2*S*,3*S*,4*S*,5*S*)-2,3-Epoxy-5-hydroxy-4-methylhexyl]tetrahydro-3,4-dihydroxy-*β*-methyl-2*H*-pyran-2-crotonic acid, ester with 9-hydroxynonanoic acid, calcium salt (2:1), dihydrate [115074-43-6].

DEFINITION

Mupirocin Calcium contains the equivalent of NLT 865 µg/mg and NMT 936 µg/mg of mupirocin (C₂₆H₄₄O₉).

IDENTIFICATION**Change to read:**

- **A. Infrared Absorption** (197M)

Sample: Do not dry or

■ 2S (USP39)

grind extensively.

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **C. Identification Tests—General** (191), *Calcium*: ~~When moistened with hydrochloric acid, it meets the requirements of the flame test.~~
 - Meets the requirements ■ 2S (USP39)

ASSAY

• **Procedure**

Solution A: 7.7 g/L of *ammonium acetate* in water, adjusted with *glacial acetic acid* to a pH of 5.7 before diluting to the final volume

Mobile phase: *Tetrahydrofuran* and *Solution A* (32:68)

Standard solution: 125 µg/mL of USP Mupirocin Lithium RS prepared as follows. Transfer a suitable amount of USP Mupirocin Lithium RS to a suitable volumetric flask, dissolve in *methanol*, using 2.5% of the final volume, and dilute with *Solution A* to volume.

System suitability solution: Adjust 10 mL of the *Standard solution* with 6 N *hydrochloric acid* to a pH of 2.0, and allow to stand for 20 h.

Sample solution: Transfer 25 mg of Mupirocin Calcium to a 200-mL volumetric flask, dissolve in 5 mL of *methanol*, and dilute with *Solution A* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 7.0 between the second of the two peaks corresponding to mupirocin rearrangement products and the peak corresponding to mupirocin, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of mupirocin (C₂₆H₄₄O₉) in the portion of Mupirocin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak area of mupirocin from the *Sample solution*

r_S = peak area of mupirocin from the *Standard solution*

C_S = concentration of USP Mupirocin Lithium RS in the *Standard solution* (mg/mL)

C_U = concentration of Mupirocin Calcium in the *Sample solution* (mg/mL)

P = potency of mupirocin in USP Mupirocin Lithium RS ($\mu\text{g}/\text{mg}$)

Acceptance criteria: 865–936 $\mu\text{g}/\text{mg}$

IMPURITIES

• Chloride and Sulfate (221), Chloride

Analysis: Dissolve 50 mg in a mixture of 1 mL of 2 N nitric acid and 15 mL of methanol. Add 1 mL of silver nitrate TS.

Acceptance criteria: The turbidity does not exceed that produced by 0.70 mL of 0.020 N hydrochloric acid (0.5%).

• Organic Impurities

Solution A: Prepare as directed in the Assay.

Solution B: 13.6 g/L of sodium acetate in water, adjusted with glacial acetic acid to a pH of 4.0 before diluting to the final volume

Mobile phase: Tetrahydrofuran and Solution A (30:70)

Diluent: Methanol and Solution B (1:1)

Standard solution: 125 $\mu\text{g}/\text{mL}$ of USP Mupirocin Lithium RS in Diluent

System suitability solution: Adjust 10 mL of the Standard solution with 6 N hydrochloric acid to a pH of 2.0, allow to stand for 20 h, and adjust with 5 N sodium hydroxide to a pH of 4.0.

Sample solution: 5 mg/mL of Mupirocin Calcium in Diluent

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L7

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: Standard solution and System suitability solution

[Note—The relative retention times for two mupirocin rearrangement products and mupirocin in the System suitability solution are 0.63, 0.67, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 7.0 between the mupirocin rearrangement product, with a relative retention time of about 0.67, and mupirocin, System suitability solution

Column efficiency: NLT 3000 theoretical plates for the mupirocin peak, Standard solution

Tailing factor: NMT 2 for the mupirocin peak, *Standard solution*

Relative standard deviation: NMT 5% for the mupirocin peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each related compound in the portion of Mupirocin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area of any impurity from the *Sample solution*

r_S = peak area of mupirocin from the *Standard solution*

C_S = concentration of USP Mupirocin Lithium RS in the *Standard solution* (mg/mL)

C_U = concentration of Mupirocin Calcium in the *Sample solution* (mg/mL)

P = potency of mupirocin in USP Mupirocin Lithium RS ($\mu\text{g}/\text{mg}$)

F = conversion factor, 0.001 mg/ μg

Acceptance criteria: See *Table 1*. Disregard any peak with an area less than 0.05 times the area of the mupirocin peak in the *Standard solution*.

Table 1

Name	Relative Retention Time	Acceptance Criteria NMT (%)
Pseudomonic acid D ^a	0.75	2.5
Mupirocin	1.0	—
Any other unspecified impurity	—	1
Total impurities	—	4.5
^a (E)-9-{{(E)-4-[(2S,3R,4R,5S)-3,4-Dihydroxy-5-{{(2S,3S)-3-[(2S,3S)-3-hydroxybutan-2-yl]oxiran-2-yl}methyl)tetrahydro-2H-pyran-2-yl]-3-methylbut-2-enoyloxy}non-4-enoic acid.		

SPECIFIC TESTS

- **Optical Rotation** (781S), *Specific Rotation*
Sample solution: 50 mg/mL in *methanol*
Acceptance criteria: -16° to -20°
- **Water Determination** (921), *Method I*: 3.0%–4.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at 25° , excursions permitted between 15° and 30° .
- **USP Reference Standards** (11)
 USP Mupirocin Calcium RS
 USP Mupirocin Lithium RS

BRIEFING

Nebivolol Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated test methods, is proposed.

1. The proposed liquid chromatographic procedures in the *Assay* and the *Dissolution* test were validated using the Hypersil BDS C18 brand of L1 column. The test for *Organic Impurities* was validated using the Sunfire C18 brand of L1 column. Another suitable column for the *Assay* and the test for *Organic Impurities* is the Eclipse plus C-18 brand of L1 column. The typical retention times for nebivolol are about 5.5 and 9 min, respectively, based on the conditions specified in the *Assay* and in the test for *Organic Impurities*.

This monograph is contingent on FDA approval of a product that meets the proposed monograph.

(CHM2: S. Ramakrishna.)

Correspondence Number—C109140

Comment deadline: November 30, 2015

Add the following:

► **Nebivolol Tablets**

DEFINITION

Nebivolol Tablets contain an amount of nebivolol hydrochloride equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of nebivolol ($C_{22}H_{25}F_2NO_4$) free base.

IDENTIFICATION

- **A.** The UV absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Dilute phosphoric acid: Dilute 1.0 mL of phosphoric acid with water to 1 L.

Buffer: Dissolve 2.72 g of monobasic potassium phosphate in 1 L of water and add 2.0 mL of triethylamine. Adjust with *Dilute phosphoric acid* to a pH of 3.0.

Mobile phase: Acetonitrile, methanol, and *Buffer* (25:30:45)

Diluent: Acetonitrile and *Dilute phosphoric acid* (55:45)

Standard stock solution: 0.55 mg/mL of USP Nebivolol Hydrochloride RS in *Diluent*. Initially fill with *Diluent* to about 70% of the total volume and sonicate. Further dilute with *Diluent* to volume.

Standard solution: 0.044 mg/mL of USP Nebivolol Hydrochloride RS in *Diluent* from the *Standard stock solution*

Sample stock solution: Nominally equivalent to 0.4 mg/mL of nebivolol in *Diluent*, prepared from a sufficient number of Tablets as follows. To an appropriate number of Tablets (NLT 10) in a suitable volumetric flask, add water to fill 10% of the volume of the flask, let stand for 10 min, and sonicate to disperse. Add *Diluent* to fill 80% of the total volume and sonicate for 30 min with intermittent shaking. Allow to cool to room temperature and dilute with *Diluent* to volume. Centrifuge the sample solution at 3500 rpm for 10 min in a stoppered centrifuge tube.

Sample solution: Nominally equivalent to 0.044 mg/mL of nebivolol in *Diluent* from the *Sample stock solution*. Pass through a suitable filter of 0.45- μ m pore size, discarding the first few mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector

Assay: UV 281 nm

Identification A: Diode array, UV 200–400 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Autosampler temperature: 10°

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: NLT 1.8 times the retention time of the nebivolol peak

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nebivolol ($C_{22}H_{25}F_2NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of nebivolol from the *Sample solution*

r_S = peak response of nebivolol from the *Standard solution*

C_S = concentration of USP Nebivolol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of nebivolol in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of nebivolol, 405.44

M_{r2} = molecular weight of nebivolol hydrochloride, 441.90

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **Dissolution** (711)

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Buffer: Proceed as directed in the *Assay*.

Mobile phase: Acetonitrile and *Buffer* (45:55)

Diluent: Acetonitrile and water (50:50)

Standard stock solution 1: 0.6 mg/mL of USP Nebivolol Hydrochloride RS in *Diluent*.

Initially add *Diluent* to fill about 70% of the total volume, sonicate to dissolve, and dilute with *Diluent* to volume.

Standard stock solution 2: 0.06 mg/mL of USP Nebivolol Hydrochloride RS in *Medium* from *Standard stock solution 1*

Standard solution: Prepare solutions with concentrations shown in *Table 1* using *Medium* from *Standard stock solution 2*.

Table 1

Tablet Strength (mg)	Concentration (µg/mL)
2.5	3
5	6
10	12
20	24

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.

Chromatographic system: Proceed as directed in the *Assay* except for the *Run time*.

Run time: NLT 1.5 times the retention time of the nebivolol peak

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nebivolol (C₂₂H₂₅F₂NO₄) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of nebivolol from the *Sample solution*

r_S = peak response of nebivolol from the *Standard solution*

C_S = concentration of USP Nebivolol Hydrochloride RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

M_{r1} = molecular weight of nebivolol, 405.44

M_{r2} = molecular weight of nebivolol hydrochloride, 441.90

Tolerances: NLT 80% (Q) of the labeled amount of nebivolol ($C_{22}H_{25}F_2NO_4$) is dissolved.

- **Uniformity of Dosage Units** (905): Meet the requirements

IMPURITIES

- **Organic Impurities**

Buffer: Dissolve 3.4 g of tetrabutylammonium hydrogen sulfate and 2.72 g of potassium dihydrogen phosphate in 1000 mL of water, and add 0.3 mL of diethylamine.

Solution A: Acetonitrile and *Buffer* (25:75)

Solution B: Acetonitrile and water (90:10)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	100	0
50	80	20
60	80	20
65	100	0
70	100	0

Dilute phosphoric acid: Dilute 1.0 mL of phosphoric acid with water to 1 L.

Diluent: Acetonitrile and *Dilute phosphoric acid* (30:70)

Standard stock solution: 0.44 mg/mL of USP Nebivolol Hydrochloride RS in *Diluent*. Add *Diluent* to fill about 70% of the total volume, sonicate to dissolve, and dilute with *Diluent* to volume.

Standard solution: 2.2 µg/mL of USP Nebivolol Hydrochloride RS in *Diluent* from the *Standard stock solution*

Sample solution: Nominally equivalent to 1 mg/mL of nebivolol in *Diluent*, prepared as follows from crushed and finely powdered Tablets (see *Table 3*)

Table 3

Tablet Strength (mg)	Number of Tablets
2.5	50
5	25
10	20
20	20

Transfer an appropriate quantity from finely powdered Tablets to a suitable volumetric flask. Add *Diluent* to about 70% of the flask volume and sonicate for 30 min with intermittent shaking. Allow the solution to cool to room temperature and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- μ m pore size and discard the first 5 mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Temperatures

Autosampler: 15°

Column: 35°

Flow rate: 2 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of nebivolol from the *Standard solution*

C_S = concentration of USP Nebivolol Hydrochloride RS in the *Standard solution* (μ g/mL)

C_U = nominal concentration of nebivolol in the *Sample solution* (μ g/mL)

M_{r1} = molecular weight of nebivolol, 405.44

M_{r2} = molecular weight of nebivolol hydrochloride, 441.90

Acceptance criteria

Individual degradation impurities: NMT 0.2%

Total degradation impurities: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store at controlled room temperature in tight, light-resistant containers.
- **USP Reference Standards** <11>
USP Nebivolol Hydrochloride RS

◀(TBD)

BRIEFING

Nebivolol Hydrochloride. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated test methods, is proposed.

1. The liquid chromatographic procedures for the *Assay* and the test for *Organic Impurities* were validated using the Hypersil BDS C-18 brand of L1 column. The typical retention time for nebivolol is about 11 min under the conditions specified.
2. The liquid chromatographic procedure for the *Content of Nebivolol Related Compound C and Nebivolol Related Compound D* was validated using the Hypersil BDS C-18 brand of L1 column. The typical retention times for the nebivolol related compound C and nebivolol related compound D peaks are about 25 and 29 min, respectively.

This monograph is contingent on FDA approval of a product that meets the proposed monograph.

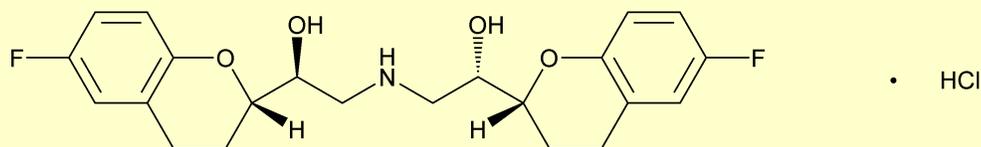
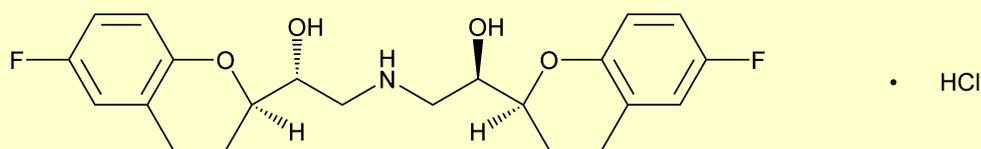
(CHM2: S. Ramakrishna.)

Correspondence Number—C108485

Comment deadline: November 30, 2015

Add the following:

►Nebivolol Hydrochloride



$C_{22}H_{25}F_2NO_4 \cdot HCl$ 441.90

2*H*-1-Benzopyran-2-methanol, α,α' -[iminobis(methylene)]bis[6-fluoro-3,4-dihydro]-, hydrochloride, (*aR,a'R,2R,2'S*)-*rel*-; (*1RS,1'RS*)-1,1'-[(*2RS,2'SR*)-Bis(6-fluoro-3,4-dihydro-2*H*-1-benzopyran-2-yl)]-2,2'-

iminodiethanol hydrochloride [152520-56-4].

DEFINITION

Nebivolol Hydrochloride contains NLT 98.0% and NMT 102.0% of nebivolol hydrochloride ($C_{22}H_{25}F_2NO_4 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K): Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General** (191), *Chloride*: Meets the requirements

ASSAY

• Procedure

Buffer: Dissolve 3.4 g of tetrabutylammonium hydrogen sulfate in 1 L of water. Add 0.3 mL of diethylamine and mix.

Mobile phase: Acetonitrile and *Buffer* (25:75)

Diluent: Dilute 1.0 mL of phosphoric acid with water to 1 L.

Standard stock solution: 0.5 mg/mL of USP Nebivolol Hydrochloride RS prepared as follows. To an appropriate amount of USP Nebivolol Hydrochloride RS in a suitable volumetric flask, add acetonitrile to fill 50% of the total volume followed by *Diluent* to fill 75% of the total volume. Sonicate to dissolve and dilute with *Diluent* to volume.

Standard solution: 0.1 mg/mL of USP Nebivolol Hydrochloride RS from the *Standard stock solution* prepared as follows. To an appropriate volume of the *Standard stock solution* in a suitable volumetric flask, add acetonitrile to fill 50% of the total volume and dilute with *Diluent* to volume.

Sample stock solution: 0.5 mg/mL of Nebivolol Hydrochloride prepared as follows. To an appropriate amount of Nebivolol Hydrochloride in a suitable volumetric flask, add acetonitrile to fill 50% of the total volume followed by *Diluent* to fill 75% of the total volume. Sonicate to dissolve and dilute with *Diluent* to volume.

Sample solution: 0.1 mg/mL of Nebivolol Hydrochloride from the *Sample stock solution* prepared as follows. To an appropriate volume of the *Sample stock solution* in a suitable volumetric flask, add acetonitrile to fill 50% of the total volume and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5- μ m packing L1

Temperatures

Autosampler: 15°

Column: 30°

Flow rate: 2 mL/min

Injection volume: 20 μ L

Run time: NLT 2 times the retention time of the nebivolol peak

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nebivolol hydrochloride ($C_{22}H_{25}F_2NO_4 \cdot HCl$) in the portion of Nebivolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nebivolol from the *Sample solution*

r_S = peak response of nebivolol from the *Standard solution*

C_S = concentration of USP Nebivolol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Nebivolol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.10%
- **Organic Impurities**

Buffer, Mobile phase, and Diluent: Prepare as directed in the *Assay*.

Standard stock solution 1: 0.125 mg/mL each of USP Nebivolol Related Compound A RS, USP Nebivolol Related Compound B RS, and USP Nebivolol Related Compound E RS prepared as follows. To appropriate amounts of USP Nebivolol Related Compound A RS, USP Nebivolol Related Compound B RS, and USP Nebivolol Related Compound E RS in a suitable volumetric flask, add acetonitrile to fill 50% of the total volume followed by *Diluent* to fill 75% of the total volume. Sonicate to dissolve, and dilute with *Diluent* to volume.

System suitability solution: 0.5 mg/mL of USP Nebivolol Hydrochloride RS and 0.75 μ g/mL each of USP Nebivolol Related Compound A RS, USP Nebivolol Related Compound B RS, and USP Nebivolol Related Compound E RS, in *Diluent* prepared as follows. To an appropriate amount of USP Nebivolol Hydrochloride RS in a suitable volumetric flask, add a suitable amount of *Standard stock solution 1* and acetonitrile to fill 50% of the total volume, followed by *Diluent* to fill 75% of the total volume. Sonicate to dissolve, and dilute with *Diluent* to volume.

Standard stock solution 2: 15 μ g/mL of USP Nebivolol Hydrochloride RS prepared as follows. To an appropriate amount of USP Nebivolol Hydrochloride RS in a suitable volumetric flask, add acetonitrile to fill 50% of the total volume followed by *Diluent* to fill 75% of the total volume. Sonicate to dissolve, and dilute with *Diluent* to volume.

Standard solution: 1.5 µg/mL of USP Nebivolol Hydrochloride RS and 0.75 µg/mL each of USP Nebivolol Related Compound A RS, USP Nebivolol Related Compound B RS, and USP Nebivolol Related Compound E RS from *Standard stock solution 1* and *Standard stock solution 2* prepared as follows. To an appropriate volume of *Standard stock solution 2* in a suitable volumetric flask, add a suitable volume of *Standard stock solution 1* and acetonitrile to fill 50% of the total volume. Dilute with *Diluent* to volume.

Sample solution: 0.5 mg/mL of Nebivolol Hydrochloride prepared as follows. To an appropriate amount of Nebivolol Hydrochloride in a suitable volumetric flask, add acetonitrile to fill 50% of the total volume followed by *Diluent* to fill 75% of the total volume. Sonicate to dissolve, and dilute with *Diluent* to volume.

Chromatographic system: Proceed as directed in the *Assay* except for the *Run time*.

Run time: NLT 5.5 times the retention time of the nebivolol peak

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between nebivolol and nebivolol related compound A; NLT 2.0 between nebivolol and the second-eluting nebivolol related compound B peak (closer to the nebivolol peak, relative retention time 0.90), *System suitability solution*

Tailing factor: NMT 2.0 for the nebivolol peak, *Standard solution*

Relative standard deviation: NMT 10.0% for the nebivolol peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Nebivolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified impurity from the *Sample solution*

r_S = peak response of the corresponding nebivolol related compound from the *Standard solution*

C_S = concentration of the corresponding USP Nebivolol Related Compound RS in the *Standard solution* (mg/mL)

C_U = concentration of Nebivolol Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Nebivolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_S = peak response of nebivolol from the *Standard solution*

C_S = concentration of USP Nebivolol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Nebivolol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, (NMT %)
Nebivolol related compound E	0.76	0.15
Nebivolol related compound B	0.85 and 0.90	0.15
Nebivolol	1.00	—
Nebivolol related compound A	1.15	0.15
Any unspecified impurity	—	0.10
Total impurities	—	0.75

● **Content of Nebivolol Related Compound C and Nebivolol Related Compound D**

[Note—All solutions containing nebivolol related compound C are prepared fresh prior to use.]

Buffer, Mobile phase, and Diluent: Prepare as directed in the Assay.

Standard stock solution 1: 0.3 mg/mL of USP Nebivolol Related Compound C RS and USP Nebivolol Related Compound D RS prepared as follows. To an appropriate amount of USP Nebivolol Related Compound C RS and USP Nebivolol Related Compound D RS in a suitable volumetric flask, add acetonitrile to fill 50% of the total volume, followed by *Diluent* to fill 75% of the total volume. Sonicate to dissolve and dilute with *Diluent* to volume.

Standard stock solution 2: 15 µg/mL of USP Nebivolol Related Compound C RS and USP Nebivolol Related Compound D RS in *Diluent* from *Standard stock solution 1*

Standard solution: 0.375 µg/mL of USP Nebivolol Related Compound C RS and USP Nebivolol Related Compound D RS in *Diluent* from *Standard stock solution 2*

Sample solution: 10 mg/mL of Nebivolol Hydrochloride prepared as follows. To an appropriate amount of Nebivolol Hydrochloride in a suitable volumetric flask, add acetonitrile to fill 50% of the total volume followed by *Diluent* to fill 75% of the total volume. Sonicate to dissolve, and dilute with *Diluent* to volume. Filter if necessary using a suitable filter of 0.45-µm pore size, and discard the first 2 mL of the filtrate.

Chromatographic system: Proceed as directed in the Assay except for the *Injection volume* and *Run time*.

Injection volume: 50 µL

Run time: NLT 4 times the retention time of the nebivolol peak

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between the nebivolol related compound D and nebivolol related compound C peaks

Relative standard deviation: NMT 10.0% for both peaks

Signal-to-noise ratio: NLT 15 for both peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nebivolol related compound D and nebivolol related compound

C in the portion of Nebivolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding nebivolol related compound from the *Sample solution*

r_S = peak response of the corresponding nebivolol related compound from the *Standard solution*

C_S = concentration of the corresponding USP Nebivolol Related Compound RS in the *Standard solution* (mg/mL)

C_U = concentration of Nebivolol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (ppm)
Nebivolol	1.00	—
Nebivolol related compound C	2.63	37.5
Nebivolol related compound D	2.7	37.5
Total impurities	—	37.5

SPECIFIC TESTS

- **Water Determination** (921), *Method I*: NMT 1.0%
- **Optical Rotation** (781S), *Procedures, Specific Rotation*

Sample solution: 20 mg/mL of Nebivolol Hydrochloride in 80% tetrahydrofuran in water

Acceptance criteria: $0.0^\circ \pm 1.0^\circ$, measured at 20°

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Protect from light and moisture, and store at controlled room temperature.
- **USP Reference Standards** (11)
 - USP Nebivolol Hydrochloride RS
 - USP Nebivolol Related Compound A RS
 - A mixture of (S)-1-[(R)-6-Fluorochroman-2-yl]-2-({(R)-2-[(S)-6-fluorochroman-2-yl]-2-hydroxyethyl}amino)ethanol;
 - (R,1S,1'S)-2,2'-Azanediylobis{1-[(R)-6-fluorochroman-2-yl]ethanol};
 - (S,1R,1'R)-2,2'-Azanediylobis{1-[(S)-6-fluorochroman-2-yl]ethanol}.
 - $C_{22}H_{25}F_2NO_4 \cdot HCl$ 441.90
 - USP Nebivolol Related Compound B RS
 - A mixture of (R)-1-[(R)-6-Fluorochroman-2-yl]-2-({(S)-2-[(S)-6-fluorochroman-2-yl]-2-hydroxyethyl}amino)ethanol;
 - (R)-1-[(R)-6-Fluorochroman-2-yl]-2-({(R)-2-[(R)-6-fluorochroman-2-yl]-2-hydroxyethyl}amino)ethanol;
 - (S)-1-[(S)-6-Fluorochroman-2-yl]-2-({(S)-2-[(S)-6-fluorochroman-2-yl]-2-hydroxyethyl}amino)ethanol.

$C_{22}H_{25}F_2NO_4 \cdot HCl$ 441.90

USP Nebivolol Related Compound C RS

(*RS*)-6-Fluoro-2-[(*RS*)-oxiran-2-yl]chroman.

$C_{11}H_{11}FO_2$ 194.20

USP Nebivolol Related Compound D RS

(*RS*)-6-Fluoro-2-[(*SR*)-oxiran-2-yl]chroman.

$C_{11}H_{11}FO_2$ 194.20

USP Nebivolol Related Compound E RS

1-(Chroman-2-yl)-2-{[2-(6-fluorochroman-2-yl)-2-hydroxyethyl]amino}ethanol.

$C_{22}H_{26}FNO_4$ 387.44

◀(TBD)

BRIEFING

Niacin, page 7447 of the *First Supplement to USP 38*. On the basis of comments received, the following changes are proposed:

1. Change the acceptance criteria in *Identification* test B.
2. Remove the limit for total specific impurities in *Table 2* of the *Related Compounds* test.
3. Replace the limit for total unspecified impurities with the limit for total impurities in *Table 2* of the *Related Compounds* test.

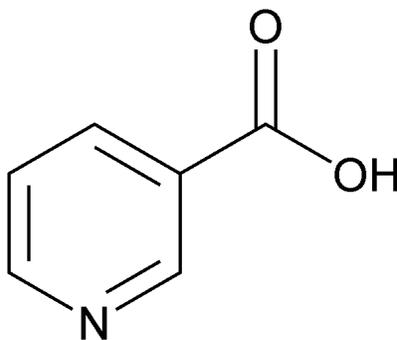
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(NBDS: H. Dinh.)

Correspondence Number—C162311

Comment deadline: November 30, 2015

Niacin



$C_6H_5NO_2$ 123.11

3-Pyridinecarboxylic acid;
Nicotinic acid [59-67-6].

DEFINITION

Change to read:

Niacin contains NLT \blacksquare 98.0% \blacksquare 1S (USP38) and NMT \blacksquare 102.0% \blacksquare 1S (USP38) of niacin ($C_6H_5NO_2$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197M)

Change to read:

- **B. Ultraviolet Absorption** (197U)

Wavelength range: 200–300 nm

\blacksquare **Buffer solution:** Dissolve 6.8 g of *monobasic potassium phosphate* in 1000 mL of water. Adjust with 50% sodium hydroxide solution to a pH of 7.0. \blacksquare 1S (USP38)

Sample solution: 20 μ g/mL in *Buffer solution*

Acceptance criteria: Meets the requirements. The A_{237}/A_{262} ratio is 0.46–0.50

\blacksquare A_{239}/A_{263} ratio is 0.46–0.52. \blacksquare 2S (USP39)

Add the following:

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. \blacksquare 1S (USP38)

ASSAY

Change to read:

- **Procedure**

\blacksquare **Diluent:** Methanol and water (82:18)

Mobile phase: Methanol and water (82:18), adjusted with *glacial acetic acid* to a pH of 3.15 ± 0.05

System suitability solution: 0.25 mg/mL of USP Niacin RS, 0.050 mg/mL of USP 6-Hydroxynicotinic Acid RS, and 0.10 mg/mL of pyridine in *Diluent*

Standard solution: 0.25 mg/mL of USP Niacin RS in *Diluent*

Sample solution: 0.25 mg/mL of Niacin in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L8

Flow rate: 1.0 mL/min

Injection volume: 25 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for pyridine, 6-hydroxynicotinic acid, and niacin are about 0.14, 0.64, and 1.0, respectively, *System suitability solution*.]

Suitability requirements

Resolution: NLT 1.5 between pyridine and 6-hydroxynicotinic acid and NLT 1.5

between 6-hydroxynicotinic acid and niacin, *System suitability solution*

Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of niacin ($C_6H_5NO_2$) in the portion of Niacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of niacin from the *Sample solution*

r_S = peak response of niacin from the *Standard solution*

C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)

C_U = concentration of Niacin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis \blacksquare_{1S} (USP38)

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%
- **Chloride and Sulfate** (221), *Chloride*

Standard: 0.15 mL of 0.020 N hydrochloric acid

Sample: 0.50 g of Niacin

Acceptance criteria: NMT 0.02%
- **Chloride and Sulfate** (221), *Sulfate*

Standard: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.50 g of Niacin

Acceptance criteria: NMT 0.02%

Delete the following:

- ~~**Heavy Metals**, Method I (231)~~

~~**Test preparation:** Mix 1 g with 4 mL of 1 N acetic acid, and dilute with water to 25 mL. Heat gently until solution is complete, and cool.~~

~~**Acceptance criteria:** NMT 20 ppm (Official 1-Dec-2015)~~

Delete the following:

- ~~**Ordinary Impurities** (466)~~

~~**Standard solutions and Test solution:** Use water as the solvent.~~

~~**Eluant:** A mixture of methanol and 0.1 N hydrochloric acid (9:1)~~

~~**Visualization:** \pm~~

~~**Analysis:** Proceed as directed in the chapter.~~

~~**Acceptance criteria:** NMT 2.0% of total ordinary impurities \blacksquare_{1S} (USP38)~~

Add the following:

- **Related Compounds**

Solution A: Dissolve 0.6 g of *glacial acetic acid* in 1 L of water, and adjust with 10% *ammonium hydroxide* solution to a pH of 5.6.

Solution B: Acetonitrile and methanol (1:1)

Mobile phase: Gradient elution. See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10 ^a	100	0
30	20	80
35	20	80
36	100	0
48	100	0

^a The gradient start time may be adjusted to achieve the required resolution between the 6-methylnicotinic acid and 6,6'-dinicotinic acid peaks of the *System suitability solution*.

System suitability solution: Transfer 3 mg each of USP 6-Methylnicotinic Acid RS, USP 6,6'-Dinicotinic Acid RS, and pyridine to a 100-mL volumetric flask, dissolve, and dilute with *Solution A* to volume. Transfer 2.0 mL of the resultant solution to a 5-mL volumetric flask, and dilute with *Solution A* to volume.

Standard solution: 0.012 mg/mL of USP Niacin RS in *Solution A*

Sample solution: Transfer 120 mg of Niacin to a 10-mL volumetric flask, add 200 μ L of 10% *ammonium hydroxide* solution, and dilute with *Solution A* to volume. Shake the flask until the Niacin is completely dissolved.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm \times 25-cm; 4- μ m packing *L1*

Column temperature: 15°

Flow rate: 1.0 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for 6-methylnicotinic acid, 6,6'-dinicotinic acid, and pyridine are about 1.0, 1.03, and 1.4, respectively, *System suitability solution*.]

Suitability requirements

Resolution: NLT 1.5 between 6-methylnicotinic acid and 6,6'-dinicotinic acid peaks, *System suitability solution*

Relative standard deviation: NMT 10.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of each impurity in the portion of Niacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of niacin from the *Standard solution*

C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)

C_U = concentration of Niacin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Isocinchomeric acid	0.38	0.05
6-Hydroxynicotinic acid	0.63	0.05
Isonicotinic acid	0.92	0.05
Niacin	1.00	—
6-Methylnicotinic acid	2.61	0.05
6,6'-Dinicotinic acid	2.68	0.05
5-Nitronicotinic acid	2.76	0.05
Pyridine	3.76	0.05
Nitropyridine ■ 3-Nitropyridine ■ 2S (USP39)	3.83	0.05
3,5-Dinitropyridine	4.03	0.05
3-Ethylpyridine	4.72	0.05
5-Ethyl-2-methylpyridine	5.00	0.05
Total specific impurities	—	0.05 ■ 2S (USP39)
Any individual unspecified impurity	—	0.05
Total unspecified impurities ■ 2S (USP39)	—	0.20

■ 1S (USP38)

SPECIFIC TESTS

- **Loss on Drying** (731)

Analysis: Dry at 105° for 1 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** (11)
 - USP 6,6'-Dinicotinic Acid RS
 - USP 6-Hydroxynicotinic Acid RS
 - USP 6-Methylnicotinic Acid RS ■ 1S (USP38)
 - USP Niacin RS

BRIEFING

Phenol, *USP 38* page 4839. As part of the USP monograph modernization effort, the following revisions are proposed:

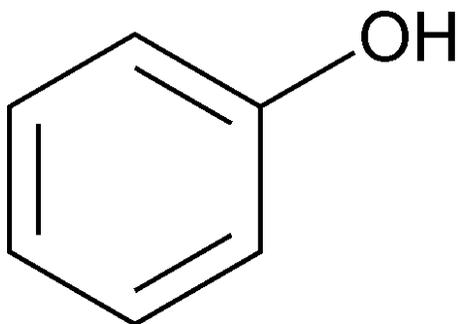
1. Replace the wet chemistry-based *Identification* tests *A* and *B* with an IR procedure and a retention time agreement based on the proposed *Assay*.
2. Replace the titration procedure in the *Assay* with a more specific HPLC procedure. The HPLC procedure is based on the analysis performed with the Dionex Acclaim Organic Acid brand of L60 column. The typical retention time for phenol is about 4 min.
3. Widen the *Definition* and *Assay* acceptance criteria from 99.0%–100.5% to 98.0%–102.0%, which is typical for a chromatographic assay.
4. Delete the *Clarity of Solution and Reaction* and *Congearing Temperature* tests in the *Specific Tests* section because the monograph contains sufficient tests to ensure the quality of the material.
5. Add USP Phenol RS to the *USP Reference Standards* section to support the proposed *Assay*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM6: D.A. Porter.)

Correspondence Number—C130686

Comment deadline: November 30, 2015

Phenol

C₆H₆O 94.11

Phenol [108-95-2].

DEFINITION**Change to read:**

Phenol contains NLT 99.0%

■ 98.0% ■_{2S} (USP39)

and NMT 100.5%

■ 102.0% ■_{2S} (USP39)

of phenol (C₆H₆O), calculated on the anhydrous basis. It may contain a suitable stabilizer.

IDENTIFICATION

[**Caution**—Avoid contact with skin because serious burns may result.]

Delete the following:

■ ● ~~A.~~

~~**Analysis:** To a solution add bromine TS.~~

~~**Acceptance criteria:** A white precipitate is formed, and it dissolves at first but becomes permanent as more of the reagent is added. ■_{2S} (USP39)~~

Add the following:

■ ● **A. Infrared Absorption** (197K) ■_{2S} (USP39)

Delete the following:

■ ● ~~B.~~

~~**Sample solution:** 1 in 100~~

~~**Analysis:** To 10 mL of the *Sample solution* add 1 drop of ferric chloride TS.~~

~~**Acceptance criteria:** A violet color is produced. ■_{2S} (USP39)~~

Add the following:

■ ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■_{2S} (USP39)

ASSAY**Change to read:**

● **Procedure**

~~**Sample:** 2 g~~

~~**Titrimetric system**~~

~~(See *Titrimetry* (541).)~~

~~**Mode:** Residual titration~~

~~**Titrant:** 0.1 N bromine VS~~

~~**Back-titrant:** 0.1 N sodium thiosulfate VS~~

~~**Endpoint detection:** Visual~~

~~**Analysis:** Place the *Sample* in a 1000 mL volumetric flask, and dilute with water to volume. Pipet 20 mL of the solution into an iodine flask, add 30.0 mL of *Titrant*, then add 5 mL of hydrochloric acid, and immediately insert the stopper. Shake the flask repeatedly~~

during 30 min, allow it to stand for 15 min, quickly add 5 mL of potassium iodide solution (1 in 5), taking precautions against the escape of bromine vapor, and at once insert the stopper. Shake thoroughly, remove the stopper, and rinse it and the neck of the flask with a small quantity of water so that the washing flows into the flask. Add 1 mL of chloroform, and shake the mixture. Titrate the liberated iodine with *Back-titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination. Each mL of 0.1 N bromine is equivalent to 1.569 mg of phenol (C_6H_6O).

Acceptance criteria: 99.0%–100.5% on the anhydrous basis

▪ **Solution A:** 0.1% Phosphoric acid in water

Mobile phase: Acetonitrile and *Solution A* (50:50)

Standard solution: 0.1 mg/mL of USP Phenol RS in *Solution A*

Sample solution: 0.1 mg/mL of Phenol in *Solution A*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.0-mm × 150-cm; 3- μ m packing L60

Column temperature: 30°

Flow rate: 0.35 mL/min

Injection volume: 5 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of phenol (C_6H_6O) in the portion of Phenol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Phenol RS in the *Standard solution* (mg/mL)

C_U = concentration of Phenol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis ■ 2S (USP39)

SPECIFIC TESTS

Delete the following:

- • **Clarity of Solution and Reaction**

Sample solution: 1 in 15

Acceptance criteria: The ~~Sample solution~~ is clear, and it is neutral or acid to litmus paper.
 ■ 2S (USP39)

Delete the following:

- ● **Congealing Temperature** ~~(651)~~ NLT 39° ■ 2S (USP39)
- **Water Determination** (921), *Method I*: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **Labeling:** Label it to indicate the name and amount of any substance added as a stabilizer.

Add the following:

- ● **USP Reference Standards** (11)

USP Phenol RS

■ 2S (USP39)

BRIEFING

Promethazine Hydrochloride Oral Solution, USP 38 page 5028. As part of the USP monograph modernization efforts, the following changes are proposed:

1. Replace the current *Identification* test *A* with the HPLC retention time agreement from the proposed *Assay* procedure. The current test uses toxic carbon disulfide in determining the IR absorption spectrum.
2. Add *Identification* test *B* based on the photodiode array spectrum agreement from the proposed *Assay*.
3. Replace the current UV absorbance procedure in the *Assay* with the same HPLC procedure that is proposed in the test for *Organic Impurities*. The procedure is based on analyses performed with the Inertsil ODS-3V brand of L1 column. The typical retention time for promethazine is about 10 min.
4. Add a test for *Organic Impurities*. The proposed HPLC procedure has the same chromatographic conditions as in the proposed *Assay*.
5. Add USP Promethazine Related Compound B RS to the *USP Reference Standards* section to support the proposed procedures in the *Assay* and the test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM5: W. Yang.)

Correspondence Number—C152625

Comment deadline: November 30, 2015

Promethazine Hydrochloride Oral Solution

DEFINITION

Promethazine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled

amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$).

[Note—Throughout the following procedures, protect the samples, Reference Standards, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

Change to read:

• A.

~~**Sample:** Treat 25 mL of Oral Solution as directed in the Assay, ending with “using only a current of air”.~~

~~**Analysis:** Dissolve the Sample in 2.5 mL of carbon disulfide, filter through paper if necessary, and determine the IR absorption spectrum as directed under *Identification: Organic Nitrogenous Bases* (181), obtaining the spectrum of USP Promethazine Hydrochloride RS as directed.~~

~~**Acceptance criteria:** Meets the requirements~~

■ The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ■ 2S (USP39)

Add the following:

■ • B. The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ■ 2S (USP39)

ASSAY

Change to read:

• Procedure

[Note—Use low-actinic glassware in this assay.]

~~**Standard solution:** 50 µg/mL of USP Promethazine Hydrochloride RS in dilute sulfuric acid (1 in 100)~~

~~**Sample solution:** Transfer a volume of Oral Solution, containing nominally 25 mg of promethazine hydrochloride to a 250-mL separator. Add 10 mL of ammonium hydroxide, and extract the promethazine base with six 40-mL portions of chloroform. Wash the combined chloroform extracts with 25 mL of dilute hydrochloric acid (1 in 9). Wash the acid solution with 25 mL of chloroform, and add the washings to the main chloroform extract. Evaporate the chloroform extract on a steam bath, with the aid of a current of air, to a volume of 5–10 mL, and finally evaporate, using only a current of air, to dryness. Dissolve the residue, with slight warming, in dilute sulfuric acid (1 in 100), and transfer to a 500-mL volumetric flask with the aid of additional acid. Cool, add dilute sulfuric acid (1 in 100) to volume, mix, and filter, rejecting the first half of the filtrate.~~

~~Instrumental conditions~~

~~**Mode:** UV~~

~~**Analytical wavelength:** Maximum absorbance at about 298 nm~~

~~**Cell:** 1 cm~~

~~**Blank:** Dilute sulfuric acid (1 in 100)~~

Analysis**Samples:** ~~Standard solution and Sample solution~~

Concomitantly determine the absorbances and calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) in the portion of the Oral Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the ~~Sample solution~~

A_S = absorbance of the ~~Standard solution~~

C_S = concentration of USP Promethazine Hydrochloride RS in the ~~Standard solution~~ ($\mu\text{g/mL}$)

C_U = nominal concentration of promethazine hydrochloride in the ~~Sample solution~~ ($\mu\text{g/mL}$)

Acceptance criteria: ~~90.0%–110.0%~~

▪ **Buffer:** 3.7 g/L of ammonium acetate in water

Solution A: Acetonitrile and Buffer (30:70)**Solution B:** Acetonitrile**Mobile phase:** See Table 1.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	60	40
18	60	40
18.1	100	0
25	100	0

Diluent: 0.1% Triethylamine in methanol**System suitability solution:** 1.0 $\mu\text{g/mL}$ each of USP Promethazine Hydrochloride RS and USP Promethazine Related Compound B RS in *Diluent***Standard solution:** 0.05 mg/mL of USP Promethazine Hydrochloride RS in *Diluent***Sample solution:** Nominally 0.05 mg/mL of promethazine hydrochloride from a volume of Oral Solution in *Diluent*. Centrifuge for 10 min and use the supernatant.

[Note—Sonication may be used in the preparation of the *System suitability solution*, *Standard solution*, and *Sample solution*.]

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** HPLC**Detector:** UV 254 nm. For *Identification test B*, use a diode-array detector in the range of 200–400 nm.**Column:** 4.6-mm \times 15-cm; 5- μm packing *L1*

Temperatures**Autosampler:** 4°**Column:** 30°**Flow rate:** 1.4 mL/min**Injection volume:** 15 µL**System suitability****Samples:** *System suitability solution* and *Standard solution*

[Note—The relative retention times for promethazine and promethazine related compound B are 1.0 and 1.3, respectively.]

Suitability requirements**Resolution:** NLT 5.0 between promethazine and promethazine related compound B peaks, *System suitability solution***Tailing factor:** NMT 2.0, *Standard solution***Relative standard deviation:** NMT 1.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0% $\pm 2S$ (USP39)**IMPURITIES****Add the following:**

- ● **Organic Impurities**

Buffer, Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system:Proceed as directed in the *Assay*.**Standard solution:** 1.0 µg/mL each of USP Promethazine Hydrochloride RS and USP Promethazine Related Compound B RS in *Diluent***Sample solution:** Nominally 500 µg/mL of promethazine hydrochloride from a volume of Oral Solution in *Diluent*. Centrifuge for 10 min and use the supernatant.[Note—Sonication may be used in the preparation of the *Standard solution* and the *Sample solution*.]**System suitability****Sample:** *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between promethazine and promethazine related compound B peaks

Relative standard deviation: NMT 5.0% for promethazine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of promethazine from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Promethazine sulfoxide ^a	0.3	0.26	0.2
Desmethyl promethazine ^b	0.6	1.0	0.2
Promethazine	1.0	—	—
Promethazine related compound B ^c	1.3	—	—
Phenothiazine ^d	1.5	2.2	0.2
Any individual unspecified degradation product	—	1.0	0.2

^a *N,N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.

^b *N*-Methyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine.

^c This is a process impurity which is controlled in the drug substance and is included in the table for identification only. It is not to be reported or included in the total degradation products.

^d 10*H*-Phenothiazine.

■ 2S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

- **USP Reference Standards** (11)

USP Promethazine Hydrochloride RS

- **USP Promethazine Related Compound B RS**

Isopromethazine;

N,N-Dimethyl-2-(10*H*-phenothiazin-10-yl)propan-1-amine.

$C_{17}H_{20}N_2S$ 284.42

- **2S (USP39)**

BRIEFING

Quetiapine Extended-Release Tablets. Because there is no existing *USP* monograph for this dosage form, a new monograph based on validated methods of analysis is proposed. The isocratic liquid chromatographic procedures in the *Assay* and the test for *Organic Impurities* are based on analyses performed with the Zorbax RX C8 brand of L7 column. The typical retention time for quetiapine is about 15 min.

(CHM4: R. Ravichandran.)

Correspondence Number—C143427

Comment deadline: November 30, 2015

Add the following:

- **Quetiapine Extended-Release Tablets**

DEFINITION

Quetiapine Extended-Release Tablets contain quetiapine fumarate $[(C_{21}H_{25}N_3O_2S)_2 \cdot (C_4H_4O_4)]$ equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$).

IDENTIFICATION

- **A. Infrared Absorption** (197F)

Standard solution: Transfer 10 mg of USP Quetiapine Fumarate RS to a suitable vial. Add 10 mL of acetone and cap the vial. Sonicate for about 10 min. Allow the solution to equilibrate to room temperature. Evaporate the acetone completely. Add 2 mL of chloroform. Gently swirl for several min. Pass through a suitable filter of 0.45- μ m pore size. Use the filtrate.

Sample solution: Grind NLT 10 Tablets. Transfer an amount of powder equivalent to NLT 10 mg of quetiapine fumarate to a suitable vial. Add 10 mL of acetone and cap the vial. Sonicate for about 10 min. Allow the solution to equilibrate to room temperature. Evaporate the acetone completely. Add 2 mL of chloroform. Gently swirl for several min. Pass through a suitable filter of 0.45- μ m pore size. Use the filtrate.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• Procedure

Buffer: Dissolve 2.6 g/L of *dibasic ammonium phosphate* in water.

Mobile phase: Methanol, acetonitrile, and *Buffer* (54:7:39)

Diluent: Acetonitrile and water (50:50)

System suitability stock solution: 0.5 mg/mL of USP Quetiapine System Suitability RS in *Mobile phase* prepared as follows. Transfer the required quantity of USP Quetiapine System Suitability RS to a suitable volumetric flask. Add 70% of the flask volume of *Mobile phase* and sonicate to dissolve. Dilute with *Mobile phase* to volume.

System suitability solution: 0.005 mg/mL of USP Quetiapine Related Compound H RS in *System suitability stock solution*

Standard solution: 0.2 mg/mL of USP Quetiapine Fumarate RS in *Mobile phase*

Sample stock solution: Transfer NLT 5 Tablets to a homogenizer vessel. Add 50 mL of acetonitrile, swirl to wet, and allow to stand for approximately 10 min. Add an additional 160 mL of *Diluent* and extract for about 10 min. Transfer the contents of the homogenizer to a 500-mL volumetric flask. Dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45- μ m pore size and use the filtrate.

Sample solution: Nominally 0.16–0.18 mg/mL of quetiapine from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Flow rate: 1.3 mL/min

Injection volume: 30 μ L

Run time: NLT 2.5 times the retention time of quetiapine

System suitability

Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between quetiapine related compound G and quetiapine related compound H; NLT 2.0 between the quetiapine desthoxy and quetiapine peaks; *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times N \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Quetiapine Fumarate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of quetiapine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of quetiapine free base, 383.51

M_{r2} = molecular weight of quetiapine fumarate, 883.09

N = number of moles of quetiapine free base per mole of quetiapine fumarate, 2

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution (711)

Medium 1: Citrate buffer, pH 4.8. Dissolve 9.6 g of *anhydrous citric acid* in 600 mL of water. Add 90 mL of *1 N sodium hydroxide*. Dilute with water to 1 L, 900 mL.

Medium 2: Dissolve 17.9 g/L of *dibasic sodium phosphate dodecahydrate* in 400 mL of water. Add 460 mL of *1 N sodium hydroxide* and dilute with water to 1 L, 100 mL.

[Note—It is recommended to check the pH of the mixture of 90 mL of *Medium 1* and 10 mL of *Medium 2*, which should be between 6.4 and 6.8. If the pH of the mixture is less than 6.4, 10 mL/L of *1 N sodium hydroxide* may be added to *Medium 2*. If the pH of the mixture is greater than 6.8, 10 mL/L of *1 N hydrochloric acid* may be added to *Medium 2*.]

Start the test with 900 mL of *Medium 1*. Add 100 mL of *Medium 2* to the vessel after 5 h of the test and continue the test.

Apparatus 1: 200 rpm

Times: 1, 6, 12, and 20 h

Diluent: *Medium 1* and *Medium 2* (90:10)

Standard solution: ($L/400$) mg/mL of USP Quetiapine Fumarate RS in *Diluent*, where L is the label claim in mg/Tablet

Sample solution: Pass a suitable portion of the solution under test through a suitable filter.

Instrumental conditions

Mode: UV

Analytical wavelength: About 290 nm

Blank: *Diluent*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, C_i , of quetiapine ($C_{21}H_{25}N_3O_2S$) in *Medium* (mg/mL) after time point (i):

$$C_i = (A_U/A_S) \times C_S \times (M_{r1}/M_{r2}) \times N$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

M_{r1} = molecular weight of quetiapine free base, 383.51

M_{r2} = molecular weight of quetiapine fumarate, 883.09

N = number of moles of quetiapine free base per mole of quetiapine fumarate, 2

Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$C_1, C_2,$
 C_3, C_4 = concentration of quetiapine in *Medium* in the portion of sample withdrawn at time points 1, 2, 3, and 4, respectively (mg/mL)

V = volume of *Medium*, 900 mL for 1 h; 1000 mL for 6, 12, and 20 h time points

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn from the vessel and replaced with *Medium* (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (i)	Time (h)	Amount Dissolved
1	1	NMT 20%
2	6	47%–69%
3	12	65%–95%
4	20	NLT 85%

The percentages of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

- **Uniformity of Dosage Units** (905): Meet the requirements

IMPURITIES

- **Organic Impurities**

Buffer, Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Sample: *System suitability solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between quetiapine related compound G and quetiapine related compound H; NLT 2.0 between the quetiapine desthoxy and quetiapine peaks

Analysis

Sample: *Sample solution*[Note—See *Table 2* for relative retention times.]

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

 r_U = peak response of each degradation product from the *Sample solution* r_S = peak response of quetiapine from the *Sample solution* F = relative response factor for the corresponding degradation product from *Table 2***Acceptance criteria:** See *Table 2*. Disregard peaks less than 0.05%.**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fumaric acid ^a	0.1	—	—
Quetiapine related compound G	0.48	1.4	0.2
Quetiapine related compound H	0.57	1.0	0.2
Quetiapine desethoxy ^b	0.87	—	—
Quetiapine	1.0	—	—
Quetiapine related compound B ^b	1.9	—	—
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	0.4

^a Counter ion peak, not to be included in the total impurities.

^b Process impurity controlled in the drug substance. Included for identification purposes only. Not reported for the drug product and not included in the total impurities.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP Reference Standards** <11>
 - USP Quetiapine Fumarate RS
 - USP Quetiapine Related Compound H RS
4-(Dibenzo[*b,f*][1,4]thiazepin-11-yl)-1-[2-(2-hydroxyethoxy)ethyl]piperazine 1-oxide.
C₂₁H₂₅N₃O₃S 399.51
 - USP Quetiapine System Suitability RS
 It contains quetiapine fumarate and at least 0.1% of each of the following impurities:
 - Quetiapine related compound B: 11-(Piperazin-1-yl)dibenzo[*b,f*][1,4]thiazepine;
 - Quetiapine related compound G: Dibenzo[*b,f*][1,4]thiazepin-11(10*H*)-one; and
 - Quetiapine desethoxy: 2-[4-(Dibenzo[*b,f*][1,4]thiazepin-11-yl)piperazin-1-

yl]ethanol.

■ 2S (USP39)

BRIEFING

Ropinirole Extended-Release Tablets. Because there is no existing *USP* monograph for this dosage form, a new monograph, based on validated methods of analysis, is proposed.

1. The isocratic liquid chromatographic procedure in the *Assay* is based on analyses performed with the Kromasil C8 brand of L7 column. The typical retention time for ropinirole is about 4 min.
2. The isocratic liquid chromatographic procedure in the *Dissolution* test is based on analyses performed with the Kromasil C8 brand of L7 column. The typical retention time for ropinirole is about 6 min.
3. The gradient liquid chromatographic procedure in the *Organic Impurities* test is based on analyses performed with the Kromasil C8 brand of L7 column. The typical retention time for ropinirole is about 18 min.

(CHM4: R. Ravichandran.)

Correspondence Number—C137240

Comment deadline: November 30, 2015

Add the following:■ **Ropinirole Extended-Release Tablets****DEFINITION**

Ropinirole Extended-Release Tablets contain ropinirole hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ropinirole free base ($C_{16}H_{24}N_2O$).

IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY● **Procedure**

Buffer: Dissolve 4.5 g of *dibasic sodium phosphate dihydrate* in 900 mL of water. Adjust with *phosphoric acid* to a pH of 7.0. Dilute with water to 1 L.

Mobile phase: Methanol and *Buffer* (75:25)

Dilute phosphoric acid: Dissolve 0.7 mL of *phosphoric acid* in 1 L of water.

Diluent: Acetonitrile and *Dilute phosphoric acid* (80:20)

System suitability solution: 0.1 mg/mL of USP Ropinirole Hydrochloride RS and 0.003 mg/mL of USP Ropinirole Related Compound B RS in *Diluent*. Sonication may be used to aid dissolution.

Standard solution: 0.11 mg/mL of USP Ropinirole Hydrochloride RS in *Diluent*. Sonication may be used to aid dissolution.

Sample solution: 0.05–0.2 mg/mL of ropinirole prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask containing 75% of the flask volume of *Diluent*. Sonicate for NLT 30 min. Allow to cool to room temperature. Dilute with *Diluent* to volume. Pass a portion of the solution through a nylon filter of 0.45- μ m pore size and use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm. For *Identification* test A, use a diode-array detector in the range of 200–400 nm.

Column: 4.6-mm \times 12.5-cm; 5- μ m packing L7

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 10 μ L

Run time: NLT 1.5 times the retention time of ropinirole

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between ropinirole related compound B and ropinirole, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ropinirole ($C_{16}H_{24}N_2O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of ropinirole from the *Sample solution*

r_S = peak response of ropinirole from the *Standard solution*

C_S = concentration of USP Ropinirole Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ropinirole in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of ropinirole free base, 260.37

M_{r2} = molecular weight of ropinirole hydrochloride, 296.84

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Dissolution** (711)

Solution A: 121.2 g/L of *trishydroxymethylaminomethane* in water

Buffer 1: Dissolve 2.1 g of *citric acid monohydrate* in 900 mL of water. Adjust with *Solution A* to a pH of 4.0.

Buffer 2: Dissolve 3.9 g of *ammonium acetate* in 900 mL of water. Adjust with *phosphoric acid* to a pH of 2.5. Dilute with water to 1 L.

Medium: *Buffer 1*; 500 mL. Deaerate as appropriate.

Apparatus 2: 100 rpm with tablet holder. See *Figure 1*.

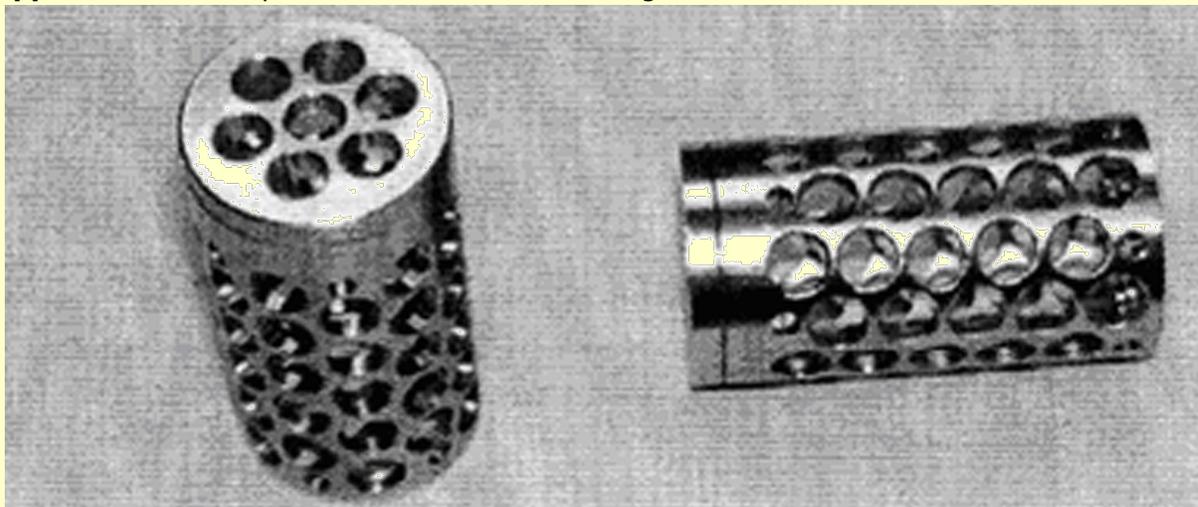


Figure 1. 37-mm(l) × 19-mm(d) stainless steel sinker; screw cap drilled with seven 4-mm holes, bottom drilled with seven 5-mm holes, 12 longitudinal series of 5-mm holes alternately starting and ending with one 3-mm hole, polished electrochemically or with a suitably validated alternative

Times: 2, 12, and 24 h

Mobile phase: Acetonitrile, methanol, and *Buffer 2* (14:6:80)

Standard solution: ($L/400$) mg/mL of USP Ropinirole Hydrochloride RS in *Medium* where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 10- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 12.5-cm; 5- μ m packing $L7$

Flow rate: 1 mL/min

Injection volume: 20 μ L for 12-mg Tablets; 100 μ L for all other strengths

Run time: NLT 2 times the retention time of ropinirole

System suitability

Sample: *Standard solution*

Suitability requirements**Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ropinirole ($C_{16}H_{24}N_2O$) dissolved at each time point (*i*):

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Ropinirole Hydrochloride RS in the *Standard solution* (mg/mL) V = volume of *Medium*, 500 mL L = label claim, mg/Tablet M_{r1} = molecular weight of ropinirole free base, 260.37 M_{r2} = molecular weight of ropinirole hydrochloride, 296.84**Tolerances:** See *Table 1*.**Table 1**

Time Point (<i>i</i>)	Time (h)	Amount Dissolved (%)
1	2	NMT 20
2	12	45–65
3	24	NLT 80

The percentage of the labeled amount of ropinirole ($C_{16}H_{24}N_2O$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

- **Uniformity of Dosage Units** (905): Meet the requirements

IMPURITIES

- **Organic Impurities**

Solution A: 0.05% (v/v) *Trifluoroacetic acid* in water**Solution B:** Acetonitrile and methanol (80:20)**Mobile phase:** See *Table 2*.**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	84	16
23	84	16
36	40	60
36.1	84	16
50	84	16

Diluent 1: Acetonitrile and *Solution A* (80:20)

Diluent 2: *Diluent 1* and *Solution A* (20:80)

System suitability solution: 0.03 mg/mL of USP Ropinirole Hydrochloride RS and 0.001 mg/mL of USP Ropinirole Related Compound B RS in *Diluent 2*. Sonication may be used to aid dissolution.

Sensitivity solution: 0.015 µg/mL of USP Ropinirole Hydrochloride RS in *Diluent 2*

Standard solution: 0.15 µg/mL of USP Ropinirole Related Compound B RS in *Diluent 2*

Sample stock solution: Nominally 0.13–0.14 mg/mL of ropinirole from a suitable number of Tablets containing 20–50 mg of ropinirole prepared as follows. Homogenize an appropriate number of Tablets in a suitable volume of *Diluent 1*. Pass a portion of the solution through a nylon filter of 0.45-µm pore size and use the filtrate.

Sample solution: Nominally 26–28 µg/mL of ropinirole from the *Sample stock solution* and *Solution A*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 25-cm; 5-µm packing *L7*

Column Temperature: 40°

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between ropinirole related compound B and ropinirole, *System suitability solution*

Relative standard deviation: NMT 10% for ropinirole related compound B, *Standard solution*

Signal-to-noise ratio: NLT 10 for ropinirole, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ropinirole related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of ropinirole related compound B from the *Sample solution*

r_S = peak response of ropinirole related compound B from the *Standard solution*

C_S = concentration of USP Ropinirole Related Compound B RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of ropinirole related compound B free base in the *Sample solution* ($\mu\text{g/mL}$)

M_{r1} = molecular weight of ropinirole related compound B free base, 274.36

M_{r2} = molecular weight of ropinirole related compound B hydrochloride, 310.82

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/F)/[\Sigma(r_U/F) + r_R] \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

F = relative response factor for the corresponding degradation product from *Table 3*

r_R = peak response of ropinirole from the *Sample solution*

Acceptance criteria: See *Table 3*. Disregard peaks less than 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ropinirole monopropyl ^a	0.42	1.0	0.5
Ropinirole related compound B	0.89	—	0.5
Ropinirole <i>N</i> -hydroxymethyl ^b	0.94	0.71	0.5
Ropinirole	1.00	—	—
Ropinirole <i>N</i> -oxide ^c	1.31	1.0	0.5
Ropinirole methylene dimer ^d	1.82	1.0	0.5
Propylidene ropinirole ^e	1.96	2.0	0.5
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.5

^a 4-[2-(Propylamino)ethyl]indolin-2-one.
^b 4-[2-(Dipropylamino)ethyl]-1-(hydroxymethyl)indolin-2-one.
^c *N*-[2-(2-Oxoindolin-4-yl)ethyl]-*N*-propylpropan-1-amine oxide.
^d {1,1'-Methylenebis{4-[2-(dipropylamino)ethyl]indolin-2-one}.
^e (*Z*)-4-[2-(Dipropylamino)ethyl]-3-propylideneindolin-2-one.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP Reference Standards** <11>
USP Ropinirole Hydrochloride RS

USP Ropinirole Related Compound B RS

4-[2-(Dipropylamino)ethyl]indoline-2,3-dione hydrochloride.

 $C_{16}H_{22}N_2O_2 \cdot HCl$ 310.82

■ 2S (USP39)

BRIEFING

Solifenacin Succinate. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated test methods, is proposed.

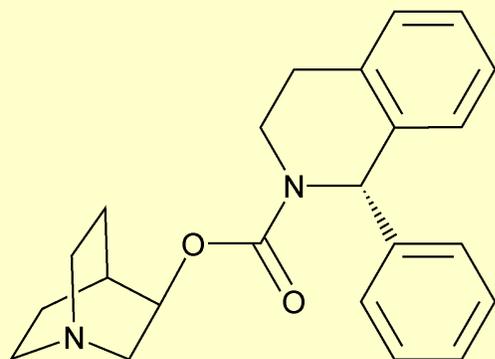
1. The liquid chromatographic procedures used in the *Assay* are based on analyses performed with the Zorbax SB Phenyl brand of *L11* column. The typical retention time for solifenacin is about 5 min.
2. The liquid chromatographic procedures used in the test for *Organic Impurities* are based on analyses performed with the Zorbax SB Phenyl brand of *L11* column. The typical retention time for solifenacin is about 15.5 min.
3. The test for *Stereoisomer Impurities* is adopted from the Solifenacin Succinate proposal published in *Pharmeuropa* 26.2. The liquid chromatographic procedure is based on analyses performed with the Chiralpak AD-H brand of *L51* column. The typical retention time for solifenacin is about 20 min.

This monograph is contingent on FDA approval of a product that meets the proposed monograph.

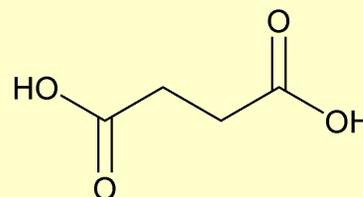
(CHM3: R.S. Prasad, E. Gonikberg.)

Correspondence Number—C134276

Comment deadline: November 30, 2015

Add the following:**► Solifenacin Succinate**

$C_{23}H_{26}N_2O_2 \cdot C_4H_6O_4$ 480.55



Butanedioic acid, compound with (1*S*)-(3*R*)-1-azabicyclo[2.2.2]oct-3-yl 3,4-dihydro-1-phenyl-2(1*H*)-isoquinolinecarboxylate (1:1);

(*R*)-Quinuclidin-3-yl (*S*)-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate succinate salt (1:1). [242478-38-2].

Solifenacin $C_{23}H_{26}N_2O_2$ 362.46

[242478-37-1].

DEFINITION

Solifenacin Succinate contains NLT 98.0% and NMT 102.0% of solifenacin succinate ($C_{23}H_{26}N_2O_2 \cdot C_4H_6O_4$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification for Succinate**
 - Solution A:** 200 mg/mL of *cupric sulfate* in water
 - Solution B:** *Solution A* and *pyridine* (5:1)
 - Sample solution:** 10 mg/mL of Solifenacin Succinate in water
 - Analysis:** To 10 mL of the *Sample solution* add 1 mL of 1 N *sodium hydroxide* and filter. Add 1 mL of *Solution B* dropwise to the filtrate.
 - Acceptance criteria:** A precipitate is formed in the blue solution within 1 min.

ASSAY

- **Procedure**

Buffer: Dissolve 4.1 g of *monobasic potassium phosphate* in 1 L of water and add 2 mL of *triethylamine*. Adjust with *phosphoric acid* to a pH of 2.5.

Mobile phase: *Acetonitrile*, *methanol*, and *Buffer* (30:30:40)

Standard solution: 0.2 mg/mL of USP Solifenacin Succinate RS in *Mobile phase*

Sample solution: 0.2 mg/mL of Solifenacin Succinate in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5- μ m packing *L11*

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of solifenacin succinate ($C_{23}H_{26}N_2O_2 \cdot C_4H_6O_4$) in the portion of Solifenacin Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of solifenacin from the *Sample solution*

r_S = peak response of solifenacin from the *Standard solution*

C_S = concentration of USP Solifenacin Succinate RS in the *Standard solution* (mg/mL)

C_U = concentration of Solifenacin Succinate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

- **Organic Impurities**

Buffer: Dissolve 4.1 g of *monobasic potassium phosphate* in 1 L of water and add 1 mL of *triethylamine*. Adjust with *phosphoric acid* to a pH of 3.3.

Solution A: *Acetonitrile*, *methanol*, and *Buffer* (15:15:70)

Solution B: *Acetonitrile*, *methanol*, and *Buffer* (35:35:30)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
5	75	25
25	30	70
50	35	65
55	75	25
60	75	25

Diluent: *Acetonitrile* and *Buffer* (50:50)

System suitability solution: 0.5 mg/mL of USP Solifenacin Identification Mixture A RS in *Diluent*

Standard solution: 0.0015 mg/mL of USP Solifenacin Succinate RS in *Diluent*

Sample solution: 1.5 mg/mL of Solifenacin Succinate in *Diluent*

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between the phenyldihydroisoquinoline and phenyltetrahydroisoquinoline peaks; NLT 2.0 between the solifenacin and phenethylbenzamide peaks, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Solifenacin Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of solifenacin from the *Standard solution*

C_S = concentration of USP Solifenacin Succinate RS in the *Standard solution* (mg/mL)

C_U = concentration of Solifenacin Succinate in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: For individual impurities, see *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Succinic acid	0.17	—	disregard
Phenyldihydroisoquinoline	0.33	0.78	0.10
Phenyltetrahydroisoquinoline	0.38	1.1	0.10
Solifenacin	1.0	—	—
Phenethylbenzamide	1.12	2.0	0.10
Solifenacin <i>N</i> -oxide	1.63	1.0	0.10
Phenyltetrahydroisoquinoline ethyl carbamate	2.21	1.9	0.10
Any individual unspecified impurity	—	1.0	0.10

• Stereoisomer Impurities

Mobile phase: *Chromatographic n-Heptane, absolute alcohol, and diethylamine* (800:200:1)

System suitability solution: 2 mg/mL of USP Solifenacin Identification Mixture B RS in *Mobile phase*

Standard solution: 0.002 mg/mL of USP Solifenacin Succinate RS in *Mobile phase*

Sample solution: 2 mg/mL of Solifenacin Succinate in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-μm packing *L51*

Flow rate: 0.8 mL/min

Injection volume: 10 μ L

Run time: 1.5 times the retention time of solifenacin

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 3* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between the solifenacin *R,S*-enantiomer and solifenacin *S,S*-diastereomer peaks and NLT 2.0 between the solifenacin *R,R*-enantiomer and solifenacin peaks, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each stereoisomer impurity in the portion of Solifenacin Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each stereoisomer impurity from the *Sample solution*

r_S = peak response of solifenacin from the *Standard solution*

C_S = concentration of USP Solifenacin Succinate RS in the *Standard solution* (mg/mL)

C_U = concentration of Solifenacin Succinate in the *Sample solution* (mg/mL)

Acceptance criteria: For individual stereoisomer impurities, see *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Solifenacin <i>R,S</i> -enantiomer	0.67	0.10
Solifenacin <i>S,S</i> -diastereomer	0.73	0.15
Solifenacin <i>R,R</i> -diastereomer	0.84	0.10
Solifenacin	1.00	—

SPECIFIC TESTS

- **Loss on Drying** (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers and protect from light. Store at a temperature NMT 30°.
- **USP Reference Standards** (11)
USP Solifenacin Succinate RS

USP Solifenacin Identification Mixture A RS

It contains Solifenacin Succinate and small amounts of the following impurities:

Phenethylbenzamide;

N-Phenethylbenzamide.

$C_{15}H_{15}NO$ 225.29

Phenyldihydroisoquinoline;

1-Phenyl-3,4-dihydroisoquinoline.

$C_{15}H_{13}N$ 207.28

Phenyltetrahydroisoquinoline;

S-1-Phenyl-1,2,3,4-tetrahydroisoquinoline.

$C_{15}H_{15}N$ 209.29

Phenyltetrahydroisoquinoline ethyl carbamate;

Ethyl (*S*)-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate.

$C_{18}H_{19}NO_2$ 281.35

Solifenacin *N*-oxide;

(*R*)-3-{[(*S*)-1-Phenyl-1,2,3,4-tetrahydroisoquinoline-2-carbonyl]oxy}quinuclidine 1-oxide.

$C_{23}H_{26}N_2O_3$ 378.46

USP Solifenacin Identification Mixture B RS

It contains Solifenacin Succinate and small amounts of the following impurities:

Solifenacin *R,R*-diastereomer;

(*R*)-Quinuclidin-3-yl (*R*)-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate hydrochloride.

$C_{23}H_{26}N_2O_2 \cdot HCl$ 398.93

Solifenacin *R,S*-enantiomer;

(*S*)-Quinuclidin-3-yl (*R*)-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate succinate.

$C_{23}H_{26}N_2O_2 \cdot C_4H_6O_4$ 480.55

Solifenacin *S,S*-diastereomer;

(*S*)-Quinuclidin-3-yl (*S*)-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate hydrochloride.

$C_{23}H_{26}N_2O_2 \cdot HCl$ 398.93

◀(TBD)

BRIEFING

Sulconazole Nitrate, USP 38 page 5371. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Add *Identification* test C based on the HPLC retention time agreement for sulconazole from the proposed Assay.
2. Replace the existing HPLC Assay procedure with a validated stability-indicating reversed-phase UPLC method similar to the proposed *Organic Impurities* procedure. The proposed liquid chromatographic procedure is based on analyses performed with the Acquity UPLC BEH C18 brand of L1 column. The typical retention time for sulconazole nitrate is about 5.3 min.
3. Replace the current *Ordinary Impurities* method with a UHPLC procedure. The proposed liquid chromatographic procedure is used in the proposed Assay.
4. Add the new Reference Standard, USP Sulconazole Related Compound A RS, introduced

by the proposed UPLC procedure for *Organic Impurities*, to the *USP Reference Standards* section.

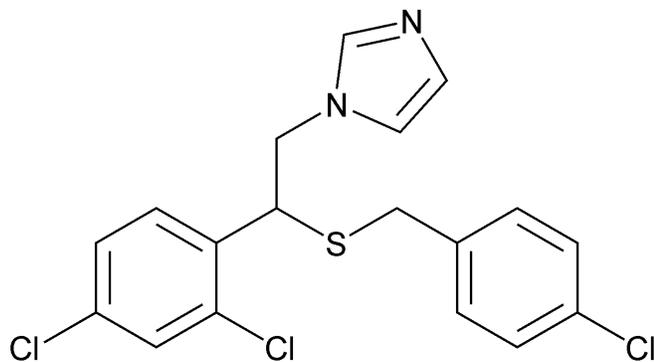
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM1: C. Anthony.)

Correspondence Number—C120149

Comment deadline: November 30, 2015

Sulconazole Nitrate



• HNO₃

C₁₈H₁₅Cl₃N₂S·HNO₃ 460.76

1*H*-Imidazole, 1-[2-[[[(4-chlorophenyl)methyl]thio]-2-(2,4-dichlorophenyl)ethyl]-, mononitrate, (±)-;
(±)-1-[2,4-Dichloro-β-[(*p*-chlorobenzyl)thio]phenethyl]imidazole mononitrate [61318-91-0].

DEFINITION

Sulconazole Nitrate contains NLT 98.0% and NMT 102.0% of sulconazole nitrate (C₁₈H₁₅Cl₃N₂S·HNO₃), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B. Identification Tests—General** (191), *Nitrate*: Meets the requirements for the ferrous sulfate–sulfuric acid test

Add the following:

- • **C.** The retention time of the major peak for sulconazole of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■_{2S} (USP39)

ASSAY

Change to read:

- **Procedure**

Mobile phase: Dissolve 1.9 g of sodium 1-pentane sulfonate in 300 mL of water and add 700 mL of methanol. Adjust with 2-N sulfuric acid to an apparent pH of 3.8 ± 0.1, filter, and degas.

Standard solution: ~~0.2 mg/mL of USP Sulconazole Nitrate RS in Mobile phase~~

Sample solution: ~~0.2 mg/mL of Sulconazole Nitrate in Mobile phase~~

Chromatographic system

~~(See Chromatography (621), System Suitability.)~~

Mode: ~~LC~~

Detector: ~~UV 230 nm~~

Column: ~~4.6 mm × 25 cm; packing L1~~

Column temperature: ~~40 ± 1.0°~~

Flow rate: ~~2 mL/min~~

Injection volume: ~~10 µL~~

System suitability

Sample: ~~Standard solution~~

Suitability requirements

Column efficiency: ~~NLT 1500 theoretical plates~~

Tailing factor: ~~NMT 2.3~~

Relative standard deviation: ~~NMT 1.5%~~

Analysis

Samples: ~~Standard solution and Sample solution~~

Calculate the percentage of sulconazole nitrate ($C_{18}H_{15}Cl_3N_2S \cdot HNO_3$) in the portion of Sulconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the ~~Sample solution~~

r_S = peak response from the ~~Standard solution~~

C_S = concentration of USP Sulconazole Nitrate RS in the ~~Standard solution~~ (mg/mL)

C_U = concentration of Sulconazole Nitrate in the ~~Sample solution~~ (mg/mL)

Acceptance criteria: ~~98.0%–102.0% on the dried basis~~

- **Buffer:** Dissolve 7.7 g of ammonium acetate in 992 mL of water and add 3.3 mL of glacial acetic acid. Pass through a suitable filter of 0.45-µm pore size under vacuum and degas with helium purging.

Solution A: Methanol, water, and Buffer (200:640:160)

Solution B: Methanol and acetonitrile (40:60)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	60	40
8.0	10	90
8.1	60	40
10.0	60	40

Diluent: Methanol and water (60:40)

Standard solution: 0.15 mg/mL of USP Sulconazole Nitrate RS in *Diluent*, prepared as follows. Transfer an appropriate quantity of USP Sulconazole Nitrate RS to a suitable volumetric flask, dissolve in 80% of the flask volume of *Diluent*, sonicate until dissolved, and dilute with *Diluent* to volume.

Sample solution: 0.15 mg/mL of Sulconazole Nitrate in *Diluent*, prepared as follows. Transfer an appropriate quantity of Sulconazole Nitrate to a suitable volumetric flask, dissolve in 80% of the flask volume of *Diluent*, sonicate until dissolved, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: 230 nm

Column: 2.1-mm × 10-cm; 1.7-μm packing L1

Flow rate: 0.5 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sulconazole nitrate ($C_{18}H_{15}Cl_3N_2S \cdot HNO_3$) in the portion of Sulconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sulconazole Nitrate RS in the *Standard solution* (mg/mL)

C_U = concentration of Sulconazole Nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ■_{2S} (USP39)

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

Delete the following:

- • **Ordinary Impurities** (466)

Standard solutions: Separate solutions of 0.02 mg/mL, 0.1 mg/mL, 0.2 mg/mL, and 0.4 mg/mL of USP Sulconazole Nitrate RS in a mixture of dichloromethane and methanol (2:1)

Sample solution: 20 mg/mL in a mixture of dichloromethane and methanol (2:1)

Eluant: Methylene chloride, cyclohexane, and diethylamine (50:45:5)

Visualization: 22

Acceptance criteria: Meets the requirements ■ 2S (USP39)

Add the following:

- • **Organic Impurities**

Buffer, Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system:

Proceed as directed in the *Assay*.

Standard solution: 1.5 µg/mL each of USP Sulconazole Nitrate RS and USP Sulconazole Related Compound A RS in *Diluent*, prepared as follows. Transfer appropriate quantities of USP Sulconazole Nitrate RS and USP Sulconazole Related Compound A RS to a suitable volumetric flask, dissolve in 80% of the flask volume of *Diluent*, sonicate until dissolved, and dilute with *Diluent* to volume.

Sample solution: 1.5 mg/mL of Sulconazole Nitrate in *Diluent*, prepared as follows. Transfer an appropriate quantity of Sulconazole Nitrate to a suitable volumetric flask, dissolve in 80% of the flask volume of *Diluent*, sonicate until dissolved, and dilute with *Diluent* to volume.

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Relative standard deviation: NMT 2.0% for sulconazole nitrate and sulconazole related compound A

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sulconazole related compound A in the portion of Sulconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sulconazole related compound A from the *Sample solution*

r_S = peak response of sulconazole related compound A from the *Standard solution*

C_S = concentration of USP Sulconazole Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Sulconazole Nitrate in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Sulconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_S = peak response of sulconazole nitrate from the *Standard solution*

C_S = concentration of USP Sulconazole Nitrate RS in the *Standard solution* (mg/mL)

C_U = concentration of Sulconazole Nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sulconazole related compound A	0.61	0.1
Sulconazole nitrate	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

■ 2S (USP39)

SPECIFIC TESTS

- **Loss on Drying** (731)

Analysis: Dry under vacuum at 80° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light.

Change to read:

- **USP Reference Standards** (11)

USP Sulconazole Nitrate RS

- **USP Sulconazole Related Compound A RS**

1-[2-(4-Chlorobenzyl)sulfinyl-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.

C₁₈H₁₅Cl₃N₂OS 413.74 ■ 2S (USP39)

BRIEFING

Sulfisoxazole, *USP 38* page 5407. As part of USP monograph modernization efforts, it is proposed to make the following changes:

1. Replace the current *Identification* test *C* that uses wet chemistry with a retention time agreement based on the proposed method in the *Assay*.
2. Replace the current *Assay* titration procedure with a stability-indicating HPLC method. The proposed liquid chromatographic procedure is based on analyses using the Zorbax Eclipse Plus C18 brand of L1 column. The typical retention time for sulfisoxazole is about 4 min.
3. Revise the acceptance criterion in the *Definition* and the *Assay* from NLT 99.0% and

NMT 101.0% to NLT 98.0% and NMT 102.0%, which is typical for chromatographic procedures.

4. Replace the test for *Ordinary Impurities* with an HPLC procedure using the same HPLC parameters as in the proposed *Assay* method.
5. Delete the *Melting Range or Temperature* test. The proposed test for *Organic Impurities* is sufficient to monitor the purity of the drug substance.

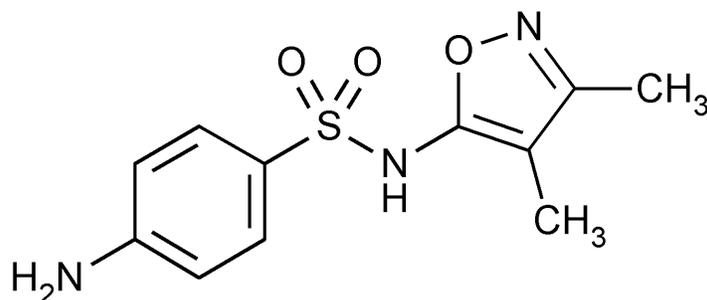
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM1: D.A. Porter.)

Correspondence Number—C149857

Comment deadline: November 30, 2015

Sulfisoxazole



$C_{11}H_{13}N_3O_3S$ 267.31

Benzenesulfonamide, 4-amino-*N*-(3,4-dimethyl-5-isoxazolyl)-;
*N*¹-(3,4-Dimethyl-5-isoxazolyl)sulfanilamide [127-69-5].

DEFINITION

Change to read:

Sulfisoxazole contains ~~NLT 99.0% and NMT 101.0%~~

■ NLT 98.0% and NMT 102.0% of sulfisoxazole ■ 2S (USP39)

($C_{11}H_{13}N_3O_3S$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.**

Sample: 100 mg of Sulfisoxazole

Analysis: Prepare a solution (1 in 100,000) in *pH 7.5 Phosphate Buffer* (see *Reagents, Indicators, and Solutions—Buffer Solutions*) by dissolving the *Sample* in 10 mL of *sodium hydroxide* solution (1 in 250) and adding sufficient quantities of the buffer solution to make 100 mL, then diluting 10 mL of the resulting solution with the buffer solution to 1000 mL.

Acceptance criteria: The UV absorption spectrum exhibits maxima and minima at the same

wavelengths as that of a similar solution of USP Sulfisoxazole RS, concomitantly measured.

Delete the following:

■ ● **C. Procedure**

Sample: 10 mg of Sulfisoxazole

Analysis: Dissolve the *Sample* in 2 mL of 3 N *hydrochloric acid* with the aid of heat, and cool for 5 min in an ice bath. Add 3 drops of *sodium nitrite* solution (1 in 100), and dilute with water to 4 mL.

Acceptance criteria: A yellow solution is produced. Add 1 mL of *sodium hydroxide* (1 in 10) containing 10 mg of *2-naphthol*; an orange-red precipitate is formed. ■_{2S} (USP39)

Add the following:

- ● **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■_{2S} (USP39)

ASSAY

Change to read:

● **Procedure**

Sample: 800 mg of Sulfisoxazole

Analysis: Dissolve the *Sample* in 50 mL of *dimethylformamide*, shake thoroughly to dissolve the solid, and add 5 drops of a solution (1 in 100) of *thymol blue* in *dimethylformamide*. Titrate with 0.1 N lithium methoxide in toluene VS to a blue endpoint, taking precautions against absorption of atmospheric carbon dioxide. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N lithium methoxide is equivalent to 26.73 mg of $C_{11}H_{13}N_3O_3S$.

Acceptance criteria: 99.0%–101.0% on the dried basis

■ **Solution A:** 0.1% (v/v) *phosphoric acid* in water

Mobile phase: *Spectrophotometric acetonitrile* and *Solution A* (35:65)

Diluent: *Spectrophotometric acetonitrile* and water (50:50)

Standard solution: 0.1 mg/mL of USP Sulfisoxazole RS in *Diluent*. Sonicate to dissolve prior to final dilution.

Sample solution: 0.1 mg/mL of sulfisoxazole in *Diluent*. Sonicate to dissolve prior to final dilution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 248 nm

Column: 4.6-mm × 15-cm; 3.5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

Run time: At least 1.5 times the retention time of sulfisoxazole

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sulfisoxazole ($C_{13}H_{15}N_3O_4S$) in the portion of Sulfisoxazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sulfisoxazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Sulfisoxazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis $\pm 2S$ (USP39)

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

- **Selenium** (291)

Sample: 200 mg

Acceptance criteria: NMT 30 ppm

Delete the following:

- ~~**Heavy Metals, Method II** (231): NMT 20 ppm~~ (Official 1-Dec-2015)

Delete the following:

- ● ~~**Ordinary Impurities** (466)~~

~~**Standard solution:** *Ethyl acetate*~~

~~**Sample solution:** *Ethyl acetate*~~

~~**Eluant:** A mixture of *acetone, cyclohexane, and glacial acetic acid (5:4:1)*~~

~~**Visualization:** $\pm 2S$ (USP39)~~

Add the following:

- ● **Organic Impurities**

Solution A, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 1 $\mu\text{g/mL}$ of USP Sulfisoxazole RS in *Diluent*.

[Note—Sonicate if necessary to dissolve.]

Sample solution: 1 mg/mL of Sulfisoxazole in *Diluent*.

[Note—Sonicate if necessary to dissolve.]

System suitability

Sample: *Standard solution*

[Note—See *Table 1* for relative retention times.]

Suitability requirements

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Sulfisoxazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sulfisoxazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Sulfisoxazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sulfisoxazole acetyl related compound A ^{a,b}	0.54	—
Sulfisoxazole	1	—
Total impurities	—	2.0

^a 5-Amino-3,4-dimethylisoxazole.

^b Included for identification purposes. Quantities of this impurity will be counted as part of the total impurities.

■ 2S (USP39)

SPECIFIC TESTS

Delete the following:

■ ● ~~Melting Range or Temperature (741): 194°–199°~~ ■ 2S (USP39)

● **Loss on Drying** (731)

Analysis: Dry a sample at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

● **Packaging and Storage:** Preserve in tight, light-resistant containers.

● **USP Reference Standards** (11)

USP Sulfisoxazole RS

BRIEFING

Sulfisoxazole Acetyl, *USP 38* page 5408. As part of USP monograph modernization efforts,

it is proposed to make the following changes:

1. Replace the current *Assay* titration procedure with a stability-indicating liquid chromatographic method. The proposed liquid chromatographic procedure is based on analyses using the Zorbax Eclipse Plus C18 brand of L1 column. The typical retention time for sulfisoxazole acetyl is about 4 min.
2. Revise the acceptance criterion in the *Definition* and the *Assay* from NLT 98.0% and NMT 100.5% to NLT 98.0% and NMT 102.0%, which is typical for chromatographic procedures.
3. Add a liquid chromatographic procedure to the *Organic Impurities* test that is consistent with the proposed *Assay* method.
4. Delete the *Melting Range or Temperature* test. The proposed selective test for *Organic Impurities* is sufficient to monitor the purity of the drug substance.
5. Delete the *Ordinary Impurities* test. The proposed selective test for *Organic Impurities* is sufficient to monitor the impurities in the drug substance.

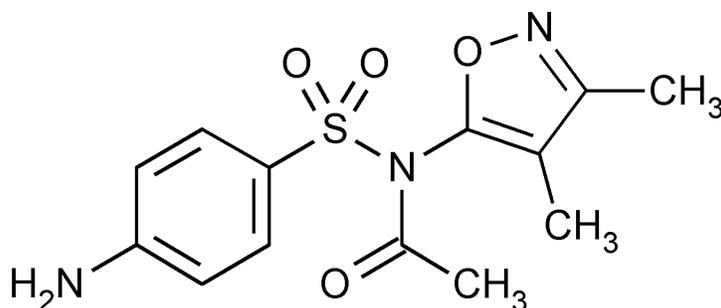
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM1: D.A. Porter.)

Correspondence Number—C127201

Comment deadline: November 30, 2015

Sulfisoxazole Acetyl



$C_{13}H_{15}N_3O_4S$ 309.34

Acetamide, *N*-[(4-aminophenyl)sulfonyl]-*N*-(3,4-dimethyl-5-isoxazolyl)-; *N*-(3,4-Dimethyl-5-isoxazolyl)-*N*-sulfanilylacamide. [80-74-0].

DEFINITION

Change to read:

Sulfisoxazole Acetyl contains NLT 98.0% and NMT ~~100.5%~~

■ 102.0% ■ 2S (*USP39*)

of sulfisoxazole acetyl ($C_{13}H_{15}N_3O_4S$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B. Ultraviolet Absorption** (197U)

Analytical wavelength: 290 nm

Sample solution: 10 µg/mL in alcohol

Acceptance criteria: NMT 3.0%, calculated on the dried basis

ASSAY

Change to read:

- **Procedure**

Sample: ~~1 g~~

Analysis: ~~Dissolve the *Sample* in 15 mL of glacial acetic acid, then add 25 mL of hydrochloric acid and 80 mL of water. Proceed as directed under *Nitrite Titration* (451), beginning with "Cool to about 15°". Each mL of 0.1 M sodium nitrite is equivalent to 30.94 mg of $C_{13}H_{15}N_3O_4S$.~~

Acceptance criteria: ~~98.0%–100.5% on the dried basis~~

▪ **Solution A:** 0.1% (v/v) phosphoric acid in water

Mobile phase: Acetonitrile and *Solution A* (45:55)

Diluent: Acetonitrile and water (75:25)

Standard solution: 0.1 mg/mL of USP Sulfisoxazole Acetyl RS in *Diluent*. Sonicate to dissolve prior to final dilution. Store at 4°.

Sample solution: 0.1 mg/mL of Sulfisoxazole Acetyl in *Diluent*. Sonicate to dissolve prior to final dilution. Store at 4°.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 272 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Autosampler temperature: 4°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

Run time: 2 times the retention time of sulfisoxazole acetyl

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sulfisoxazole acetyl ($C_{13}H_{15}N_3O_4S$) in the portion of Sulfisoxazole Acetyl taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sulfisoxazole Acetyl RS in the *Standard solution* (mg/mL)

C_U = concentration of Sulfisoxazole Acetyl in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%, calculated on the dried basis ■ 2S (USP39)

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

- **Selenium** (291)

Sample: 200 mg

Acceptance criteria: NMT 30 ppm

Delete the following:

- ~~**Heavy Metals, Method II** (231): NMT 20 ppm~~ (Official 1-Dec-2015)

Change to read:

- **Organic Impurities**

~~**Ordinary Impurities** (466)~~

~~**Sample solution:** Methanol~~

~~**Standard solution:** Methanol~~

~~**Eluant:** Acetone and toluene (1:1)~~

~~**Visualization:** 1~~

■ **Solution A:** 0.1% (v/v) phosphoric acid in water

Mobile phase: Acetonitrile and *Solution A* (45:55)

Diluent: Acetonitrile and water (75:25)

Standard solution: 1 µg/mL of USP Sulfisoxazole Acetyl RS in *Diluent*. Sonicate if necessary to dissolve. Store at 4°.

Sample solution: Proceed as directed in the *Assay*.

Chromatographic system: Proceed as directed in the *Assay*, except for the *Detector*.

Detector: UV 248 nm

System suitability

Sample: *Standard solution*

[Note—See *Table 1* for relative retention times.]

Suitability requirements

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Sulfisoxazole Acetyl taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sulfisoxazole Acetyl RS in the *Standard solution* (mg/mL)

C_U = concentration of Sulfisoxazole Acetyl in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sulfisoxazole acetyl related compound A ^{a,b}	0.5	—
Sulfisoxazole ^c	0.7	—
Sulfisoxazole acetyl	1.0	—
Total impurities	—	2.0

^a 5-Amino-3,4-dimethylisoxazole.
^b Included for identification purposes. Quantities of this impurity will be counted as part of the total impurities.
^c Benzenesulfonamide, 4-amino-*N*-(3,4-dimethyl-5-isoxazolyl)-; *N*¹-(3,4-Dimethyl-5-isoxazolyl)sulfanilamide.

■ 2S (USP39)

SPECIFIC TESTS

Delete the following:

■ ● **Melting Range or Temperature** (741): 192°–195° ■ 2S (USP39)

● **Loss on Drying** (731)

Sample: Dry a sample at 105° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

● **Packaging and Storage:** Preserve in tight, light-resistant containers.

● **USP Reference Standards** (11)

USP Sulfisoxazole Acetyl RS

BRIEFING

Theophylline Oral Solution, USP 38 page 5527. As a part of USP monograph modernization efforts, the following revisions are proposed:

1. Replace the TLC procedure in *Identification* test A with a procedure in which the UV

spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the proposed *Assay*.

2. Replace the HPLC procedure in the *Assay* with the same procedure that is proposed in the added test for *Organic Impurities*. The liquid chromatographic procedure is performed using the Acquity BEH C8 brand of L7 column. The typical retention time for theophylline is about 3.8 min.
3. Add a stability-indicating liquid chromatographic procedure for the *Organic Impurities* test.
4. Revise the storage requirement in the *Packaging and Storage* section to be consistent with FDA-approved drug products.
5. Add four Reference Standards, USP Theophylline Related Compound D RS (a known degradant), USP Saccharin Sodium RS (potential excipient, for use in establishing system suitability), USP Alcohol Determination—Acetonitrile RS, and USP Alcohol Determination—Alcohol RS to the *USP Reference Standards* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM5: D.A. Porter.)

Correspondence Number—C143817

Comment deadline: November 30, 2015

Theophylline Oral Solution

DEFINITION

Theophylline Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of theophylline ($C_7H_8N_4O_2$).

IDENTIFICATION

Delete the following:

~~A. Thin-Layer Chromatographic Identification Test (201)~~

~~Standard solution:~~ 1 mg/mL of USP Theophylline RS in methanol

~~Sample solution:~~ Nominally 1 mg/mL of theophylline prepared as follows. Transfer a suitable quantity of Oral Solution, equivalent to 100 mg of theophylline, to a separatory funnel. Extract with two 25-mL portions of chloroform, collecting the extracts in a 100-mL volumetric flask. Dilute with methanol to volume.

~~Application volume:~~ 20 μ L

~~Developing solvent system:~~ Chloroform, methanol, and acetic acid (89:10:1)

~~Analysis~~

~~Samples:~~ *Standard solution* and *Sample solution*

Apply the *Standard solution* and the *Sample solution* as directed in the chapter, and dry the plate in a current of cool air. Place the plate in a suitable chromatographic chamber lined with filter paper and previously equilibrated with the *Developing solvent system*. Upon removing the plate from the chamber, dry with a current of warm air in a suitable hood.

Acceptance criteria: Meets the requirements ■_{2S} (USP39)

Add the following:

- ● **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■_{2S} (USP39)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

● **Procedure**

Mobile phase: ~~Methanol, acetic acid, and water (22.5: 1: 76.5) containing 0.2 g/L of sodium 1-octanesulfonate~~

System suitability solution: ~~0.68 mg/mL each of USP Theophylline RS and caffeine in water~~

Standard solution: ~~0.68 mg/mL of USP Theophylline RS~~

Sample solution: ~~Nominally 0.68 mg/mL of theophylline prepared as follows. Transfer a suitable quantity of Oral Solution, equivalent to 68 mg of theophylline, to a 100-mL volumetric flask. Dilute with water to volume.~~

Chromatographic system

~~(See Chromatography (621), System Suitability.)~~

Mode: ~~LC~~

Detector: ~~UV 254 nm~~

Column: ~~4.0 mm × 30 cm; packing L1~~

Flow rate: ~~2.0 mL/min~~

Injection volume: ~~10 µL~~

System suitability

Sample: ~~System suitability solution~~

~~[Note—The relative retention times for theophylline and caffeine are about 0.6 and 1.0, respectively.]~~

Suitability requirements

Resolution: ~~NLT 2.0 between theophylline and caffeine~~

Tailing factor: ~~NMT 2~~

Relative standard deviation: ~~NMT 2.0%~~

- **Solution A:** 10 mM *ammonium acetate* prepared as follows. Transfer 771 mg/L of *ammonium acetate* to a suitable flask, and dissolve in water. Adjust with *glacial acetic acid* to a pH of 5.4 and dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.

Solution B: *Methanol*

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	93.5	6.5
2.5	93.5	6.5
5.0	10	90
5.1	93.5	6.5
7.0	93.5	6.5

Standard solution: 0.2 mg/mL of USP Theophylline RS in water

Sample solution: Nominally 0.2 mg/mL of theophylline from Oral Solution in water. Centrifuge and use the supernatant.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm. For *Identification* test A, use a photodiode array detector in the range of 210–400 nm.

Column: 2.1-mm × 10-cm; 1.7- μ m packing L7

Column temperature: 40 \pm 2°

Flow rate: 0.4 mL/min

Injection volume: 1 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 1.0%

■ 2S (USP39)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline (C₇H₈N₄O₂) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline from the *Sample solution*

r_S = peak response of theophylline from the *Standard solution*

C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

Add the following:**Organic Impurities**

Solution A, Solution B, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

Impurity stock solution: 0.1 mg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS in water

System suitability solution: 10 µg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS from *Impurity stock solution*, and 10 µg/mL of USP Saccharin Sodium RS in water

Standard solution: 2.0 µg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS from *Impurity stock solution* in water

Sample solution: Nominally 1.0 mg/mL of theophylline from a portion of Oral Solution in water. Centrifuge and use the supernatant.

System suitability

Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between theophyllidine and saccharin, *System suitability solution*

Relative standard deviation: NMT 3.0% for theophylline and theophylline related compound D peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of theophylline related compound D in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline related compound D from the *Sample solution*

r_S = peak response of theophylline related compound D from the *Standard solution*

C_S = concentration of USP Theophylline Related Compound D RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified degradation product from the *Sample solution*

r_S = peak response of theophylline from the *Standard solution*

C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound D	0.45	0.2
Saccharin ^a	0.50	—
Theophylline	1.0	—
Any other individual unspecified degradation product	—	0.2
Total impurities	—	0.5

^a Included as a potential excipient. Do not include in the calculation of total impurities.

■ 2S (USP39)

SPECIFIC TESTS

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*. The total aerobic microbial count does not exceed 1×10^2 cfu/mL, and the total combined molds and yeasts count does not exceed 5×10^1 cfu/mL.
- **pH** (791): 3.0–4.7
- **Alcohol Determination** (611), *Method II* (if present)
 - Analysis:** Use acetone as the internal standard.
 - Acceptance criteria:** 90.0%–115.0% of the labeled amount of alcohol (C₂H₅OH)

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat
 - and store at controlled room temperature. ■ 2S (USP39)
- **Labeling:** Label it to indicate the alcohol content (if present).

Change to read:

- **USP Reference Standards** (11)
 - USP Alcohol Determination—Acetonitrile RS
 - USP Alcohol Determination—Alcohol RS
 - USP Saccharin Sodium RS
- 2S (USP39)
 - USP Theophylline RS
 - USP Theophylline Related Compound D RS
 - Theophyllidine;
 - N*-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide.
 - C₆H₁₀N₄O 154.17

■ 2S (USP39)

Trihexyphenidyl Hydrochloride Tablets, *USP 38* page 5688. As part of the USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Add a test for *Organic Impurities* with a stability-indicating HPLC procedure. The validation of the proposed HPLC procedure is performed using the Phenomenex Kinetex XB-C18 brand of L1 column. The typical retention time for trihexyphenidyl is about 15 min.
2. Replace the current HPLC procedure in the *Assay* with the one that has the same HPLC parameters as proposed in the test for *Organic Impurities*.
3. Replace *Identification* test *A*, which requires chloroform for extraction, with the UV spectrum agreement of the major peak from the proposed *Assay*.
4. Delete *Identification* test *B* since chloride identification is already required for the drug substance.
5. Change the designation of *Identification* test *C* to *Identification* test *B*.
6. Add additional storage requirements in the *Packaging and Storage* section based on the information in the drug product package insert.
7. Add a new Reference Standard to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM4: D. Min.)

Correspondence Number—C117812

Comment deadline: November 30, 2015

Trihexyphenidyl Hydrochloride Tablets

DEFINITION

Trihexyphenidyl Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$).

IDENTIFICATION

Delete the following:

■ ● ~~A.~~

~~**Sample:** Reduce a number of Tablets, equivalent to 20 mg of trihexyphenidyl hydrochloride, to a fine powder, and triturate with 25 mL of chloroform. Filter the mixture, and evaporate the filtrate to about 10 mL by gently heating. Add the solution to 100 mL of *n*-hexane: a white precipitate is formed. Allow the mixture to stand for 30 min, and collect the precipitate on a solvent-resistant membrane filter of 1- μ m pore size. Wash the crystals with a small portion of *n*-hexane, and allow them to air-dry.~~

~~**Acceptance criteria:** the IR absorption spectrum of a potassium bromide dispersion of the *Sample* so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Trihexyphenidyl Hydrochloride RS. ■ 2S (*USP39*)~~

Add the following:

- ● **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■_{2S} (USP39)

Delete the following:

- ● **B. Identification Tests—General, Chloride** (191): The precipitate obtained in *Identification test A* meets the requirements. ■_{2S} (USP39)

Change to read:● **C.**

- **B.** ■_{2S} (USP39)

The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**Change to read:**● **Procedure**

Mobile phase: ~~Acetonitrile, triethylamine and water (920: 0.2: 80). Adjust with phosphoric acid to a pH of 4.0.~~

Standard solution: 0.2 mg/mL of USP Trihexyphenidyl Hydrochloride RS in *Mobile phase*

Sample solution: Nominally 0.2 mg/mL of trihexyphenidyl hydrochloride in *Mobile phase* prepared as follows. Transfer 20 Tablets into a volumetric flask of appropriate size. Add a volume of 0.1 N hydrochloric acid equivalent to 10% of the capacity of the volumetric flask, and sonicate with occasional shaking until the Tablets have disintegrated. Add a volume of *Mobile phase* equivalent to about one-half of the capacity of the volumetric flask, sonicate with frequent shaking for 10 min, and shake by mechanical means for 10 min. Cool, dilute with *Mobile phase* to volume, mix, and filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6 mm × 8 cm; 3- μ m packing L1

Flow rate: 2 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1300 theoretical plates

Tailing factor: NMT 3.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: ~~Standard solution and Sample solution~~

Calculate the percentage of the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Trihexyphenidyl Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of trihexyphenidyl hydrochloride the *Sample solution* (mg/mL)

- **Solution A:** 1.4 g/L of *monobasic potassium phosphate* in water. Adjust with *phosphoric acid* to a pH of 4.0. Pass the solution through a suitable filter of 0.22- μ m pore size.

Solution B: 0.5 mL of *phosphoric acid* in 1 L of *acetonitrile*

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	60	40
22	60	40
22.1	95	5
24	95	5

Diluent: *Methanol* and water (80:20)

Standard solution: 0.1 mg/mL USP Trihexyphenidyl Hydrochloride RS in *Diluent*

Sample stock solution: Nominally 0.5 mg/mL of trihexyphenidyl hydrochloride prepared as follows. Transfer a suitable amount of Tablets (NLT 10), equivalent to 50 mg of trihexyphenidyl hydrochloride, to a 100-mL volumetric flask. Add 10 mL of 0.1 N *hydrochloric acid* and sonicate. Add another 80 mL of *Solution B* and sonicate again. Dilute with *Solution B* to volume. Centrifuge and use the supernatant.

Sample solution: Nominally 0.1 mg/mL of trihexyphenidyl hydrochloride in *Diluent* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm. For *Identification test A*, use a diode array detector in the range of 190–300 nm.

Column: 2.1-mm \times 10-cm; 2.6- μ m packing L1

Column temperature: 30°

Flow rate: 0.3 mL/min

Injection volume: 3.0 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 3.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trihexyphenidyl from the *Sample solution*

r_S = peak response of trihexyphenidyl from the *Standard solution*

C_S = concentration of USP Trihexyphenidyl Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of trihexyphenidyl hydrochloride in the *Sample solution* (mg/mL)

■ 2S (USP39)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

● Dissolution <711>

Medium: pH 4.5 acetate buffer, prepared by mixing ~~2.99 g~~

■ 3.0 g ■ 2S (USP39)

of *sodium acetate trihydrate*

■ 2S (USP39)

and ~~1.66 mL~~

■ 1.7 mL ■ 2S (USP39)

of *glacial acetic acid* with water to obtain 1000 mL of solution with a pH of 4.50 ± 0.05 ; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: A known concentration of USP Trihexyphenidyl Hydrochloride RS in *Medium*

Sample solution: Filtered portion of the solution under test

Bromocresol green solution: Dissolve 250 mg of bromocresol green in a mixture of 15 mL of water and 5 mL of 0.1 N *sodium hydroxide*, dilute with *Medium* to 500 mL, and mix. Extract 250 mL portions of this solution with two 100 mL portions of *chloroform* and discard the *chloroform* extracts.

Instrumental conditions**Mode:** UV-Vis**Analytical wavelength:** 415 nm**Blank:** *Medium***Analysis****Samples:** *Standard solution* and *Sample solution*

Transfer an accurately measured volume of the *Sample solution*, estimated to contain about 50 µg of trihexyphenidyl hydrochloride, to a 50-mL centrifuge tube. Transfer an equal, accurately measured volume of the *Standard solution* to a second 50-mL centrifuge tube. Transfer an equal, accurately measured volume of *Medium* to a third 50-mL centrifuge tube to provide a *Blank*. Add 5 mL of the *Bromocresol green solution* and 10.0 mL of *chloroform* to each tube, insert the stoppers into the tubes, and shake vigorously for NLT 20 s. Centrifuge the mixtures to separate the layers. Aspirate and discard the upper aqueous layers. Filter each *chloroform* layer through a separate phase-separating filter paper. Determine the amount of trihexyphenidyl hydrochloride (C₂₀H₃₁NO·HCl) dissolved from the absorbance of the filtrate from the *Sample solution* in comparison with that from the *Standard solution*. The filtrate from the *Blank* is used to set the instrument.

Tolerances: NLT 75% (Q) of the labeled amount of trihexyphenidyl hydrochloride (C₂₀H₃₁NO·HCl) is dissolved.

- **Uniformity of Dosage Units** (905): Meet the requirements

IMPURITIES**Add the following:****• Organic Impurities**

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 0.1 mg/mL of USP Trihexyphenidyl Hydrochloride RS and 0.01 mg/mL of USP Trihexyphenidyl Related Compound A RS in *Diluent*

Standard solution: 0.001 mg/mL of USP Trihexyphenidyl Hydrochloride RS in *Diluent*

Sample solution: Nominally 1 mg/mL of trihexyphenidyl hydrochloride in *Diluent* prepared as follows. Transfer a suitable amount of Tablets (NLT 10), equivalent to 50 mg of trihexyphenidyl hydrochloride, to a 50-mL volumetric flask. Add 10 mL of 0.1 N *hydrochloric acid* and sonicate. Add another 80 mL of *Solution B* and sonicate again. Dilute with *Solution B* to volume. Centrifuge and use the supernatant.

System suitability

Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 50, *Standard solution*

Analysis**Samples:** *Standard solution and Sample solution*

Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of trihexyphenidyl from the *Standard solution*

C_S = concentration of USP Trihexyphenidyl Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of trihexyphenidyl hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.10%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Trihexyphenidyl related compound A ^a	0.4	—
Trihexyphenidyl	1.0	—
Any individual unspecified degradation product	—	1
Total degradation products	—	2

^a This impurity included for identification only and is not to be included in the calculation of total degradation products.

■ 2S (USP39)

ADDITIONAL REQUIREMENTS**Change to read:**

- **Packaging and Storage:** Preserve in tight containers.
 - Store at controlled room temperature. ■ 2S (USP39)

Change to read:

- **USP Reference Standards** (11)

USP Trihexyphenidyl Hydrochloride RS

- USP Trihexyphenidyl Related Compound A RS

1-Phenyl-3-(piperidin-1-yl)propan-1-one hydrochloride.

C₁₄H₁₉NO·HCl 253.77 ■ 2S (USP39)

BRIEFING

Valproic Acid, *USP 38* page 5736. On the basis of comments received, the *Assay* procedure is revised to be consistent with the assay procedures in the *Valproic Acid Capsules* and the *Valproic Acid Oral Solution* monographs.

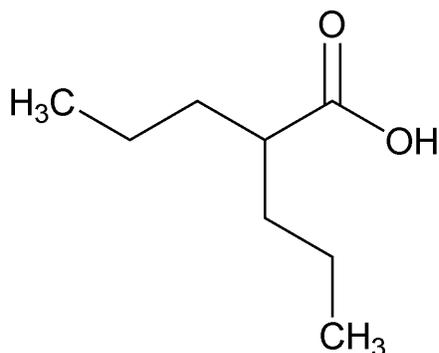
Additionally, minor editorial changes have been made to update the monograph to current *USP*

style.

(CHM4: W. Yang, R. Ravichandran.)
Correspondence Number—C160870

Comment deadline: November 30, 2015

Valproic Acid



$C_8H_{16}O_2$ 144.21

Pentanoic acid, 2-propyl-;
Propylvaleric acid [99-66-1].

DEFINITION

Valproic Acid contains NLT 98.0% and NMT 102.0% of valproic acid ($C_8H_{16}O_2$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** (197F)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

Buffer: 3.5 g/L of *monobasic sodium phosphate* in water. Adjust with *phosphoric acid* to a pH of 3.5.

Mobile phase: *Acetonitrile* and *Buffer*(50:50)

▪ (45:55)

Diluent: *Acetonitrile* and *water* (45:55) ■ 2S (USP39)

System suitability solution: 50 µg/mL of USP Valproic Acid Related Compound B RS and 0.5 mg/mL of USP Valproic Acid RS in *Mobile phase*

▪ *Diluent* ■ 2S (USP39)

Standard solution: 0.5 mg/mL of USP Valproic Acid RS in *Mobile phase*

▪ *Diluent* ■ 2S (USP39)

Sample solution: 0.5 mg/mL of Valproic Acid in Mobile phase

▪ **Diluent** 2S (USP39)

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15.0-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between valproic acid related compound B and valproic acid, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of valproic acid (C₈H₁₆O₂) in the portion of Valproic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Valproic Acid in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

Delete the following:

- **Heavy Metals, Method II** (231): NMT 20 ppm (Official 1-Dec-2015)

- **Organic Impurities**

System suitability solution: 0.1 µL/mL of USP Valproic Acid Related Compound A RS and 1.0 µL/mL each of butyric acid and valeric acid in Valproic Acid

Sample solution: Valproic Acid

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 60-m; coated with a 0.3-μm film of phase G25

Temperatures

Injection port: 240°

Detector: 260°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
145	0	145	48
145	5	190	—

Carrier gas: Helium

Flow rate: 150 mL/min

Injection volume: 0.5 μL

Injection type: Split flow ratio, 100:1

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for butyric acid, valeric acid, valproic acid, and valproic acid related compound A are 0.38, 0.52, 1.0, and 1.64, respectively.]

Suitability requirements

Resolution: NLT 23.0 between butyric acid and valeric acid

Column efficiency: NLT 100,000 theoretical plates for valeric acid

Tailing factor: NMT 1.5 for valeric acid

Retention time: The related compound A peak must elute between 41 and 50 min.

Peak area: The related compound A peak area must be NLT 0.01% relative to the valproic acid peak area.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Valproic Acid (C₈H₁₆O₂) taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity

r_T = sum of the responses for all the peaks

Acceptance criteria

Individual impurities: NMT 0.1%

Total impurities: NMT 0.3%

SPECIFIC TESTS

- **Water Determination** (921), *Method I*: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight glass, stainless steel, or polyethylene (HDPE) containers.

Change to read:

- **USP Reference Standards** (11)

USP Valproic Acid RS

USP Valproic Acid Related Compound A RS

▪ 2-Allylpent-4-enoic acid;

Also known as **2S** (USP39)

Diallylacetic acid.

$C_8H_{12}O_2$ 140.18

USP Valproic Acid Related Compound B RS

▪ 2-Isopropylpentanoic acid;

Also known as **2S** (USP39)

(2*RS*)-2-(1-Methylethyl)pentanoic acid.

$C_8H_{16}O_2$ 144.21

BRIEFING

Ziprasidone Capsules. Because there is no existing *USP* monograph for this drug product, a new monograph is proposed based on validated methods of analyses.

1. The isocratic liquid chromatographic procedure used in the *Assay* and *Dissolution* tests is based on validations performed with the Inertsil ODS 3V brand of L1 column manufactured by GL Sciences. The typical retention times for ziprasidone are about 15 and 10 min in the *Assay* and *Dissolution* tests, respectively.
2. The gradient liquid chromatographic procedure used in the *Organic Impurities* test is based on validations performed with the Inertsil ODS 3V brand of L1 column manufactured by GL Sciences. The typical retention time for ziprasidone is about 21 min.

(CHM4: R. Ravichandran.)

Correspondence Number—C133990

Comment deadline: November 30, 2015

Add the following:

- **Ziprasidone Capsules**

DEFINITION

Ziprasidone Capsules contain an amount of ziprasidone hydrochloride equivalent to NLT 90.0%

and NMT 110.0% of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• Procedure

Buffer: 0.3% (v/v) of *triethylamine* in water

Mobile phase: Acetonitrile and *Buffer* (35:65). Adjust with *glacial acetic acid* to a pH of 6.0.

Diluent: Acetonitrile, water, and *glacial acetic acid* (70:30:5)

Standard stock solution: 1.0 mg/mL of USP Ziprasidone Hydrochloride RS in *Diluent*

Standard solution: 0.2 mg/mL of USP Ziprasidone Hydrochloride RS from the *Standard stock solution* in *Mobile phase*

Sample stock solution: Nominally 1 mg/mL of ziprasidone prepared as follows. Empty the contents of NLT 20 Capsules into a container. Blend the contents. Transfer an amount of the contents, equivalent to NLT 50 mg of ziprasidone, to a suitable volumetric flask. Dissolve the contents in 60% of the flask volume of *Diluent*. Sonicate for NLT 5 min. Dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45- μ m pore size and use the filtrate to prepare the *Sample solution*.

Sample solution: Nominally 0.2 mg/mL of ziprasidone prepared from the filtered *Sample stock solution* and *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm. For *Identification* test B, a diode array detector may be used in the wavelength range of 200–300 nm.

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 2.0 mL/min

Injection volume: 20 μ L

Run time: 1.5 times the retention time of ziprasidone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of ziprasidone from the *Sample solution*

r_S = peak response of ziprasidone from the *Standard solution*

C_S = concentration of USP Ziprasidone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ziprasidone in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of ziprasidone free base, 412.94

M_{r2} = molecular weight of ziprasidone hydrochloride, 467.41 for the monohydrate, 449.40 for the anhydrous form

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Dissolution <711>

Tier I

pH 7.5 phosphate buffer: Dissolve 7.8 g of *monobasic sodium phosphate dihydrate* and 20 g of *sodium dodecylsulfate* in 1 L water. Sonicate to dissolve and adjust with *phosphoric acid* or *sodium hydroxide* to a pH of 7.5.

Medium: *pH 7.5 phosphate buffer*; 900 mL

Apparatus 2: 75 rpm. Use a suitable sinker, if necessary.

Time: 45 min

Buffer: 0.3% (v/v) of *triethylamine* in water, adjusted with *glacial acetic acid* to a pH of 6.0

Mobile phase: Acetonitrile and *Buffer* (45:55)

Diluent: Acetonitrile, water, and *glacial acetic acid* (70:30:5)

Standard stock solution: 0.24 mg/mL of USP Ziprasidone Hydrochloride RS prepared as follows. Dissolve a suitable amount of USP Ziprasidone Hydrochloride RS in a suitable volumetric flask first in 60% of the flask volume of *Diluent*, and dilute with *Diluent* to volume.

Standard solution: 0.024 mg/mL of USP Ziprasidone Hydrochloride RS in *Medium* from the *Standard stock solution*

Sample solution: Pass a portion of the solution through a suitable filter of 0.45- μ m pore size. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing *L1*

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

Run time: 1.5 times the retention time of ziprasidone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of ziprasidone from the *Sample solution*

r_S = peak response of ziprasidone from the *Standard solution*

C_S = concentration of USP Ziprasidone Hydrochloride RS in the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of *Medium*, 900 mL

M_{r1} = molecular weight of ziprasidone free base, 412.94

M_{r2} = molecular weight of ziprasidone hydrochloride, 467.41 for the monohydrate, 449.40 for the anhydrous form

Tolerances: NLT 75% (Q) of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$) is dissolved.

If the above tolerance cannot be met, proceed to *Tier II*.

Tier II

Solution A: Dissolve 7.8 g of *monobasic sodium phosphate dihydrate* in 1 L of water.

Sonicate to dissolve and adjust with *phosphoric acid* or *sodium hydroxide* to a pH of 7.5.

Dissolve 10 g of *pancreatin* in the resulting solution.

Solution B: Dissolve 7.8 g of *monobasic sodium phosphate dihydrate* in 1 L of water.

Adjust with *phosphoric acid* or *sodium hydroxide* to a pH of 7.5. Dissolve 90 g of *sodium dodecylsulfate* in the resulting solution. Sonicate to dissolve.

Medium: Transfer 700 mL of *Solution A* to the dissolution vessel and equilibrate at 37° for 15 min. Add 200 mL of *Solution B*; 900 mL.

Apparatus 2: 75 rpm. Use a suitable sinker, if necessary.

Time: 45 min

Analyze the *Sample solution* using the liquid chromatographic procedure described in *Tier I*.

Tolerances: NLT 75% (Q) of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$) is dissolved.

- **Uniformity of Dosage Units** (905): Meet the requirements

IMPURITIES

- **Organic Impurities**

Buffer: 0.05 M *monobasic potassium phosphate*

Solution A: Methanol and *Buffer* (33:67). Adjust with *phosphoric acid* to a pH of 3.0.

Solution B: Acetonitrile, methanol, and *Buffer* (55:5:40). Adjust with *potassium hydroxide* to a pH of 6.0.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
20	85	15
30	85	15
40	55	45
55	40	60
65	25	75
70	20	80
71	100	0
75	100	0

Diluent: Acetonitrile, methanol, and water (40:10:50). Adjust with *phosphoric acid* to a pH of 2.5.

System suitability solution: 0.5 mg/mL of USP Ziprasidone Hydrochloride RS and 0.05 mg/mL each of USP Ziprasidone Related Compound B RS and USP Ziprasidone Related Compound F RS in *Diluent*

Standard solution: 0.002 mg/mL each of USP Ziprasidone Hydrochloride RS and USP Ziprasidone Related Compound B RS in *Diluent*. Sonication may be used to aid in dissolution.

Sample solution: Nominally 1.0 mg/mL of ziprasidone in *Diluent* from a portion of contents of Capsules (NLT 20) prepared as follows. Transfer a suitable amount of Capsule contents to a suitable volumetric flask. Add 60% of the flask volume of *Diluent*. Sonicate for 10 min. Dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 229 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between ziprasidone related compound B and related compound F;
NLT 2.0 between ziprasidone related compound F and ziprasidone, *System suitability solution*

Tailing factor: NMT 1.5 for ziprasidone, *Standard solution*

Relative standard deviation: NMT 5.0% for both ziprasidone and ziprasidone related compound B, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ziprasidone related compound B in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ziprasidone related compound B from the *Sample solution*

r_S = peak response of ziprasidone related compound B from the *Standard solution*

C_S = concentration of USP Ziprasidone Related Compound B RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ziprasidone in the *Sample solution* (mg/mL)

Calculate the percentage of any other unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of ziprasidone from the *Standard solution*

C_S = concentration of USP Ziprasidone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ziprasidone in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of ziprasidone free base, 412.94

M_{r2} = molecular weight of ziprasidone hydrochloride, 467.41 for the monohydrate, 449.40 for the anhydrous form

Acceptance criteria: See *Table 2*. Disregard any peak with an area below 0.05% in the *Sample solution*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ziprasidone related compound A ^{a,b}	0.22	—
Chloroindolinone ^{a,c}	0.59	—
Ziprasidone related compound B	0.70	0.20
Ziprasidone related compound F ^a	0.84	—
Ziprasidone	1.0	—
Ziprasidone related compound C ^{a,d}	1.84	—
Ziprasidone related compound D ^{a,e}	2.18	—
Any individual unspecified degradation product	—	0.20
Total degradation products	—	0.50

^a Process impurity included in the table for identification only; controlled in the drug substance. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities for the drug product.
^b 3-(Piperazin-1-yl)benzo[*d*]isothiazole.
^c 6-Chloroindolin-2-one.
^d 5,5'-Bis{2-[4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl]ethyl}-6,6'-dichloro-3-hydroxy-3,3'-biindoline-2,2'-dione.
^e 3-(Benzo[*d*]isothiazol-3-yl)-5-{2-[4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl]ethyl}-6-chloroindolin-2-one.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP Reference Standards** <11>
 - USP Ziprasidone Hydrochloride RS
 - USP Ziprasidone Related Compound B RS
 - 5-{2-[4-(Benzo[*d*]isothiazol-3-yl)piperazin-1-yl]ethyl}-6-chloroindoline-2,3-dione.
C₂₁H₁₉ClN₄O₂S 426.92
 - USP Ziprasidone Related Compound F RS
 - 2-(2-Amino-5-{2-[4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl]ethyl}-4-chlorophenyl)acetic acid.
C₂₁H₂₃ClN₄O₂S 430.95

■ 2S (USP39)

BRIEFING

Ashwagandha Root, page 7775 of the *Second Supplement* to USP 38. The monograph proposes a new HPTLC procedure as *Identification* test A. In addition to the savings in time, solvent use, and improved experimental control, the proposed procedure enhances system suitability based on two chemical markers, withanolide A and β-sitosterol; simplifies sample preparation; and utilizes a different derivatization reagent.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C162480

Comment deadline: November 30, 2015

Ashwagandha Root

DEFINITION

Ashwagandha Root is the dried mature root of *Withania somnifera* (L.) Dunal (Fam. Solanaceae). It contains NLT 0.3% of withanolides, calculated on the dried basis as the sum of withanolide aglycones calculated as withanolide A, and withanolide glycosides calculated as withanoside IV.

IDENTIFICATION

Change to read:

- **A.**

- **HPTLC for Articles of Botanical Origin (203)** ■ *2S (USP39)*

Standard solution: ~~About 200 mg of USP Powdered Ashwagandha Root Extract RS in 10 mL of methanol. Heat gently for 10–15 min, centrifuge, and use the supernatant. [Note—Save the remaining supernatant for use in the test for *Content of Withanolides*.]~~

Sample solution: ~~Transfer about 5.0 g of Ashwagandha Root, finely powdered and accurately weighed, to a 250 mL flask fitted with a reflux condenser. Add 50 mL of methanol, reflux on a water bath for 10–15 min, cool to room temperature, and decant the supernatant. Repeat until the extract is colorless. Combine the extracts, filter, concentrate under vacuum to about 40 mL, and adjust the volume with methanol to 50.0 mL. [Note—Save the remaining volume of the *Sample solution* for use in the test for *Content of Withanolides*.]~~

Adsorbent: ~~0.25-mm layer of chromatographic silica gel (TLC plates)~~

Application volume: ~~25 µL~~

Developing solvent system: ~~A mixture of ethyl acetate, toluene, and acetic acid (45:55:3)~~

Derivatization reagent: ~~Mix 0.5 mL of anisaldehyde, 10 mL of glacial acetic acid, 85 mL of methanol, and 5 mL of concentrated sulfuric acid in the order given.~~

Analysis

Samples: ~~*Standard solution* and *Sample solution*~~

~~Apply the *Samples* as bands (see *Chromatography* (621)). Use a saturated chamber. Develop until the solvent front has moved up about 90% of the length of the plate. Dry the plate, spray with *Derivatization reagent*, heat for 5–10 min at 100°, and examine under white light.~~

Acceptance criteria: ~~The *Sample solution* exhibits five major grayish-blue bands with R_f~~

values of approximately 0.12, 0.29, 0.47, 0.67, and 0.73 that are similar in position and color to the bands in the *Standard solution*. Additional, less intense, bands may be observed in the *Sample solution* and the *Standard solution*.

■ **Standard solution A:** 0.2 mg/mL each of USP β -Sitosterol RS and USP Withanolide A RS in *methanol*

Standard solution B: 20 mg/mL of USP Powdered Ashwagandha Root Extract RS in *methanol*. Sonicate for 10 min, centrifuge, and use the supernatant. [Note—Retain the supernatant for use in the test for *Content of Withanolides*.]

Sample solution: Suspend about 1 g of Ashwagandha Root, finely powdered, in 10 mL of *methanol*, and sonicate for 15 min. Centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 μ m (HPTLC plate).¹

Application volume: 2 μ L each of *Standard solution A* and *Standard solution B*, and 10 μ L of the *Sample solution*, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient, not to exceed 30°

Developing solvent system: *Toluene*, *ethyl acetate*, and *glacial acetic acid* (55:45:3)

Developing distance: 6 cm

Derivatization reagent: 20 mL of *sulfuric acid* combined with 180 mL of ice-cold *methanol*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the samples as bands and dry in air. Develop in a saturated chamber and dry in air. Treat the plate with the *Derivatization reagent*, heat at 100° for 5 min, and examine under UV light at 365 nm and under white light.

System suitability: Under UV light at 365 nm, the derivatized chromatogram of *Standard solution A* displays, in its lower third, a blue band due to withanolide A, and in the middle third a grayish-blue band due to β -sitosterol. The chromatogram of *Standard solution B* displays a light-gray to whitish band due to withanone below the withanolide A band, and a faint light-gray band above the β -sitosterol band; there is also a light-gray band close to the solvent front. A reddish band slightly above the application line is due to withaferin A. Under white light, the bands due to β -sitosterol and withanolide A appear violet-gray. Additional faint bands may appear.

Acceptance criteria: Under UV light at 365 nm and under white light, the chromatogram of the *Sample solution* displays the bands similar in position and color to those seen in *Standard solution B*. Additional bands may be observed, in particular a band just above that due to withanolide A (light-brown under UV light at 365 nm, dark-brown under white light), and a thin band below that due to β -sitosterol (light-blue under UV light at 365 nm, gray-violet under white light). Bands vary in intensity, and some of those seen in the chromatogram of *Standard solution B* may be very faint or absent from the *Sample solution*. ■ 2S (USP39)

Change to read:

- **B. HPLC**

Analysis: Proceed as directed in the test for *Content of Withanolides*.

Acceptance criteria: The *Sample solution* shows main peaks at retention times corresponding to those of withanolide A and withanoside IV in *Standard solution A* and *Standard solution B*, respectively. Identify other withanolide peaks in the *Sample solution* by comparison with *Standard solution C* and the reference chromatogram provided with the lot of USP Powdered Ashwagandha Root Extract RS being used. The *Sample solution* shows additional withanolide peaks: physagulin D, 27-hydroxywithanone, withanoside V, withanoside VI, withaferin A, withastramonolide, withanone, and withanolide B.

■ The *Sample solution* shows some of the withanolide peaks listed in Table 2. ■2S (USP39)

COMPOSITION

Change to read:

- **Content of Withanolides**

Solution A: Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL of water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL, and mix.

Solution B: Acetonitrile, filtered and degassed

~~**Standard solution A:** Dissolve, using gentle heat, a quantity of USP Withanolide A RS in methanol to obtain a solution having a known concentration of about 0.1 mg/mL.~~

~~**Standard solution B:** Dissolve, using gentle heat, a quantity of USP Withanoside IV RS in methanol to obtain a solution having a known concentration of about 0.1 mg/mL.~~

~~**Standard solution C:** Dilute 5 mL of the *Standard solution* prepared in *Identification test A* with methanol to 10 mL, and mix. Before injection, pass through a membrane filter of 0.45- μ m or finer pore size.~~

■ **Standard solution A:** A composite solution containing 0.1 mg/mL of USP Withanolide A RS and 0.1 mg/mL of USP Withanoside IV RS in *methanol*, accurately calculated. Use gentle heat to aid dissolution.

Standard solution B: Dilute two-fold a portion of *Standard solution B* from *Identification test A* with *methanol*, and mix well. Before injection, pass through a membrane filter of 0.45- μ m or finer pore size. ■2S (USP39)

~~**Sample solution:** Use the *Sample solution* prepared in *Identification test A*. Before injection, pass through a membrane filter of 0.45- μ m or finer pore size, discarding the first few mL of the filtrate.~~

■ Transfer about 5.00 g of Ashwagandha Root, finely powdered and accurately weighed, to a 250-mL round-bottom flask fitted with a reflux condenser. Add 50 mL of *methanol*, reflux on a water bath for 15 min, cool to room temperature, decant and retain the solvent. Repeat until the solvent is colorless. Combine the retained solvents, filter, concentrate under vacuum to about 40 mL, transfer to a 50-mL volumetric flask, and adjust the volume with *methanol*. Before injection, pass through a filter of 0.45- μ m or finer pore size, discarding the first few mL of the filtrate. ■2S (USP39)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	55	45
25	20	80
28	20	80
30	95	5
40	95	5

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 227 nm

Column: 4.6-mm × 25-cm, end-capped; packing *L1*

Column temperature: 27°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Using the chromatogram of *Standard solution B* and the reference chromatogram provided with the lot of USP Powdered Ashwagandha Root Extract RS being used, identify the retention times of the peaks corresponding to withanolide aglycones and glycosides. The approximate relative retention times are provided in *Table 2*.

Table 2

Analyte	Relative Retention Time
Withanoside IV	0.70
Physagulin D	0.75
27-Hydroxywithanone	0.80
Withanoside V	0.89
Withanoside VI	0.89
Withaferin A	0.92
Withastramonolide	0.96
Withanolide A	1.00
Withanone	1.01
Withanolide B	1.14

Suitability requirements

The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of USP Powdered Ashwagandha Root Extract RS being used.

Resolution: NLT 1.0 between the withanolide A and withanone peaks, and NLT 3.0 between the withaferin A peak and the peak corresponding to coeluting withanoside V

and withanoside VI, *Standard solution B*

Tailing factor: NMT 1.5 for the withanolide A peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for replicate injections, withanolide A peak, *Standard solution A*

Analysis

Samples: *Standard solution A, Standard solution B, and Sample solution*

Calculate the percentage of withanolide aglycones in the portion of Ashwagandha Root taken:

$$\text{Result} = 5(r_T/r_S)(C_S/W)$$

r_T = sum of the peak responses for withaferin A, withastramonolide, withanolide A, withanone, and withanolide B from the *Sample solution*

r_S = peak response of withanolide A from *Standard solution A*

C_S = concentration of USP Withanolide A RS in *Standard solution A* (mg/mL)

W = weight of Ashwagandha Root taken to prepare the *Sample solution* (g)

Calculate the percentage of withanolide glycosides in the portion of Ashwagandha Root taken:

$$\text{Result} = 5(r_T/r_S)(C_S/W)$$

r_T = sum of the peak responses for withanoside IV, withanoside V, and withanoside VI from the *Sample solution*

r_S = peak response of USP Withanoside IV RS from *Standard solution B*

C_S = concentration of USP Withanoside IV RS in *Standard solution B* (mg/mL)

W = weight of Ashwagandha Root taken to prepare the *Sample solution* (g)

- Calculate the percentage of withanolide aglycones in the portion of Ashwagandha Root taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = sum of the peak areas of withaferin A, withastramonolide, withanolide A, withanone, and withanolide B from the *Sample solution*

r_S = peak area of withanolide A from *Standard solution A*

C_S = concentration of USP Withanolide A RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Ashwagandha Root taken to prepare the *Sample solution* (mg)

Calculate the percentage of withanolide glycosides in the portion of Ashwagandha Root taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = sum of the peak areas of withanoside IV, withanoside V, and withanoside VI from the *Sample solution*

r_S = peak area of withanoside IV from *Standard solution A*

C_S = concentration of USP Withanoside IV RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Ashwagandha Root taken to prepare the *Sample solution* (mg) ■ 2S (USP39)

Acceptance criteria: The sum of the percentages of withanolide aglycones and withanolide glycosides is NLT 0.3%, calculated on the dried basis. [Note—Because of inherent variation, some of the withanolides mentioned in this test may be present in minor quantities or may be totally absent. The sample will be deemed compliant if the sum of the withanolides is NLT 0.3%.]

IMPURITIES

Delete the following:

- ● **Heavy Metals**, *Method II (231)*: NMT 20 ppm ■ 2S (USP38)

Add the following:

- ● **Articles of Botanical Origin (561)**, *Limits of Elemental Impurities*: Meets the requirements ■ 2S (USP38)
- **Articles of Botanical Origin (561)**, *Pesticide Residue Analysis*: Meets the requirements
- **Articles of Botanical Origin (561)**, *Methods of Analysis, Foreign Organic Matter*: NMT 2.0%
- **Articles of Botanical Origin (561)**, *Test for Aflatoxins*: Meets the requirements
- **Microbial Enumeration Tests (2021)**: The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacterial count does not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms (2022)**, *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

● Botanical Characteristics

Macroscopic: Primary roots are not branched and are straight, conical, or fingerlike in shape and variable in thickness with age; some carry a crown, consisting of a number of remains of stem base; the outer surface is buff to grayish-yellow with longitudinal wrinkles; fracture is short and uneven; secondary roots are thin and fibrous.

Microscopic: Transverse section of root shows a narrow band of yellowish crumpled cork, moderate-size cortex, and a wide wood. The cork cells are rectangular, radially flattened, nonlignified, and filled with starch grains and reddish brown content; cork cambium is 2–4 diffused rows of cells; secondary cortex is formed of 20–25 rows of thin-walled parenchymatous cells, filled with starch grains, and shows occasional microsphenoidal crystals of calcium oxalate; phloem consists of sieve tubes, companion cells, and phloem parenchyma; vascular cambium consists of tangentially elongated parenchymatous cells; vessels and tracheids are in radial rows toward the periphery of the wood; medullary rays are uniseriate to 2- to 3-seriate, and are filled with starch grains; scattered vessels in groups are embedded in the parenchyma; vessels have pitted and scalariform thickenings, and generally the end walls are perforated; a few fibers with thick lignified walls are also found scattered in the wood.

● Loss on Drying (731)

Sample: 1.0 g of finely powdered Ashwagandha Root

Analysis: Dry the *Sample* at 105° for 3 h.

Acceptance criteria: NMT 12.0%

- **Articles of Botanical Origin** (561), *Methods of Analysis, Total Ash*

Sample: 1.0 g of finely powdered Ashwagandha Root

Acceptance criteria: NMT 7.0%

- **Articles of Botanical Origin** (561), *Methods of Analysis, Acid-Insoluble Ash:* NMT 1.0%
- **Articles of Botanical Origin** (561), *Methods of Analysis, Alcohol-Soluble Extractives, Method 2:* NLT 10.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling:** The label states the Latin binomial and, following the official name, the part of the plant contained in the article.

Change to read:

- **USP Reference Standards** (11)
USP Powdered Ashwagandha Root Extract RS
 - USP β -Sitosterol RS ■ 2S (USP39)
 USP Withanolide A RS
 USP Withanoside IV RS

■ 1 Suitable commercially available plates are HPTLC Silica Gel 60 F₂₅₄ from EMD Millipore (e.g., Part No. 1.05642.0001). ■ 2S (USP39)

BRIEFING

Powdered Ashwagandha Root, page 7777 of the *Second Supplement to USP 38*. The monograph proposes a new HPTLC procedure as *Identification* test A. In addition to the savings in time, solvent use, and improved experimental control, the proposed procedure enhances system suitability based on two chemical markers, withanolide A and β -sitosterol; simplifies sample preparation; and utilizes a different derivatization reagent.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C162481

Comment deadline: November 30, 2015

Powdered Ashwagandha Root

DEFINITION

Change to read:

Powdered Ashwagandha Root is Ashwagandha Root reduced to a fine or very fine powder. ■ It contains NLT 0.3% of withanolides, calculated on the dried basis as the sum of withanolide

aglycones calculated as withanolide A, and withanolide glycosides calculated as withanoside IV.

■ 2S (USP38)

IDENTIFICATION

Change to read:

● A. Thin-Layer Chromatography

■ HPTLC for Articles of Botanical Origin (203) ■ 2S (USP39)

Standard solution: Heat gently, for 10–15 min, about 200 mg of USP Powdered Ashwagandha Root Extract RS in 10 mL of methanol, centrifuge, and use the supernatant. [Note—Save the remaining supernatant for use in the test for *Content of Withanolides*.]

Sample solution: Transfer about 5.0 g of accurately weighed Powdered Ashwagandha Root to a 250 mL flask fitted with a reflux condenser. Add 50 mL of methanol, reflux on a water bath for 10–15 min, cool to room temperature, and decant the supernatant. Repeat until the extract is colorless. Combine the extracts, filter, concentrate under vacuum to about 40 mL, and adjust the volume with methanol to 50.0 mL. [Note—Save the remaining volume of the *Sample solution* for use in the test for *Content of Withanolides*.]

Adsorbent: 0.25 mm layer of chromatographic silica gel (TLC plates)

Application volume: 25 µL

Developing solvent system: A mixture of ethyl acetate, toluene, and acetic acid (45:55:3)

Derivatization reagent: Mix 0.5 mL of anisaldehyde, 10 mL of glacial acetic acid, 85 mL of methanol, and 5 mL of concentrated sulfuric acid in the order mentioned.

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands (see *Chromatography* (621)). Use a saturated chamber. Develop the chromatograms until the solvent front has moved up about 90% of the plate. Remove the plate from the chamber, dry, spray with *Derivatization reagent*, heat for 5–10 min at 100°, and examine under white light.

Acceptance criteria: The *Sample solution* exhibits five major grayish-blue bands with R_f values of approximately 0.12, 0.29, 0.47, 0.67, and 0.73 that are similar in position and color to the bands in the *Standard solution*. Additional, less intense, bands may be observed in the *Sample solution* and *Standard solution*.

■ **Standard solution A:** 0.2 mg/mL each of USP β -Sitosterol RS and USP Withanolide A RS in *methanol*

Standard solution B: 20 mg/mL of USP Powdered Ashwagandha Root Extract RS in *methanol*. Sonicate for 10 min, centrifuge, and use the supernatant. [Note—Retain the supernatant for use in the test for *Content of Withanolides*.]

Sample solution: Suspend about 1 g of Powdered Ashwagandha Root in 10 mL of *methanol*, and sonicate for 15 min. Centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 µm (HPTLC)

plate).¹

Application volume: 2 μ L each of *Standard solution A* and *Standard solution B*, and 10 μ L of the *Sample solution*, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient, not to exceed 30°

Developing solvent system: *Toluene, ethyl acetate, and glacial acetic acid* (55:45:3)

Developing distance: 6 cm

Derivatization reagent: 20 mL of *sulfuric acid* combined with 180 mL of ice-cold *methanol*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the samples as bands and dry in air. Develop in a saturated chamber and dry in air. Treat the plate with the *Derivatization reagent*, heat at 100° for 5 min, and examine under UV light at 365 nm and under white light.

System suitability: Under UV light at 365 nm, the derivatized chromatogram of *Standard solution A* displays, in its lower third, a blue band due to withanolide A, and in the middle third a grayish-blue band due to β -sitosterol. The chromatogram of *Standard solution B* displays a light-gray to whitish band due to withanone below the withanolide A band, and a faint light-gray band above the β -sitosterol band; there is also a light-gray band close to the solvent front. A reddish band slightly above the application line is due to withaferin A. Under white light, the bands due to β -sitosterol and withanolide A appear violet-gray. Additional faint bands may appear.

Acceptance criteria: Under UV light at 365 nm and under white light, the chromatogram of the *Sample solution* displays the bands similar in position and color to those seen in *Standard solution B*. Additional bands may be observed, in particular a band just above that due to withanolide A (light-brown under UV light at 365 nm, dark-brown under white light), and a thin band below that due to β -sitosterol (light-blue under UV light at 365 nm, gray-violet under white light). Bands vary in intensity, and some of those seen in the chromatogram of *Standard solution B* may be very faint or absent from the *Sample solution*. ■2S (USP39)

Change to read:

• B. HPLC

Analysis: Proceed as directed in the test for *Content of Withanolides*.

Acceptance criteria: The *Sample solution* shows main peaks at retention times corresponding to those of withanolide A and withanoside IV in *Standard solution A*. ~~and *Standard solution B*, respectively. Identify other withanolide peaks in the *Sample solution* by comparison with *Standard solution C* and the reference chromatogram provided with the lot of USP Powdered Ashwagandha Root Extract RS being used. The *Sample solution* shows additional withanolide peaks: physagulin D, 27-hydroxywithanone, withanoside V, withanoside VI, withaferin A, withastramonolide, withanone, and withanolide B~~

■ The *Sample solution* shows some of the withanolide peaks listed in Table 2. ■2S (USP39)

COMPOSITION

Change to read:

- **Content of Withanolides**

Solution A: Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL of water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL, and mix.

Solution B: Acetonitrile, filtered and degassed

~~**Standard solution A:** Dissolve, using gentle heat, a quantity of USP Withanolide A RS in methanol to obtain a solution having a known concentration of about 0.1 mg/mL.~~

~~**Standard solution B:** Dissolve, using gentle heat, a quantity of USP Withanoside IV RS in methanol to obtain a solution having a known concentration of about 0.1 mg/mL.~~

~~**Standard solution C:** Dilute 5 mL of the *Standard solution* prepared in *Identification test A* with methanol to 10 mL, and mix. Before injection, pass through a membrane filter of 0.45- μ m or finer pore size.~~

■ **Standard solution A:** A composite solution containing 0.1 mg/mL of USP Withanolide A RS and 0.1 mg/mL of USP Withanoside IV RS in *methanol*, accurately calculated. Use gentle heat to aid dissolution.

Standard solution B: Dilute two-fold a portion of the *Standard solution B* from *Identification test A* with *methanol*, and mix well. Before injection, pass through a membrane filter of 0.45- μ m or finer pore size. ■2S (USP39)

~~**Sample solution:** Use the *Sample solution* prepared in *Identification test A*. Before injection, pass through a membrane filter of 0.45- μ m or finer pore size, discarding the first few mL of the filtrate.~~

■ Transfer about 5.00 g of accurately weighed Powdered Ashwagandha Root to a 250-mL round-bottom flask fitted with a reflux condenser. Add 50 mL of *methanol*, reflux on a water bath for 15 min, cool to room temperature, decant and retain the solvent. Repeat until the solvent is colorless. Combine the retained solvents, filter, concentrate under vacuum to about 40 mL, transfer to a 50-mL volumetric flask, and adjust the volume with *methanol*. Before injection, pass through a filter of 0.45- μ m or finer pore size, discarding the first few mL of the filtrate. ■2S (USP39)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	55	45
25	20	80
28	20	80
30	95	5
40	95	5

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 227 nm

Column: 4.6-mm × 25-cm, end-capped; packing *L1*

Column temperature: 27°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Using the chromatogram of *Standard solution B* and the reference chromatogram provided with the lot of USP Powdered Ashwagandha Root Extract RS being used, identify the retention times of the peaks corresponding to withanolide aglycones and glycosides. The approximate relative retention times are provided in *Table 2*.

Table 2

Analyte	Relative Retention Time
Withanoside IV	0.70
Physagulin D	0.75
27-Hydroxywithanone	0.80
Withanoside V	0.89
Withanoside VI	0.89
Withaferin A	0.92
Withastramonolide	0.96
Withanolide A	1.00
Withanone	1.01
Withanolide B	1.14

Suitability requirements

The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of USP Powdered Ashwagandha Root Extract RS being used.

Resolution: NLT 1.0 between the withanolide A and withanone peaks, and NLT 3.0 between the withaferin A peak and the peak corresponding to coeluting withanoside V and withanoside VI, *Standard solution B*

Tailing factor: NMT 1.5 for the withanolide A peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for replicate injections, withanolide A peak, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of withanolide aglycones in the portion of Powdered Ashwagandha Root taken:

$$\text{Result} = 5(r_T/r_S)(C_S/W)$$

r_T = sum of the peak responses for withaferin A, withastramonolide, withanolide A, withanone, and withanolide B from the *Sample solution*

r_S = peak response of withanolide A from *Standard solution A*

C_S = concentration of USP Withanolide A RS in *Standard solution A* (mg/mL)

W = weight of Powdered Ashwagandha Root taken to prepare the *Sample solution* (g)

Calculate the percentage of withanolide glycosides in the portion of Powdered Ashwagandha Root taken:

$$\text{Result} = 5(r_T/r_S)(C_S/W)$$

r_T = sum of the peak responses for withanoside IV, withanoside V, and withanoside VI in the *Sample solution*

r_S = peak response of USP Withanoside IV RS in *Standard solution B*

C_S = concentration of USP Withanoside IV RS in *Standard solution B* (mg/mL)

W = weight of Powdered Ashwagandha Root taken to prepare the *Sample solution* (g)

- Calculate the percentage of withanolide aglycones in the portion of Powdered Ashwagandha Root taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = sum of the peak areas of withaferin A, withastramonolide, withanolide A, withanone, and withanolide B from the *Sample solution*

r_S = peak area of withanolide A from *Standard solution A*

C_S = concentration of USP Withanolide A RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Powdered Ashwagandha Root taken to prepare the *Sample solution* (mg)

Calculate the percentage of withanolide glycosides in the portion of Powdered Ashwagandha Root taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = sum of the peak areas of withanoside IV, withanoside V, and withanoside VI from the *Sample solution*

r_S = peak area of withanoside IV from *Standard solution A*

C_S = concentration of USP Withanoside IV RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Powdered Ashwagandha Root taken to prepare the *Sample solution* (mg)

- 2S (USP39)

Acceptance criteria: The sum of the percentages of withanolide aglycones and withanolide glycosides is NLT 0.3%, calculated on the dried basis. [Note—Because of inherent variation, some of the withanolides mentioned in this test may be present in minor quantities or may be totally absent. The sample will be deemed compliant if the sum of the withanolides is NLT 0.3%.]

IMPURITIES

Delete the following:

- • **Heavy Metals**, *Method II* (231): NMT 20 ppm ■ 2S (USP38)

Add the following:

- ● **Articles of Botanical Origin** <561>, *Limits of Elemental Impurities*: Meets the requirements
 - 2S (USP38)
- **Articles of Botanical Origin** <561>, *Pesticide Residue Analysis*: Meets the requirements
- **Articles of Botanical Origin** <561>, *Test for Aflatoxins*: Meets the requirements
- **Microbial Enumeration Tests** <2021>: The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria count does not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** <2022>, *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

● Botanical Characteristics

Macroscopic: Dusty white or grey to light brown powder with a characteristic odor and a mucilaginous, bitter, acrid taste

Microscopic: Collapsed cork cells filled with starch grains and reddish-brown content; thin-walled cortex parenchyma cells filled with starch grains and occasional microsphenoidal crystals of calcium oxalate; vessels have pitted and scalariform thickenings, and generally with end walls perforated; a few fibers with thick lignified walls and simple pits; abundant starch grains, mostly simple, sometimes compound, spherical, reniform-oval with central hilum.

● Loss on Drying <731>

Sample: 1.0 g of Powdered Ashwagandha Root

Analysis: Dry the *Sample* at 105° for 3 h.

Acceptance criteria: NMT 12.0%

● Articles of Botanical Origin <561>, *Methods of Analysis, Total Ash*

Sample: 1.0 g of Powdered Ashwagandha Root

Acceptance criteria: NMT 7.0%

● Articles of Botanical Origin <561>, *Methods of Analysis, Acid-Insoluble Ash*: NMT 1.0%

● Articles of Botanical Origin <561>, *Methods of Analysis, Alcohol-Soluble Extractives, Method 2*: NLT 10.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling:** The label states the Latin binomial and, following the official name, the part of the plant contained in the article.

Change to read:

- **USP Reference Standards** <11>
USP Powdered Ashwagandha Root Extract RS
 - USP β -Sitosterol RS ■ 2S (USP39)

USP Withanolide A RS USP Withanoside IV RS

- ¹ Suitable commercially available plates are HPTLC Silica Gel 60 F₂₅₄ from EMD Millipore (e.g., Part No. 1.05642.0001). ■ _{2S} (USP39)

BRIEFING

Powdered Ashwagandha Root Extract, *USP 38* page 5886. The monograph proposes a new HPTLC procedure as *Identification* test A. In addition to the savings in time, solvent use, and improved experimental control, the proposed procedure enhances system suitability based on two chemical markers, withanolide A and β -sitosterol; simplifies sample preparation; and utilizes a different derivatization reagent.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C162482

Comment deadline: November 30, 2015

Powdered Ashwagandha Root Extract

DEFINITION

Change to read:

Powdered Ashwagandha Root Extract is prepared from Ashwagandha

- Root ■ _{2S} (USP39)

using methanol, alcohol, water, or mixtures of these solvents. It contains NLT 2.5% of withanolides, calculated on the dried basis as the sum of withanolide aglycones

- calculated as withanolide A ■ _{2S} (USP39)

and withanolide glycosides

- calculated as withanoside IV. It may contain suitable added substances. ■ _{2S} (USP39)

IDENTIFICATION

Change to read:

- **A. Thin-Layer Chromatographic Identification Test** (201)

- **HPTLC for Articles of Botanical Origin** (203) ■ _{2S} (USP39)

Standard solution: Heat gently for 10–15 min about 200 mg of USP Powdered Ashwagandha Extract Root RS in 10 mL of methanol, centrifuge, and use the supernatant. [Note—Save the remaining volume of the supernatant for use in the test for *Content of Withanolides*.]

Sample solution: Heat gently for 10–15 min about 200 mg of Powdered Ashwagandha Root Extract in 10 mL of methanol, centrifuge, and use the supernatant.

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 25 μ L

Developing solvent system: ~~A mixture of ethyl acetate, toluene, and acetic acid (45:55:3)~~

Spray reagent: ~~Mix 0.5 mL of anisaldehyde, 10 mL of glacial acetic acid, 85 mL of methanol, and 5 mL of concentrated sulfuric acid in the order mentioned.~~

Analysis

Samples: ~~Standard solution and Sample solution~~

~~Apply the Samples as bands to a suitable plate (see *Chromatography* (621)). Use a saturated chamber. Develop the chromatograms until the solvent front has moved up about 90% of the length of the plate. Remove the plate from the chamber, dry, spray with *Spray reagent*, heat for 5–10 min at 100°, and examine under visible light.~~

Acceptance criteria: ~~The Sample solution exhibits five main grayish blue bands with R_f values of approximately 0.12, 0.29, 0.47, 0.67, and 0.73 that are similar in position and color to the main bands for the Standard solution. Other less intense bands are observed for the Sample solution and the Standard solution.~~

■ **Standard solution A:** 0.2 mg/mL each of USP β -Sitosterol RS and USP Withanolide A RS in methanol

Standard solution B: 20 mg/mL of USP Powdered Ashwagandha Root Extract RS in methanol. Sonicate for 10 min, centrifuge, and use the supernatant. [Note—Retain the supernatant for use in the test for *Content of Withanolides*.]

Sample solution: Suspend about 200 mg of Powdered Ashwagandha Root Extract in 10 mL of methanol, and sonicate for 10 min. Centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 μ m (HPTLC plate)¹

Application volume: 2 μ L each of *Standard solution A*, *Standard solution B*, and *Sample solution*, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient, not to exceed 30°

Developing solvent system: *Toluene, ethyl acetate, and glacial acetic acid* (55:45:3)

Developing distance: 6 cm

Derivatization reagent: 20 mL of *sulfuric acid* combined with 180 mL of ice-cold methanol

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the samples as bands and dry in air. Develop in a saturated chamber and dry in air. Treat the plate with the *Derivatization reagent*, heat at 100° for 5 min, and examine under UV light at 365 nm and under white light.

System suitability: Under UV light at 365 nm, the derivatized chromatogram of *Standard solution A* displays, in its lower third, a blue band due to withanolide A, and in the middle third a grayish-blue band due to β -sitosterol. The chromatogram of *Standard solution B* displays a light-gray to whitish band due to withanone below the withanolide A band, and a faint light-gray band above the β -sitosterol band; there is also a light-gray band close

to the solvent front. A reddish band slightly above the application line is due to withaferin A. Under white light, the bands due to β -sitosterol and withanolide A appear violet-gray. Additional faint bands may appear.

Acceptance criteria: Under UV light at 365 nm and under white light, the chromatogram of the *Sample solution* displays the bands similar in position and color to those seen in *Standard solution B*. Additional bands may be observed, in particular a band just above that due to withanolide A (light-brown under UV light at 365 nm, dark-brown under white light), and a thin band below that due to β -sitosterol (light-blue under UV light at 365 nm, gray-violet under white light). Bands vary in intensity, and some of those seen in the chromatogram of *Standard solution B* may be very faint or absent from the *Sample solution*. ■2S (USP39)

Change to read:

• B. HPLC

Analysis: Proceed as directed in the test for *Content of Withanolides*.

Acceptance criteria: The *Sample solution* shows main peaks at retention times corresponding to those of withanolide A and withanoside IV in *Standard solution A* and *Standard solution B*, respectively. Identify other withanolide peaks in the *Sample solution* by comparison with *Standard solution C* and the reference chromatogram provided with the lot of USP Powdered Ashwagandha Root Extract RS being used. The *Sample solution* shows additional withanolide peaks: physagulin D, 27-hydroxywithanone, withanoside V, withanoside VI, withaferin A, withastramonolide, withanone, and withanolide B

■ The *Sample solution* may show additional withanolide peaks listed in Table 2. ■2S (USP39)

COMPOSITION

Change to read:

• Content of Withanolides

Solution A: Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL of water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL, and mix.

~~**Standard solution A:** Dissolve, using gentle heat, a quantity of USP Withanolide A RS in methanol to obtain a solution having a known concentration of about 0.1 mg/mL.~~

~~**Standard solution B:** Dissolve, using gentle heat, a quantity of USP Withanoside IV RS in methanol to obtain a solution having a known concentration of about 0.1 mg/mL.~~

~~**Standard solution C:** Dilute 5 mL of the *Standard solution* prepared in *Identification test A* with methanol to 10 mL, and mix. Before injection, pass through a membrane filter of 0.45- μ m or finer pore size.~~

■ **Standard solution A:** A composite solution containing 0.1 mg/mL of USP Withanolide A RS and 0.1 mg/mL of USP Withanoside IV RS in *methanol*, accurately calculated. Use gentle heat to aid dissolution.

Standard solution B: Dilute two-fold a portion of *Standard solution B* from *Identification test A* with *methanol*, and mix well. Before injection, pass through a membrane filter of 0.45- μ m or finer pore size. ■2S (USP39)

Sample solution: Transfer about 100 mg of Powdered Ashwagandha Root Extract, accurately weighed, to a 10-mL volumetric flask, add about 7 mL of *methanol*, heat gently

on a water bath for 20 min, cool, dilute with *methanol* to volume, and mix. Before injection, pass through a membrane filter of 0.45- μ m or finer pore size, discarding the first few mL of the filtrate.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	55	45
25	20	80
28	20	80
30	95	5
40	95	5

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 227 nm

Column: 4.6-mm \times 25-cm, end-capped; packing *L1*

Column temperature: 27 $^{\circ}$ \pm 1

■ ■ 2S (USP39)

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Samples: *Standard solution A* and *Standard solution B*

Using the chromatogram of *Standard solution B* and the reference chromatogram provided with the lot of USP Powdered Ashwagandha Root Extract RS being used, identify the retention times of the peaks corresponding to withanolide aglycones and glycosides. The approximate relative retention times are provided in *Table 2*.

Table 2

Analyte	Relative Retention Time
Withanoside IV	0.70
Physagulin D	0.75
27-Hydroxywithanone	0.80
Withanoside V	0.89
Withanoside VI	0.89
Withaferin A	0.92
Withastramonolide	0.96
Withanolide A	1.00
Withanone	1.01
Withanolide B	1.14

Suitability requirements

The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of USP Powdered Ashwagandha Root Extract RS being used.

Resolution: NLT 1.0 between the withanolide A and withanone peaks, and NLT 3.0 between the withaferin A peak and the peak corresponding to coeluting withanoside V and withanoside VI, *Standard solution B*

Tailing factor: NMT 1.5 for the withanolide A peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for replicate injections, withanolide A peak, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of withanolide aglycones in the portion of Powdered Ashwagandha Root Extract taken:

$$\text{Result} = (r_T/r_S)(C_S/W)$$

r_T = sum of the peak responses for withaferin A, withastramonolide, withanolide A, withanone, and withanolide B from the *Sample solution*

r_S = peak response of withanolide A from *Standard solution A*

C_S = concentration of USP Withanolide A RS in *Standard solution A* (mg/mL)

W = weight of Powdered Ashwagandha Root Extract taken to prepare the *Sample solution* (g)

Calculate the percentage of withanolide glycosides in the portion of Powdered Ashwagandha Root Extract taken:

$$\text{Result} = (r_T/r_S)(C_S/W)$$

r_T = sum of the peak responses for withanoside IV, withanoside V and withanoside VI from the *Sample solution*

r_S = peak response of USP Withanoside IV from *Standard solution B*

C_S = concentration of USP Withanoside IV RS in *Standard solution B* (mg/mL)

W = weight of Powdered Ashwagandha Root Extract taken to prepare the *Sample solution* (g)

- Calculate the percentage of withanolide aglycones in the portion of Powdered Ashwagandha Root Extract taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = sum of the peak areas of withaferin A, withastramonolide, withanolide A, withanone and withanolide B from the *Sample solution*

r_S = peak area of withanolide A from *Standard solution A*

C_S = concentration of USP Withanolide A RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Powdered Ashwagandha Root Extract taken to prepare the *Sample solution* (mg)

Calculate the percentage of withanolide glycosides in the portion of Powdered Ashwagandha Root taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = sum of the peak areas of withanoside IV, withanoside V, and withanoside VI from the *Sample solution*

r_S = peak area of withanoside IV from *Standard solution A*

C_S = concentration of USP Withanoside IV RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Powdered Ashwagandha Root Extract taken to prepare the *Sample solution* (mg) ■ 2S (USP39)

Acceptance criteria: The sum of the percentages of the withanolide aglycones and withanolide glycosides is NLT 2.5%, calculated on the dried basis. [Note—Because of inherent variation, some of the withanolides mentioned in this test may be present in minor quantities or may be totally absent. The sample will be deemed compliant if the sum of the total withanolides is NLT 2.5%.]

IMPURITIES

Delete the following:

- **Heavy Metals, Method II (231):** NMT 20 ppm (Official 1-Dec-2015)
- **Articles of Botanical Origin (561), Pesticide Residue Analysis:** Meets the requirements
- **Botanical Extracts (565), Preparations, General Pharmacopeial Requirements, Residual Solvents:** Meets the requirements
- **Articles of Botanical Origin (561), Test for Aflatoxins:** Meets the requirements
- **Microbial Enumeration Tests (2021):** The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms (2022), Test Procedures, Test for Absence of Salmonella Species and Test for Absence of Escherichia coli:** Meets the requirements

SPECIFIC TESTS

- **Loss on Drying (731)**

Sample: 2.0 g of Powdered Ashwagandha Root Extract

Analysis: Dry the *Sample* at 105° for 3 h.

Acceptance criteria: NMT 6.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at controlled room temperature.
- **Labeling:** The label states the Latin binomial and, following the official name, the part of the plant from which the article was prepared. It meets other labeling requirements under *Botanical Extracts (565)*.

Change to read:

- **USP Reference Standards** (11)
 - USP Powdered Ashwagandha Root Extract RS
 - USP β -Sitosterol RS ■ 2S (USP39)
 - USP Withanolide A RS
 - USP Withanoside IV RS

■ 1 Suitable commercially available plates are HPTLC Silica Gel 60 F₂₅₄ from EMD Millipore (e.g., Part No. 1.05642.0001). ■ 2S (USP39)

BRIEFING

Curcuminoids, *USP 38* page 5984. It is proposed to make the following revisions:

1. Implement a new HPTLC procedure as *Identification* test A, which discontinues the use of chloroform and permits differentiation from two common confounders, *Curcuma zanthorrhiza* Roxb. and *Curcuma aromatica* Salisb.
2. Update the formula in the test for *Content of Curcuminoids* for clarity.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C162479

Comment deadline: November 30, 2015

Curcuminoids

DEFINITION

Curcuminoids is a partially purified natural complex of diaryl heptanoid derivatives isolated from Turmeric, *Curcuma longa* L. It contains NLT 95.0% of curcuminoids, calculated on the dried basis, as the sum of curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin. It contains NLT 70.0% and NMT 80.0% of curcumin, NLT 15.0% and NMT 25.0% of desmethoxycurcumin, and NLT 2.5% and NMT 6.5% of bisdesmethoxycurcumin.

IDENTIFICATION

Change to read:

- **A. Thin-Layer Chromatographic Identification Test**

- **HPTLC for Articles of Botanical Origin** (203) ■ 2S (USP39)

Standard solution: 0.2 mg/mL of USP Curcuminoids RS in acetone

Sample solution: 2 mg/mL of Curcuminoids in acetone

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, typically 20-cm long (TLC plates)

Application volume: 10 μ L, as bands

Developing solvent system: ~~Chloroform, methanol, and formic acid (96:4:1)~~

Analysis

Samples: ~~Standard solution and Sample solution~~

~~Apply the samples as bands to a suitable thin-layer chromatographic plate (see *Chromatography* (621), *Thin-Layer Chromatography*). Use saturated chamber. Develop the chromatograms until the solvent front has moved up about three-fourths of the length of the plate. Remove the plate from the chamber, dry, and examine under UV light at 365 nm.~~

Acceptance criteria: ~~The *Sample solution* chromatogram shows yellowish-brown bands due to bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin at R_f values of about 0.4, 0.6, and 0.7, respectively, corresponding in position and color to those obtained from the *Standard solution*.~~

▪ **Standard solution:** 1 mg/mL of USP Curcuminoids RS in *methanol*

Sample solution: Suspend about 5 mg of curcuminoids in 5 mL of *methanol*, and sonicate briefly.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 μm (HPTLC plate).¹

Application volume: 2 μL each of the *Standard solution* and the *Sample solution* as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient, not to exceed 30°

Developing solvent system: *Toluene* and *glacial acetic acid* (4:1)

Developing distance: 6 cm

Derivatization reagent: 85 mL of ice-cold *methanol* combined with 10 mL of *glacial acetic acid*, 5 mL of *sulfuric acid*, and 0.5 mL of *p-anisaldehyde*

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber and dry in air. Treat with *Derivatization reagent*, heat at 100° for 3 min, and examine under long-wave UV light (365 nm) and under white light.

System suitability: Under long-wave UV light (365 nm), the derivatized chromatogram of the *Standard solution* exhibits, in its lower half, three bands, in the order of increasing R_f : an orange band due to bisdesmethoxycurcumin, an orange band due to desmethoxycurcumin, and the red band due to curcumin. Under white light, the two lower bands appear orange, while the topmost band is reddish-pink.

Acceptance criteria: Under long-wave UV light (365 nm), the derivatized chromatogram of the *Sample solution* displays two orange bands and one red band, similar in position and color to those observed in the *Standard solution*. At the bottom part of the upper half of the plate, two purple bands are seen. Under white light, two orange bands and a darker red band are seen coincident with the bands due to bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin in the *Standard solution*, in the order of increasing

R_f . In the upper half of the plate, the lower of the two bands appears purple, while the upper band is brown. No bands appear in the topmost quarter of the plate, which are characteristic of *Curcuma zanthorrhiza* Roxb. and *Curcuma aromatica* Salisb. These confounders, and occasional adulterants, also lack the lower orange band corresponding to bisdesmethoxycurcumin. Additional weak bands may be observed in the *Sample solution* under either illumination conditions. ■2S (USP39)

Change to read:

• **B.**

■ **HPLC** ■2S (USP39)

The retention times of the peaks for curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin of the *Sample solution* chromatogram correspond to those of the *Standard solution* for the appropriate USP Reference Standard, as obtained in the test for *Content of Curcuminoids*.

■ **Analysis:** Proceed as directed in the test for *Content of Curcuminoids*.

Acceptance criteria: The retention times of the peaks for curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin of the *Sample solution* correspond to those of *Standard solution A* and *Standard solution B*. ■2S (USP39)

COMPOSITION

Change to read:

• **Content of Curcuminoids**

Mobile phase: Tetrahydrofuran and 1 mg/mL of citric acid in water (4:6)

[~~Note—Sonication may be necessary to dissolve the RS in each *Standard solution*; all solutions should be passed through a filter of 0.45- μ m pore size before injection. USP Curcumin RS, USP Desmethoxycurcumin, and USP Bisdesmethoxycurcumin RS can also be prepared in one standard solution containing the final concentration specified below for each.]~~

■ ■2S (USP39)

Standard solution A: 40 μ g/mL of USP Curcuminoids RS in *Mobile phase*

~~**Standard solution B:** 40 μ g/mL of USP Curcumin RS in *Mobile phase*~~

~~**Standard solution C:** 10 μ g/mL of USP Desmethoxycurcumin RS in *Mobile phase*~~

~~**Standard solution D:** 2 μ g/mL of USP Bisdesmethoxycurcumin RS in *Mobile phase*~~

■ **Standard solution B:** A composite solution containing 40 μ g/mL of USP Curcumin RS, 10 μ g/mL of USP Desmethoxycurcumin RS, and 2.0 μ g/mL of USP Bisdesmethoxycurcumin RS in *Mobile phase*. Use sonication if necessary. Before injection, pass through a filter of 0.45- μ m pore size, and discard the initial 10 mL of the filtrate. ■2S (USP39)

~~**Sample solution:** Transfer about 20 mg of Curcuminoids to a 50-mL volumetric flask, add about 30 mL of acetone, and sonicate for 30 min. Dilute with acetone to volume and centrifuge. Transfer 5 mL to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.~~

■ **Sample stock solution:** Transfer about 20 mg of Curcuminoids, accurately weighed, to a 50-mL volumetric flask, add 30 mL of acetone, and sonicate for 30 min. Dilute with acetone to volume, mix, and centrifuge.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Before injection, pass through a filter of 0.45- μ m pore size, and discard the initial 10 mL of the filtrate. ■2S (USP39)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV

■ Vis ■2S (USP39)

420 nm

Column: 4.6-mm \times 20-cm; 5- μ m packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

System suitability

Samples: *Standard solution A*

■ and *Standard solution B* ■2S (USP39)

[Note—The relative retention times for the curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin peaks are 1.0, 1.2, and 1.4, respectively.]

Suitability requirements

Chromatogram similarity: The chromatogram of *Standard solution A* is similar to the reference chromatogram provided with USP Curcuminoids RS.

Resolution: NLT 2.0, between curcumin and desmethoxycurcumin peaks and desmethoxycurcumin and bisdesmethoxycurcumin peaks,

■ *Standard solution B* ■2S (USP39)

Tailing factor: NMT 1.5 for bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin peaks,

■ *Standard solution B* ■2S (USP39)

Relative standard deviation: NMT 2.0% for the desmethoxycurcumin peak, in replicate injections,

■ *Standard solution B* ■2S (USP39)

Analysis

Samples: ~~*Standard solution A,*~~

■ ■2S (USP39)

~~*Standard solution B, Standard solution C, Standard solution D*~~

■ ■2S (USP39)

and *Sample solution*

Calculate the percentage of curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin in the portion of Curcuminoids taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for curcumin, desmethoxycurcumin, or bisdesmethoxycurcumin from the *Sample solution*

r_S = peak response for curcumin, desmethoxycurcumin, or bisdesmethoxycurcumin from the appropriate *Standard solution*

C_S = concentration of the appropriate *Standard solution* (mg/mL)

C_U = concentration of Curcuminoids in the *Sample solution* (mg/mL)

- Calculate the percentages of curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin in the portion of Curcuminoids taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times D \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of the relevant analyte from *Standard solution B*

C_S = concentration of the relevant analyte in *Standard solution B* (mg/mL)

V = volume of the *Sample stock solution* (mL)

W = weight of Curcuminoids used to prepare the *Sample stock solution* (mg)

D = dilution factor to obtain the *Sample solution* from the *Sample stock solution*,
10 ■ 2S (USP39)

Acceptance criteria: Curcuminoids contains NLT 95.0% of curcuminoids, calculated on the dried basis, as the sum of curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin. It contains 70.0%–80.0% of curcumin, 15.0%–25.0% of desmethoxycurcumin, and 2.5%–6.5% of bisdesmethoxycurcumin.

CONTAMINANTS

Delete the following:

- Heavy Metals, Method III (231):** NMT 20 ppm (Official 1-Dec-2015)
- Articles of Botanical Origin (561), Pesticide Residue Analysis:** Meets the requirements

Add the following:

- Botanical Extracts (565), Preparations, Residual Solvents:** Meets the requirements
■ 2S (USP39)
- Articles of Botanical Origin (561), Test for Aflatoxins:** Meets the requirements
- Microbial Enumeration Tests (2021):** The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
- Absence of Specified Microorganisms (2022), Test Procedures, Test for Absence of Salmonella Species and Test for Absence of Escherichia coli:** Meets the requirements

SPECIFIC TESTS

- Melting Range or Temperature (741), Class I:** 172°–178°
- Loss on Drying (731)**
Sample: 1.0 g of Curcuminoids
Analysis: Dry the *Sample* at 105° for 2 h.
Acceptance criteria: NMT 2.0%
- Articles of Botanical Origin (561), Methods of Analysis, Total Ash:** NMT 1.0%

ADDITIONAL REQUIREMENTS

- Packaging and Storage:** Preserve in well-closed containers; protect from light and

moisture, and store at room temperature.

- **Labeling:** The label states the content of curcuminoids and the content of the individual curcuminoids, on the dried basis; the Latin binomial and the part of the plant used to prepare the article.
- **USP Reference Standards** <11>
 - USP Bisdesmethoxycurcumin RS
 - USP Curcumin RS
 - USP Curcuminoids RS
 - USP Desmethoxycurcumin RS

■ ¹ Suitable commercially available plates are HPTLC Silica Gel 60 F₂₅₄ from EMD Millipore (e.g., Part No. 1.05642.0001). ■ _{2S} (USP39)

BRIEFING

Eleuthero, page 7818 of the *Second Supplement to USP 38*. It is proposed to change the title of this monograph to *Eleuthero Root and Rhizome* to comply with the proposed *Guideline for Assigning Titles to USP Dietary Supplement Monographs*, as outlined in the eponymous *Stimuli* article published in *PF 41(6)* [Nov–Dec. 2015].

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C162947

Comment deadline: November 30, 2015

Change to read:

Eleuthero

- **Eleuthero Root and Rhizome** ■ _{2S} (USP39)

DEFINITION

Change to read:

Eleuthero

- **Eleuthero Root and Rhizome** ■ _{2S} (USP39)

is the dried rhizome with roots of *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. (Fam. Araliaceae) [*Acanthopanax senticosus* (Rupr. & Maxim.) Harms]. It contains NLT 0.08% of the sum of eleutheroside B and eleutheroside E, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ■ HPTLC for Articles of Botanical Origin** <203> ■ _{2S} (USP38)

Solvent: Alcohol and water (1:1)

Standard solution A: 1 mg/mL of USP Eleutheroside E RS in *methanol*

Standard solution B: 1 mg/mL of USP Eleutheroside B RS in *methanol*

Standard solution C: 0.1 g of USP Powdered Eleuthero Extract RS in 5 mL of *Solvent*.

Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: Transfer about 1 g of finely powdered Eleuthero

■ Eleuthero Root and Rhizome ■_{2S} (USP39)

to a centrifuge tube, add 5 mL of *Solvent*, and mix well. Sonicate for 10 min. Centrifuge or filter the solution, and use the supernatant or the filtrate.

Adsorbent: Chromatographic silica gel with an average particle size of 5 μm (HPTLC plates)

Application volume: 10 μL, as bands

■ **Relative humidity:** Condition the plate to a relative humidity of 33%.

■ **Temperature:** Ambient, not to exceed 30° ■_{2S} (USP39)

Developing solvent system: *Chloroform*, *methanol*, and water (35:15:2)

■ **Developing distance:** 6 cm ■_{2S} (USP39)

Derivatization reagent: To 18 mL of ice-cold *methanol* slowly and carefully add 2 mL of *sulfuric acid*, and mix well. Allow the mixture to adjust to room temperature.

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

~~Before the development of the chromatogram, saturate the chamber for 20 min with *Developing solvent system*. If the ambient relative humidity exceeds 50%, condition the plate to about 30% relative humidity, using a suitable device. Develop the plate over a path of 6 cm, dry, and spray~~

■ Apply the *Samples* as bands and dry in air. Develop in a saturated chamber and dry in air. Treat the plate ■_{2S} (USP39) with *Derivatization reagent*. Heat the plate at 100° for 5 min, and examine under white light and under UV light (365 nm).

Acceptance criteria: Under white light, the *Sample solution* exhibits two brown bands due to eleutheroside E and eleutheroside B at R_F values of about 0.34 and 0.45, corresponding in color and R_F to the bands exhibited by *Standard solution A* and *Standard solution B*, respectively. The *Sample solution* also exhibits two additional brown bands near the application zone, corresponding in color and R_F to the bands exhibited by *Standard solution C*. Other bands may be observed in the *Sample solution* and *Standard solution C* chromatograms. Under UV light, the *Sample solution* shows a brown band due to eleutheroside E corresponding in color and R_F to the band exhibited by *Standard solution A*.

- **B. HPLC:** The *Sample solution* in the test for *Content of Eleutherosides B and E* shows a peak at the retention time corresponding to that of eleutheroside B in *Standard solution B* and a peak at the retention time corresponding to that of eleutheroside E in *Standard solution A*.

COMPOSITION

Change to read:

- **Content of Eleutherosides B and E**

Solvent: *Methanol* and water (1:1)

Solution A: *Acetonitrile* and water (5:95)

Solution B: *Acetonitrile* and water (60:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	97	3
5	97	3
30	60	40
31	5	95
45	5	95
45.1	97	3
60	97	3

Standard solution A: 0.1 mg/mL of USP Eleutheroside E RS in *methanol*. Transfer 2.0 mL to a 5-mL volumetric flask, and dilute with *Solvent* to volume.

Standard solution B: 0.1 mg/mL of USP Eleutheroside B RS in *methanol*. Transfer 2.0 mL to a 5-mL volumetric flask, and dilute with *Solvent* to volume.

Standard solution C: 5.0 mg/mL of USP Powdered Eleuthero Extract RS in *Solvent*.

Sonicate for 30 min, cool to room temperature, decant, and pass through a nylon filter of 0.45- μ m or finer pore size.

Sample solution: Transfer about 5.0 g of finely ground Eleuthero

■ Eleuthero Root and Rhizome, ■_{2S} (USP39)

accurately weighed, to a round-bottom flask equipped with a condenser. Add 50 mL of *Solvent*, and heat under reflux for 30 min. Filter the supernatant through cotton wool into a 100-mL volumetric flask. Transfer the cotton wool to the round-bottom flask, and repeat the extraction twice, using 22 mL of *Solvent* for each extraction. Filter through cotton wool into the volumetric flask, wash the residue and the cotton wool with *Solvent*, cool to room temperature, dilute with *Solvent* to volume, and mix. Before injection, pass through a nylon filter of 0.45- μ m or finer pore size, discarding the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.0-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *Standard solution B* and *Standard solution C*

Suitability requirements

Chromatogram similarity: The chromatogram from *Standard solution C* is similar to

the reference chromatogram provided with the lot of USP Powdered Eleuthero Extract RS being used.

Relative standard deviation: NMT 2.0% determined from the eleutheroside B peak in replicate injections, *Standard solution B*

Analysis

Samples: *Standard solution A*, *Standard solution B*, ~~*Standard solution C*~~,

■ ■ 2S (USP39)
and *Sample solution*

Identify the eleutheroside B and eleutheroside E peaks in the *Sample solution* by comparison with the chromatograms of *Standard solution B* and *Standard solution A*, respectively.

Separately calculate the percentages of eleutheroside B and eleutheroside E in the portion of Eleuthero

■ Eleuthero Root and Rhizome ■ 2S (USP39)
taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of eleutheroside E or eleutheroside B from *Standard solution A* or *Standard solution B*, respectively

C_S = concentration of USP Eleutheroside E RS or USP Eleutheroside B RS in *Standard solution A* or *Standard solution B*, respectively (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Eleuthero

■ Eleuthero Root and Rhizome ■ 2S (USP39)
taken to prepare the *Sample solution* (mg)

Acceptance criteria: NLT 0.08% for the sum of percentages of eleutheroside B and eleutheroside E on the dried basis

CONTAMINANTS

Delete the following:

- ● **Heavy Metals** (231), *Method III*: NMT 20 ppm ■ 2S (USP38)

Add the following:

- ● **Articles of Botanical Origin** (561), *Limits of Elemental Impurities*: Meets the requirements ■ 2S (USP38)
- **Articles of Botanical Origin** (561), *Pesticide Residue Analysis*: Meets the requirements
- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria do not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

- **Botanical Characteristics**

Macroscopic: The rhizome is knotty and of irregular cylindrical shape with a diameter of 15–40 mm. The heartwood area is light brown, and the connecting splint wood is pale yellow. The bark is approximately 2 mm thick and is firmly affixed to the xylem. The surface is gray-brown or black-brown, coarse, and longitudinally valliculate and plicate. A broken rhizome is coarse and fibrous, particularly inside the xylem. The fractured surface of the bark shows short, thin fibers. Numerous roots spring from the underside of the rhizome. These roots are 35–150 mm long, cylindrical and knotty, with a diameter of 3–15 mm. The surface of the roots is gray-brown to black-brown, smoother than the rhizome, and has longitudinal stripes. A 0.5-mm thin bark is tightly affixed to the pale yellow xylem. A broken root is sparsely fibrous and appears yellowish-gray where the thin epidermis is flaked off.

Microscopic: The roots have five to seven rows of brown cork cells. Secretory canals with brown contents appear in groups of four or five and are NMT 20 µm in diameter. Phloem fibers with thick lignified walls occur singly or in small groups; there are cluster crystals of calcium oxalate in the phloem parenchyma. Parenchymatous cells surround the secretory cells, and medullary ray cells contain small starch granules. The xylem shows reticulately thickened and pitted vessels. The rhizome is similar to the roots except for larger secretory canals, up to 25 µm in diameter, and the presence of a pith with parenchymatous cells containing starch granules.

- **Loss on Drying** (731)

Analysis: Dry at 105° to constant weight.

Acceptance criteria: NMT 14.0%

- **Articles of Botanical Origin** (561), *Methods of Analysis, Total Ash:* NMT 8.0%
- **Articles of Botanical Origin** (561), *Methods of Analysis, Water-Soluble Extractives, Method 2:* NLT 4.0%
- **Articles of Botanical Origin** (561), *Methods of Analysis, Foreign Organic Matter:* NMT 3.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
- **Labeling:** The label states the Latin binomial and, following the official name, the parts of the plant contained in the article.
- **USP Reference Standards** (11)
 - USP Powdered Eleuthero Extract RS
 - USP Eleutheroside B RS
 - β-d-Glucopyranoside, 4-(3-hydroxy-1-propenyl)-2,6-dimethoxyphenyl.
 - C₁₇H₂₄O₉ 372.37
 - USP Eleutheroside E RS
 - β-d-Glucopyranoside, (tetrahydro-1*H*,3*H*-furo(3,4-*c*)furan-1,4-diyl)bis(2,6-dimethoxy-4,1-phenylene)bis-
 - C₃₄H₄₆O₁₈ 742.70

Powdered Eleuthero, page 7820 of the *Second Supplement to USP 38*. It is proposed to change the title of this monograph to *Eleuthero Root and Rhizome Powder* to comply with the proposed *Guideline for Assigning Titles to USP Dietary Supplement Monographs*, as outlined in the eponymous *Stimuli* article published in *PF 41(6)* [Nov–Dec. 2015].

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C162761

Comment deadline: November 30, 2015

Change to read:

Powdered Eleuthero

■ **Eleuthero Root and Rhizome Powder** ■_{2S} (USP39)

DEFINITION

Change to read:

~~Powdered Eleuthero is Eleuthero—~~

■ **Eleuthero Root and Rhizome Powder** is Eleuthero Root and Rhizome ■_{2S} (USP39) reduced to a powder or very fine powder. It contains NLT 0.08% of the sum of eleutheroside B and eleutheroside E, calculated on the dried basis.

IDENTIFICATION

Change to read:

● **A. ■ HPTLC for Articles of Botanical Origin** (203) ■_{2S} (USP38)

Solvent: Alcohol and water (1:1)

Standard solution A: 1 mg/mL of USP Eleutheroside E RS in *methanol*

Standard solution B: 1 mg/mL of USP Eleutheroside B RS in *methanol*

Standard solution C: 0.1 g of USP Powdered Eleuthero Extract RS in 5 mL of *Solvent*.
Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: Transfer about 1 g of ~~Powdered Eleuthero~~

■ **Eleuthero Root and Rhizome Powder** ■_{2S} (USP39)

to a centrifuge tube, add 5 mL of *Solvent*, and mix well. Sonicate for 10 min. Centrifuge or filter the solution, and use the supernatant or the filtrate.

Adsorbent: Chromatographic silica gel with an average particle size of 5 μm (HPTLC plates)

Application volume: 10 μL, as bands

■ **Relative humidity:** Condition the plate to a relative humidity of 33%.

Temperature: Ambient, not to exceed 30° ■_{2S} (USP39)

Developing solvent system: *Chloroform*, *methanol*, and water (35:15:2)

■ **Developing distance:** 6 cm ■_{2S} (USP39)

Derivatization reagent: To 18 mL of ice-cold *methanol* slowly and carefully add 2 mL of *sulfuric acid*, and mix well. Allow the mixture to adjust to room temperature.

Analysis

Samples: *Standard solution A, Standard solution B, Standard solution C, and Sample solution*

~~Before the development of the chromatogram, saturate the chamber for 20 min with *Developing solvent system*. If the ambient relative humidity exceeds 50%, condition the plate to about 30% relative humidity, using a suitable device. Develop the plate over a path of 6 cm, dry, and spray~~

■ Apply the *Samples* as bands and dry in air. Develop in a saturated chamber and dry in air. Treat the plate ■ 2S (USP39) with *Derivatization reagent*. Heat the plate at 100° for 5 min, and examine under white light and under UV light (365 nm).

Acceptance criteria: Under white light, the *Sample solution* exhibits two brown bands due to eleutheroside E and eleutheroside B at R_F values of about 0.34 and 0.45, corresponding in color and R_F to the bands exhibited by *Standard solution A* and *Standard solution B*, respectively. The *Sample solution* also exhibits two additional brown bands near the application zone, corresponding in color and R_F to the bands exhibited by *Standard solution C*. Other bands may be observed in the *Sample solution* and *Standard solution C* chromatograms. Under UV light, the *Sample solution* shows a brown band due to eleutheroside E corresponding in color and R_F to the band exhibited by *Standard solution A*.

- **B. HPLC:** The *Sample solution* in the test for *Content of Eleutherosides B and E* shows a peak at the retention time corresponding to that of eleutheroside B in *Standard solution B* and a peak at the retention time corresponding to that of eleutheroside E in *Standard solution A*.

COMPOSITION

Change to read:

- **Content of Eleutherosides B and E**

Solvent: *Methanol* and water (1:1)

Solution A: *Acetonitrile* and water (5:95)

Solution B: *Acetonitrile* and water (60:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	97	3
5	97	3
30	60	40
31	5	95
45	5	95
45.1	97	3
60	97	3

Standard solution A: 0.1 mg/mL of USP Eleutheroside E RS in *methanol*. Transfer 2.0 mL to a 5-mL volumetric flask, and dilute with *Solvent* to volume.

Standard solution B: 0.1 mg/mL of USP Eleutheroside B RS in *methanol*. Transfer 2.0 mL to a 5-mL volumetric flask, and dilute with *Solvent* to volume.

Standard solution C: 5.0 mg/mL of USP Powdered Eleuthero Extract RS in *Solvent*. Sonicate for 30 min, cool to room temperature, decant, and pass through a nylon filter of 0.45- μ m or finer pore size.

Sample solution: Transfer 5.0 g of Powdered Eleuthero

■ Eleuthero Root and Rhizome Powder, ■ 2S (USP39)

accurately weighed, to a round-bottom flask equipped with a condenser. Add 50 mL of *Solvent*, and heat under reflux for 30 min. Filter the supernatant through cotton wool into a 100-mL volumetric flask. Transfer the cotton wool to the round-bottom flask, and repeat the extraction twice, using 22 mL of *Solvent* for each extraction. Filter through cotton wool into the volumetric flask, wash the residue and the cotton wool with *Solvent*, cool to room temperature, dilute with *Solvent* to volume, and mix. Before injection, pass through a nylon filter of 0.45- μ m or finer pore size, discarding the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.0-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *Standard solution B* and *Standard solution C*

Suitability requirements

Chromatogram similarity: The chromatogram from *Standard solution C* is similar to the reference chromatogram provided with the lot of USP Powdered Eleuthero Extract RS being used.

Relative standard deviation: NMT 2.0%, determined from the eleutheroside B peak in replicate injections, *Standard solution B*

Analysis

Samples: *Standard solution A*, *Standard solution B*, ~~*Standard solution C*~~,

■ ■ 2S (USP39)

and *Sample solution*

Identify the eleutheroside B and eleutheroside E peaks in the *Sample solution* by comparison with the chromatograms of *Standard solution B* and *Standard solution A*, respectively.

Separately calculate the percentages of eleutheroside B and eleutheroside E in the portion of Powdered Eleuthero

■ Eleuthero Root and Rhizome Powder ■ 2S (USP39)

taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of eleutheroside E or eleutheroside B from *Standard solution A* or *Standard solution B*, respectively

C_S = concentration of eleutheroside E or eleutheroside B in *Standard solution A* or *Standard solution B*, respectively (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Powdered Eleuthero

■ Eleuthero Root and Rhizome Powder ■ 2S (USP39)

taken to prepare the *Sample solution* (mg)

Acceptance criteria: NLT 0.08% for the sum of the percentages of eleutheroside B and eleutheroside E on the dried basis

CONTAMINANTS

Delete the following:

- • **Heavy Metals** (231), *Method III*: NMT 20 ppm ■ 2S (USP38)

Add the following:

- • **Articles of Botanical Origin** (561), *Limits of Elemental Impurities*: Meets the requirements ■ 2S (USP38)
- **Articles of Botanical Origin** (561), *Pesticide Residue Analysis*: Meets the requirements
- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria do not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

- **Botanical Characteristics:** The powder is brown with a faint aromatic odor and a slightly acrid, persistent taste. Groups of secretory canals with brown contents are surrounded by parenchymatous cells containing cluster crystals of calcium oxalate. The parenchymatous cells show small starch granules, thick-walled lignified fibers, and fragments of reticulate and pitted vessels. It turns bright yellow when mounted in sodium hydroxide solution.
- **Loss on Drying** (731)
 - Analysis:** Dry at 105° to constant weight.
 - Acceptance criteria:** NMT 14.0%
- **Articles of Botanical Origin** (561), *Methods of Analysis, Total Ash*: NMT 8.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
- **Labeling:** The label states the Latin binomial and, following the official name, the part of the

plant from which the article was derived.

- **USP Reference Standards** <11>

USP Powdered Eleuthero Extract RS

USP Eleutheroside B RS

β -d-Glucopyranoside, 4-(3-hydroxy-1-propenyl)-2,6-dimethoxyphenyl.

C₁₇H₂₄O₉ 372.37

USP Eleutheroside E RS

β -d-Glucopyranoside, (tetrahydro-1*H*,3*H*-furo(3,4-*c*)furan-1,4-diyl)bis(2,6-dimethoxy-4,1-phenylene)bis-.

C₃₄H₄₆O₁₈ 742.70

BRIEFING

Powdered Eleuthero Extract, *USP 38* page 6017. It is proposed to change the title of this monograph to *Eleuthero Root and Rhizome Dry Extract* to comply with the proposed *Guideline for Assigning Titles to USP Dietary Supplement Monographs*, as outlined in the eponymous *Stimuli* article published in *PF 41(6)* [Nov–Dec. 2015]. It is also proposed to remove the test for *Alcohol Determination* <611> as inapplicable to dry extracts.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C162762

Comment deadline: November 30, 2015

Change to read:

Powdered Eleuthero Extract

- **Eleuthero Root and Rhizome Dry Extract** ■_{2S} (*USP39*)

DEFINITION

Change to read:

~~Powdered Eleuthero Extract~~

- **Eleuthero Root and Rhizome Dry Extract** ■_{2S} (*USP39*)

is prepared from ~~Eleuthero~~

- **Eleuthero Root and Rhizome** ■_{2S} (*USP39*)

using hydroalcoholic mixtures. The ratio of the starting crude plant material to ~~Powdered~~

- **Dry** ■_{2S} (*USP39*)

Extract is between 13:1 and 25:1. It contains NLT 0.8% of eleutherosides B and E, calculated on the anhydrous basis. It may contain added substances.

IDENTIFICATION

Change to read:

- ~~A. Thin-Layer Chromatographic Identification Test~~ ⁽²⁰¹⁾
 - **HPTLC for Articles of Botanical Origin <203>** ■_{2S} (*USP39*)

Solvent: Alcohol and water (1:1)

Standard solution A: 1 mg/mL of USP Eleutheroside E RS in *methanol*

Standard solution B: 1 mg/mL of USP Eleutheroside B RS in *methanol*

Standard solution C: 0.1 g of USP Powdered Eleuthero Extract RS in 5 mL of *Solvent*.
Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: 0.1 g of Powdered Extract

▪ Eleuthero Root and Rhizome Dry Extract ■ 2S (USP39)

in 5 mL of *Solvent*. Sonicate for 10 min, centrifuge, and use the supernatant.

Adsorbent: Chromatographic silica gel with an average particle size of 5 µm (HPTLC plates)

Application volume: 10 µL, as bands

▪ **Relative humidity:** Condition the plate to a relative humidity of 33%.

▪ **Temperature:** Ambient, not to exceed 30° ■ 2S (USP39)

Developing solvent system: *Chloroform*, *methanol*, and water (35:15:2)

▪ **Developing distance:** 6 cm ■ 2S (USP39)

Derivatization reagent: To 18 mL of ice-cold *methanol* slowly and carefully add 2 mL of *sulfuric acid*, and mix well. Allow the mixture to adjust to room temperature.

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

~~Before the development of the chromatogram, saturate the chamber for 20 min with *Developing solvent system*. If the ambient relative humidity exceeds 50%, condition the plate to about 30% relative humidity, using a suitable device. Develop the plate over a path of 6 cm, dry, and spray~~

▪ Apply the *Samples* as bands and dry in air. Develop in a saturated chamber and dry in air. Treat the plate ■ 2S (USP39)

with *Derivatization reagent*, heat at 100° for 5 min, and examine under white light and under UV light (365 nm).

Acceptance criteria: Under white light, the *Sample solution* exhibits two brown bands due to eleutheroside E and eleutheroside B at R_F values of about 0.34 and 0.45, corresponding in color and R_F to the bands exhibited by *Standard solution A* and *Standard solution B*, respectively. The *Sample solution* also exhibits two additional brown bands near the application zone, corresponding in color and R_F to the bands exhibited by *Standard solution C*. Other bands may be observed in the *Sample solution* and *Standard solution C* chromatograms. Under UV light, the *Sample solution* shows a brown band due to eleutheroside E corresponding in color and R_F to the band exhibited by *Standard solution A*.

- **B. HPLC:** The *Sample solution* in the test for *Content of Eleutherosides B and E* shows a peak at a retention time corresponding to that of eleutheroside B in *Standard solution B* and a peak at a retention time corresponding to that of eleutheroside E in *Standard solution A*.

COMPOSITION

Change to read:

- **Content of Eleutherosides B and E**

Solvent: *Methanol* and water (1:1)

Solution A: *Acetonitrile* and water (5:95)

Solution B: *Acetonitrile* and water (60:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	97	3
5	97	3
30	60	40
31	5	95
45	5	95
45.1	97	3
60	97	3

Standard solution A: 0.1 mg/mL of USP Eleutheroside E RS in *methanol*. Transfer 2.0 mL to a 5-mL volumetric flask, and dilute with *Solvent* to volume.

Standard solution B: 0.1 mg/mL of USP Eleutheroside B RS in *methanol*. Transfer 2.0 mL to a 5-mL volumetric flask, and dilute with *Solvent* to volume.

Standard solution C: 5.0 mg/mL of USP Powdered Eleuthero Extract RS in *Solvent*. Sonicate for 30 min, cool to room temperature, and decant. Before injection, pass through a nylon filter of 0.45- μ m or finer pore size, discarding the first few mL of the filtrate.

Sample solution: Transfer 500 mg of Powdered Extract

▪ Eleuthero Root and Rhizome Dry Extract, accurately weighed, \pm 2S (USP39)

to a 100-mL volumetric flask, add 80 mL of *Solvent*, and sonicate for 30 min. Cool to room temperature, dilute with *Solvent* to volume, and mix. Before injection, pass through a nylon filter of 0.45- μ m or finer pore size, discarding the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.0-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *Standard solution B* and *Standard solution C*

Suitability requirements

Chromatogram similarity: The chromatogram of *Standard solution C* is similar to the reference chromatogram provided with the lot of USP Powdered Eleuthero Extract RS being used.

Relative standard deviation: NMT 2.0% determined from the eleutheroside B peak in

replicate injections, *Standard solution B*

Analysis

Samples: *Standard solution A*, *Standard solution B*, ~~*Standard solution C*~~,

■ **2S (USP39)**

and *Sample solution*

Identify the eleutheroside B and eleutheroside E peaks in the *Sample solution* by comparison with the chromatograms of *Standard solution B* and *Standard solution A*, respectively, and measure the peak responses.

Separately calculate the percentages of eleutheroside B and eleutheroside E in the portion of ~~Powdered Extract~~

■ **Eleuthero Root and Rhizome Dry Extract** ■ **2S (USP39)**

taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the relevant analyte from the ~~*Sample solution*~~

r_S = peak response of eleutheroside E or eleutheroside B from ~~*Standard solution A*~~ or ~~*Standard solution B*~~, respectively

C_S = concentration of eleutheroside E or eleutheroside B from ~~*Standard solution A*~~ or ~~*Standard solution B*~~, respectively (mg/mL)

C_U = concentration of ~~Powdered Extract~~ in the ~~*Sample solution*~~ (mg/mL)

■

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak area of eleutheroside E or eleutheroside B from the *Sample solution*

r_S = peak area of eleutheroside E or eleutheroside B from *Standard solution A* or *Standard solution B*, respectively

C_S = concentration of USP Eleutheroside E RS or USP Eleutheroside B RS in *Standard solution A* or *Standard solution B*, respectively (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Eleuthero Root and Rhizome Dry Extract used to prepare the *Sample solution* (mg) ■ **2S (USP39)**

Acceptance criteria: NLT 0.8% for the sum of percentages of eleutheroside B and eleutheroside E on the anhydrous basis

CONTAMINANTS

Delete the following:

- **Heavy Metals** (231), ~~Method II~~: NMT 20 ppm (Official 1-Dec-2015)
- **Articles of Botanical Origin** (561), *Pesticide Residue Analysis*: Meets the requirements
- **Botanical Extracts** (565), *Preparations, General Pharmacopeial Requirements, Residual Solvents*: Meets the requirements
- **Microbial Enumeration Tests** (2021): The total aerobic microbial count does not exceed 10^4 cfu/g. The total combined yeasts and molds count does not exceed 10^3 cfu/g.

- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

- **Water Determination** (921), *Method Ia*: NMT 5.0%
- **Articles of Botanical Origin** (561), *Methods of Analysis, Total Ash*: NMT 10.0%

Delete the following:

- ● **Alcohol Determination** (611), *Method II*: NMT 0.5% ■ 2S (USP39)

Delete the following:

- ● **Other Requirements**: It meets the requirements for *Residual Solvents* and *Pesticide Residues in Botanical Extracts* (565) ■ 2S (USP39)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage**: Preserve in tight, light-resistant containers.

Change to read:

- **Labeling**: The label states the Latin binomial and, following the official name, the part of the plant from which the article was prepared. The label also indicates the content of eleutherosides, the extracting solvent used for preparation, and the ratio of the starting crude plant material to Powdered
 - Dry ■ 2S (USP39)
 Extract. It meets the requirements in *Botanical Extracts* (565), *Preparations, General Pharmacopeial Requirements, Labeling*.
- **USP Reference Standards** (11)
 - USP Powdered Eleuthero Extract RS
 - USP Eleutheroside B RS
 - β -d-Glucopyranoside, 4-(3-hydroxy-1-propenyl)-2,6-dimethoxyphenyl.
 - $C_{17}H_{24}O_9$ 372.37
 - USP Eleutheroside E RS
 - β -d-Glucopyranoside, (tetrahydro-1*H*,3*H*-furo(3,4-*c*)furan-1,4-diyl)bis(2,6-dimethoxy-4,1-phenylene)bis-
 - $C_{34}H_{46}O_{18}$ 742.70

BRIEFING

Eleuthero Root and Rhizome Dry Extract Capsules. Because there is no existing *USP* monograph for this dietary supplement, a new monograph is being proposed. The liquid chromatographic procedure in the test for *Content of Eleutherosides B and E* is based on analyses performed with the Phenomenex Luna C18 brand of L1 column. The approximate retention times for eleutheroside B and eleutheroside E are 12.3 and 18.9 min, respectively.

(BDSHM: N. Davydova.)

Correspondence Number—C130331

Comment deadline: November 30, 2015

Add the following:

- **Eleuthero Root and Rhizome Dry Extract Capsules**

DEFINITION

Eleuthero Root and Rhizome Dry Extract Capsules contain Eleuthero Root and Rhizome Dry Extract. They contain NLT 95% of the labeled amount of eleutherosides, calculated as the sum of eleutheroside B and eleutheroside E. They may contain suitable added substances.

IDENTIFICATION

- **A. HPLC**

Analysis: Proceed as directed in the test for *Content of Eleutherosides B and E*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits peaks at the retention times corresponding to the peaks due to eleutheroside B and eleutheroside E in the chromatogram of *Standard solution B*.

STRENGTH

- **Content of Eleutherosides B and E**

Extraction solvent: Methanol and water (6:4)

Solution A: 0.2% *o*-Phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
2	90	10
20	70	30
25	70	30
27	90	10
37	90	10

Standard solution A: 0.005 mg/mL of USP Eleutheroside B RS and 0.01 mg/mL of USP Eleutheroside E RS in *Extraction solvent*. Sonicate to dissolve, if necessary.

Standard solution B: 0.5 mg/mL of USP Powdered Eleuthero Extract RS in *Extraction solvent*. Sonicate for 30 min, cool to room temperature, and decant. Before injection, pass through a PVDF membrane filter of 0.45- μ m or finer pore size.

Sample solution: Determine the total weight of 20 Capsules. Open the Capsules and combine their contents in an appropriate container. Weigh the empty Capsule shells and calculate the average fill weight per Capsule. Transfer a portion of the Capsule contents, equivalent to 0.4 mg of eleutherosides (sum of eleutheroside B and eleutheroside E) to a 25-mL volumetric flask. Add 20 mL of *Extraction solvent* and sonicate for 30 min with occasional shaking. Shake the flask manually for 1 min, cool to room temperature, dilute

with *Extraction solvent* to volume, mix well, and pass through a PVDF membrane filter of 0.45- μm or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L1

Column temperature: 35°

Flow rate: 0.8 mL/min

Injection volume: 20 μL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Chromatogram similarity: The chromatogram from *Standard solution B* is similar to the reference chromatogram provided with the lot of USP Powdered Eleuthero Extract RS being used.

Relative standard deviation: NMT 2.0% for the eleutheroside B and eleutheroside E peaks in replicate injections, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Identify the peaks corresponding to eleutheroside B and eleutheroside E in the *Sample solution* chromatogram by comparison with the chromatogram from *Standard solution A* and the reference chromatogram provided with the lot of USP Powdered Eleuthero Extract RS being used. Measure the areas of the analyte peaks.

Calculate the quantity, in mg, of eleutheroside B and eleutheroside E in each Capsule taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (W_{AV}/W)$$

r_U = peak area of relevant eleutheroside from the *Sample solution*

r_S = peak area of corresponding eleutheroside from *Standard solution A*

C_S = concentration of relevant eleutheroside in *Standard solution A* (mg/mL)

V = volume of the solvent taken for preparation of the *Sample solution* (mL)

W_{AV} = average fill weight per Capsule (mg)

W = weight of the sample taken for preparation of the *Sample solution* (mg)

Calculate the percentage of the labeled amount of eleutherosides, as the sum of eleutheroside B and eleutheroside E, in each Capsule taken:

$$\text{Result} = (\Sigma Q_i/L) \times 100$$

ΣQ_i = sum of the quantities of eleutherosides as determined above (mg)

L = labeled amount of eleutherosides (mg)

Acceptance criteria: NLT 95% of the labeled amount of eleutherosides, calculated as the sum of eleutheroside B and eleutheroside E

PERFORMANCE TESTS

- **Disintegration and Dissolution** (2040), *Disintegration*: Meet the requirements
- **Weight Variation** (2091): Meet the requirements

CONTAMINANTS

- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species* and *Test Procedures, Test for Absence of Escherichia coli*: Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling:** The label states the Latin binomial and, following the official name, the part of the plant from which the article was derived. The label states the amount of eleutherosides, as the sum of eleutheroside B and eleutheroside E, and the amount of Powdered Eleuthero Extract in mg/Capsule.
- **USP Reference Standards** (11)
 - USP Powdered Eleuthero Extract RS
 - USP Eleutheroside B RS
 - USP Eleutheroside E RS

■ 2S (USP39)

BRIEFING

Eleuthero Root and Rhizome Dry Extract Tablets. Because there is no existing *USP* monograph for this dietary supplement, a new monograph is being proposed. The liquid chromatographic procedure in the test for *Content of Eleutherosides B and E* is based on analyses performed with the Phenomenex Luna C18 brand of L1 column. The approximate retention times for eleutheroside B and eleutheroside E are 12.3 and 18.9 min, respectively.

(BDSHM: N. Davydova.)

Correspondence Number—C130330

Comment deadline: November 30, 2015

Add the following:

- **Eleuthero Root and Rhizome Dry Extract Tablets**

DEFINITION

Eleuthero Root and Rhizome Dry Extract Tablets contain Eleuthero Root and Rhizome Dry Extract. They contain NLT 95% of the labeled amount of eleutherosides, calculated as the sum

of eleutheroside B and eleutheroside E. They may contain added substances.

IDENTIFICATION

• A. HPLC

Analysis: Proceed as directed in the test for *Content of Eleutherosides B and E*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits peaks at the retention times corresponding to the peaks due to eleutheroside B and eleutheroside E in the chromatogram of *Standard solution B*.

STRENGTH

• Content of Eleutherosides B and E

Extraction solvent: Methanol and water (6:4)

Solution A: 0.2% *o*-Phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
2	90	10
20	70	30
25	70	30
27	90	10
37	90	10

Standard solution A: 0.005 mg/mL of USP Eleutheroside B RS and 0.01 mg/mL of USP Eleutheroside E RS in *Extraction solvent*. Sonicate to dissolve, if necessary.

Standard solution B: 0.5 mg/mL of USP Powdered Eleuthero Extract RS in *Extraction solvent*. Sonicate for 30 min, cool to room temperature, and decant. Before injection, pass through a PVDF membrane filter of 0.45- μ m or finer pore size.

Sample solution: Weigh NLT 20 Tablets, determine the average tablet weight, and finely powder. Transfer a portion of finely powdered Tablets, nominally equivalent to 0.4 mg of eleutherosides (sum of eleutheroside B and eleutheroside E) to a 25-mL volumetric flask. Add 20 mL of *Extraction solvent* and sonicate for 30 min with occasional shaking. Shake the flask manually for 1 min, cool to room temperature, dilute with *Extraction solvent* to volume, mix well, and pass through a PVDF membrane filter of 0.45- μ m or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 35°

Flow rate: 0.8 mL/min

Injection volume: 20 μ L

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Chromatogram similarity: The chromatogram from *Standard solution B* is similar to the reference chromatogram provided with the lot of USP Powdered Eleuthero Extract RS being used.

Relative standard deviation: NMT 2.0% for the eleutheroside B and eleutheroside E peaks in replicate injections, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Identify the peaks corresponding to eleutheroside B and eleutheroside E in the *Sample solution* chromatogram by comparison with the chromatogram from *Standard solution A* and the reference chromatogram provided with the lot of USP Powdered Eleuthero Extract RS being used. Measure the areas of the analyte peaks.

Calculate the quantity, in mg, of eleutheroside B and eleutheroside E in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (W_{AV}/W)$$

r_U = peak area of relevant eleutheroside from the *Sample solution*

r_S = peak area of corresponding eleutheroside from *Standard solution A*

C_S = concentration of relevant eleutheroside in *Standard solution A* (mg/mL)

V = volume of the solvent taken for preparation of the *Sample solution* (mL)

W_{AV} = average Tablet weight (mg)

W = weight of the sample taken for preparation of the *Sample solution* (mg)

Calculate the percentage of the labeled amount of eleutherosides, as the sum of eleutheroside B and eleutheroside E, in each Tablet taken:

$$\text{Result} = (\Sigma Q_i/L) \times 100$$

ΣQ_i = sum of the quantities of eleutherosides as determined above (mg)

L = labeled amount of eleutherosides (mg)

Acceptance criteria: NLT 95% of the labeled amount of eleutherosides, calculated as the sum of eleutheroside B and eleutheroside E

PERFORMANCE TESTS

- **Disintegration and Dissolution** (2040), *Disintegration*: Meet the requirements
- **Weight Variation** (2091): Meet the requirements

CONTAMINANTS

- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.

- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species* and *Test Procedures, Test for Absence of Escherichia coli*: Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling:** The label states the Latin binomial and, following the official name, the part of the plant from which the article was derived. The label states the amount of eleutherosides, as the sum of eleutheroside B and eleutheroside E, and the amount of Powdered Eleuthero Extract in mg/Tablet.
- **USP Reference Standards** (11)
 - USP Powdered Eleuthero Extract RS
 - USP Eleutheroside B RS
 - USP Eleutheroside E RS

■ 2S (USP39)

BRIEFING

Japanese Honeysuckle Flower. Because there is no existing *USP* monograph for this dietary supplement, a new monograph is being proposed. The liquid chromatographic procedures in the tests for *Content of Caffeoylquinic Acids* and *Content of Iridoids* are both conducted using the Thermo Scientific Syncronis C18 brand of *L1* column with 5- μ m packing and a UV detector at 327 and 240 nm, respectively. The typical retention times for neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid are 8.1, 10.8, 11.4, 28.7, 31.2, and 32.6 min, respectively. The typical retention times for sweroside, secoxyloganin, and centauroside are 12.9, 15.2, and 27.3 min, respectively.

(BDSHM: C. Ma.)

Correspondence Number—C159653

Comment deadline: November 30, 2015

Add the following:

- **Japanese Honeysuckle Flower**

DEFINITION

Japanese Honeysuckle Flower consists of the dried flower buds or dried flowers in the early-opening stage of *Lonicera japonica* Thunb. (Fam. Caprifoliaceae) collected in early summer. It contains NLT 3.8% of caffeoylquinic acids, calculated as the sum of chlorogenic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid on the dried basis; and NLT 0.80% of iridoids, calculated as the sum of sweroside, secoxyloganin, and centauroside on the dried basis.

IDENTIFICATION

- **A. HPTLC for Articles of Botanical Origin** (203)

Standard solution A: 0.2 mg/mL of USP Chlorogenic Acid RS, 0.25 mg/mL of USP Rutin RS, and 0.2 mg/mL of USP Luteolin-7-*O*-glucoside RS in methanol

Standard solution B: 10 mg/mL of USP *Lonicera japonica* Flower Dry Extract RS in methanol. Sonicate for 10 min, and filter. Evaporate the filtrate at about 50° under reduced pressure to dryness. Add 5 mL of water to dissolve the residue, and then add 10 mL of ethyl acetate and mix. After the solution separates into two layers, take the ethyl acetate layer. Repeat the extraction one more time. Combine the ethyl acetate extracts, evaporate the solvent at about 50° under reduced pressure to dryness, and dissolve the residue in methanol equivalent to 1/5 of the initial volume of USP *Lonicera japonica* Flower Dry Extract RS solution.

Sample solution: 500 mg of Japanese Honeysuckle Flower, finely powdered, in 10 mL of methanol. Sonicate for 10 min, and filter. Evaporate the filtrate at about 50° under reduced pressure to dryness. Add 5 mL of water to dissolve the residue, and then add 10 mL of ethyl acetate and mix. After the solution separates into two layers, take the ethyl acetate layer. Repeat the extraction one more time. Combine the ethyl acetate extracts, evaporate the solvent at about 50° under reduced pressure to dryness, and add 2 mL of methanol to dissolve the residue.

Chromatographic system

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 µm

Application volume: 5 µL, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Temperature: About 25°

Developing solvent system: The upper layer solution of a mixture of *n*-butyl acetate, formic acid, and water (7:5:5)

Developing distance: 6 cm

Derivatization reagent A: 10 mg/mL of 2-aminoethyl diphenylborinate in methanol

Derivatization reagent B: 50 mg/mL of polyethylene glycol 4000 in alcohol

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Treat the plate with *Derivatization reagent A* and allow to air-dry. Immediately, treat the plate with *Derivatization reagent B*, allow to air-dry, and examine under UV light at 366 nm.

System suitability requirements: Under UV light at 366 nm, in the lower-third section, *Standard solution B* exhibits one blue fluorescent band corresponding in R_f to the band due to chlorogenic acid and one yellow band above chlorogenic acid corresponding in R_f to the band due to luteolin-7-*O*-glucoside in *Standard solution A*. One faint blue fluorescent band is below the chlorogenic acid band and another yellow band, due to rutin, is below the faint blue fluorescent band. In the middle-third section, *Standard solution B* exhibits three

blue fluorescent bands: the band with the highest R_f is due to 3,5-di-*O*-caffeoylquinic acid; the band with the middle R_f is due to 4,5-di-*O*-caffeoylquinic acid; and the band with the lowest R_f is due to 3,4-di-*O*-caffeoylquinic acid.

Acceptance criteria: Under UV light at 366 nm, the *Sample solution* exhibits the most intense band corresponding in R_f and color to the band of chlorogenic acid and a yellow band above chlorogenic acid corresponding in R_f and color to the band due to luteolin-7-*O*-glucoside in *Standard solution A*. The *Sample solution* exhibits another yellow band in the lower-third section corresponding to rutin and two blue bands in the middle-third section due to 3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid corresponding in R_f and color to similar bands in *Standard solution B*.

- **B. Caffeoylquinic Acids HPLC Profile**

Analysis: Proceed as directed in the test for *Content of Caffeoylquinic Acids*.

Acceptance criteria: The *Sample solution* exhibits the most intense peak with a retention time corresponding to chlorogenic acid in *Standard solution A* and peaks at the retention times corresponding to 3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid in *Standard solution B*. It meets the content ratios in *Table 2*.

- **C. Iridoids HPLC Profile**

Analysis: Proceed as directed in the test for *Content of Iridoids*.

Acceptance criteria: The *Sample solution* exhibits a peak with a retention time corresponding to secoxyloganin in *Standard solution A* and two additional iridoid peaks of sweroside and centauroside at retention times corresponding to the same iridoids in *Standard solution B*.

COMPOSITION

- **Content of Caffeoylquinic Acids**

Solution A: 0.1% Phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	86	14
8	81	19
14	81	19
34	69	31
35	10	90
39.5	10	90
40	86	14
48	86	14

[Note—Protect from light and proceed under low actinic light. The *Standard solutions* and the *Sample solution* are stable for 24 h at room temperature.]

Solvent: Methanol and water (7.5: 2.5)

Standard solution A: 0.30 mg/mL of USP Chlorogenic Acid RS in methanol

Standard solution B: 2.5 mg/mL of USP *Lonicera japonica* Flower Dry Extract RS in *Solvent*. Sonicate for 15 min, and pass through a membrane filter of 0.45- μ m or finer pore size.

Sample solution: Accurately transfer about 100 mg of Japanese Honeysuckle Flower, finely powdered, into a suitable stoppered conical flask, and accurately add 10.0 mL of *Solvent*. Weigh the filled flask with a precision of ± 0.1 mg, stopper, and sonicate for 30 min. After cooling to room temperature, adjust to the initial weight by adding *Solvent*. Pass through a membrane filter of 0.45- μ m or finer pore size, and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 327 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing *L1*

Column temperature: 15 $^{\circ}$

Flow rate: 0.7 mL/min

Injection volume: 2 μ L

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Resolution: NLT 1.5 between the chlorogenic acid and cryptochlorogenic acid peaks, and the 3,4-di-*O*-caffeoylquinic acid and 3,5-di-*O*-caffeoylquinic acid peaks, *Standard solution B*

Tailing factor: NMT 2.0 for the chlorogenic acid peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the chlorogenic acid peak in repeated injections, *Standard solution A*

Chromatographic similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram for caffeoylquinic acids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatograms of *Standard solution A* and *Standard solution B*, and the reference chromatogram for caffeoylquinic acids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used, identify the retention times of the peaks of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid in the *Sample solution*. [Note—See *Table 2* for relative retention times. These values are not monograph requirements. They may vary due to differences in the chromatographic conditions allowed by the system suitability requirements.]

Table 2

Analyte	Approximate Relative Retention Time	Conversion Factor	Content Ratio
Neochlorogenic acid ^a	0.75	1.00	Minor peak ^b
Chlorogenic acid ^c	1.00	1.00	1.0
Cryptochlorogenic acid ^d	1.05	1.00	Minor peak ^b
3,4-Di- <i>O</i> -caffeoylquinic acid ^e	2.65	0.92	Minor peak ^b
3,5-Di- <i>O</i> -caffeoylquinic acid ^f	2.88	0.77	0.3–0.8
4,5-Di- <i>O</i> -caffeoylquinic acid ^g	3.01	0.77	0.04–0.2

^a (1*R*,3*R*,4*S*,5*R*)-3-{[(*E*)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy}-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

^b The peak area is lower than the peak area of 4,5-di-*O*-caffeoylquinic acid.

^c (1*S*,3*R*,4*R*,5*R*)-3-{[(*E*)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy}-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

^d (1*S*,3*R*,4*S*,5*R*)-4-{[(*E*)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy}-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

^e (1*S*,3*R*,4*R*,5*R*)-3,4-Bis{[(*E*)-3-(3,4-dihydroxyphenyl)acryloyl]oxy}-1,5-dihydroxycyclohexane-1-carboxylic acid.

^f (1*S*,3*R*,4*S*,5*R*)-3,5-Bis{[(*E*)-3-(3,4-dihydroxyphenyl)acryloyl]oxy}-1,5-dihydroxycyclohexane-1-carboxylic acid.

^g (1*R*,3*R*,4*S*,5*R*)-3,4-Bis{[(*E*)-3-(3,4-dihydroxyphenyl)acryloyl]oxy}-1,5-dihydroxycyclohexane-1-carboxylic acid.

Separately calculate the percentages of chlorogenic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid in the portion of Japanese Honeysuckle Flower taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of chlorogenic acid from *Standard solution A*

C_S = concentration of USP Chlorogenic Acid RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Japanese Honeysuckle Flower taken to prepare the *Sample solution* (mg)

F = conversion factor for the analyte (see *Table 2*)

Calculate the content of caffeoylquinic acids as the sum of the percentages of chlorogenic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid.

Acceptance criteria: NLT 3.8% on the dried basis

• **Content of Iridoids**

Solution A, Solution B, Mobile phase, Standard solution B, and Sample solution:

Prepare as directed in the test for *Content of Caffeoylquinic Acids*.

Standard solution A: 0.05 mg/mL of USP Secoxyloganin RS in methanol

Chromatographic system: Proceed as directed in the test for *Content of Caffeoylquinic Acids* except for the *Detector*.

Detector: UV 240 nm

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Resolution: NLT 1.5 between the peak of secoxyloganin and the peak before it, *Standard solution B*

Tailing factor: NMT 2.0 for the secoxyloganin peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the secoxyloganin peak in repeated injections, *Standard solution A*

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram for iridoids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatograms of *Standard solution A* and *Standard solution B*, and the reference chromatogram for iridoids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used, identify the peaks of sweroside, secoxyloganin, and centauroside in the *Sample solution*. [Note—The approximate relative retention times for the peaks of sweroside, secoxyloganin, and centauroside are 0.85, 1.00, and 1.80, respectively.]

Separately calculate the percentages of secoxyloganin, sweroside, and centauroside in the portion of Japanese Honeysuckle Flower taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of secoxyloganin from *Standard solution A*

C_S = concentration of USP Secoxyloganin RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Japanese Honeysuckle Flower taken to prepare the *Sample solution* (mg)

F = conversion factor for the analyte (1.00 for secoxyloganin, 1.03 for sweroside, and 0.89 for centauroside)

Calculate the content of iridoids as the sum of the percentages of secoxyloganin, sweroside, and centauroside.

Acceptance criteria: NLT 0.80% on the dried basis

CONTAMINANTS

- **Articles of Botanical Origin** (561), *Limits of Elemental Impurities*: Meets the requirements
- **Articles of Botanical Origin** (561), *Pesticide Residue Analysis*: Meets the requirements
- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria does not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

- **Botanical Characteristics**

Macroscopic: Flower buds are clavate, tapered downwards, slightly curved; 2–3 cm long, about 3 mm in diameter in the upper part, and 1.5 mm in diameter in the lower part with densely pubescent surface; externally yellowish white, greenish white to yellowish green, gradually darkening over time to a golden color. The foliaceous bracts are occasionally visible. The calyx is green, pubescent, five-lobed at the apex, about 2 mm long. The corollas of opening flowers are tubular and two-lipped at the apex. The stamens are epipetalous, yellow, in groups of five, and the one ovary is glabrous.

Microscopic: The powder contains numerous glandular hairs. The heads of glandular hairs are multicellular, subround or slightly oblate, usually 30–70 μm in diameter, exceptionally up to 110 μm . The stalks of glandular hairs are unicellular or multicellular with up to five cells, usually 20–70 μm long, exceptionally up to 700 μm . Non-glandular hairs occur in two types: one with thick walls, unicellular, 45–900 μm long, 15–40 μm in diameter, with fine verrucae on the surface, some have corneous spirals; another type with thin walls, slender, curved or shrunken, with fine verrucae on the surface. Clusters of calcium oxalate are usually 6–45 μm in diameter. Pollen grains are spherical, with three germinal pores, 60–90 μm in diameter.

- **Limit of Triterpenoid Saponins**

Standard solution: 5.0 mg/mL of USP *Lonicera macranthoides* Flower Dry Extract RS in methanol. Sonicate for 10 min, and filter.

Sample solution: 500 mg of Japanese Honeysuckle Flower, finely powdered, in 10 mL of methanol. Sonicate for 10 min, and filter.

Chromatographic system

(See HPTLC for Articles of Botanical Origin (203).)

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 μm

Application volume: 3 μL , as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Developing solvent system: The upper layer solution of a mixture of *n*-butanol, formic acid, and water (4:1:5)

Developing distance: 6 cm

Derivatization reagent: 10% Sulfuric acid in ethanol

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Treat the plate with *Derivatization reagent*, heat at 105° for 5 min, and examine immediately under UV light at 366 nm.

System suitability requirements: Under UV light at 366 nm, in the lower-third section, the *Standard solution* exhibits three clearly separated brown bands: the band with the highest R_f is due to dipsacocide B; the middle band is due to macranthoidin A; the band with the lowest R_f is due to macranthoidin B.

Acceptance criteria: Under UV light at 366 nm, the *Sample solution* does not exhibit any bands corresponding in R_f and color to the bands due to dipsacocide B, macranthoidin A, and macranthoidin B in the *Standard solution*.

- **Articles of Botanical Origin** (561), *Methods of Analysis, Foreign Organic Matter*: NMT 2.0%
- **Articles of Botanical Origin** (561), *Methods of Analysis, Alcohol-Soluble Extractives, Method 1*: NLT 30.0%
- **Articles of Botanical Origin** (561), *Methods of Analysis, Water-Soluble Extractives, Method 2*: NLT 35.0%
- **Loss on Drying** (731)
 - Sample:** 2 g of Japanese Honeysuckle Flower, finely powdered
 - Analysis:** Dry the *Sample* at 105° for 2 h.
 - Acceptance criteria:** NMT 12.0%
- **Articles of Botanical Origin** (561), *Methods of Analysis, Total Ash*
 - Sample:** 4 g of Japanese Honeysuckle Flower, finely powdered
 - Acceptance criteria:** NMT 10.0%
- **Articles of Botanical Origin** (561), *Methods of Analysis, Acid-Insoluble Ash*: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store in a cool place.
- **Labeling:** The label states the Latin binomial and part(s) of the plant contained in the article.
- **USP Reference Standards** (11)
 - USP Chlorogenic Acid RS
 - USP *Lonicera japonica* Flower Dry Extract RS
 - USP *Lonicera macranthoides* Flower Dry Extract RS
 - USP Luteolin-7-*O*-glucoside RS
 - USP Rutin RS
 - USP Secoxyloganin RS

■ 2S (USP39)

Japanese Honeysuckle Flower Dry Extract. Because there is no existing *USP* monograph for this dietary supplement, a new monograph is being proposed. The liquid chromatographic procedures in the tests for *Content of Caffeoylquinic Acids* and *Content of Iridoids* are both conducted using the Thermo Scientific Synchronis C18 brand of *L1* column with 5- μ m packing and a UV detector at 327 and 240 nm, respectively. The typical retention times for neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid are 8.1, 10.8, 11.4, 28.7, 31.2, and 32.6 min, respectively. The typical retention times for sweroside, secoxyloganin, and centauroside are 12.9, 15.2, and 27.3 min, respectively.

(BDSHM: C. Ma.)

Correspondence Number—C159655

Comment deadline: November 30, 2015

Add the following:

▪ **Japanese Honeysuckle Flower Dry Extract**

DEFINITION

Japanese Honeysuckle Flower Dry Extract is prepared from the dried flower buds or dried flowers in the early opening stage of *Lonicera japonica* Thunb. (Fam. Caprifoliaceae) collected in early summer and extracted with water or a hydroalcoholic mixture containing NMT 15% alcohol. Extracts may be further processed by adding alcohol, filtering, evaporating, and then adding water, filtering, and evaporating. It contains NLT 90.0% and NMT 110.0% of the labeled amount of caffeoylquinic acids, calculated as the sum of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid on the dried basis; and NLT 90.0% and NMT 110.0% of the labeled amount of iridoids, calculated as the sum of sweroside, secoxyloganin, and centauroside on the dried basis.

IDENTIFICATION

• **A. HPTLC for Articles of Botanical Origin** <203>

Standard solution A: 0.2 mg/mL of USP Chlorogenic Acid RS, 0.25 mg/mL of USP Rutin RS, and 0.2 mg/mL of USP Luteolin-7-*O*-glucoside RS in methanol

Standard solution B: 10 mg/mL of USP *Lonicera japonica* Flower Dry Extract RS in methanol. Sonicate for 10 min, and filter. Evaporate the filtrate at about 50° under reduced pressure to dryness. Add 5 mL of water to dissolve the residue, and then add 10 mL of ethyl acetate and mix. After the solution separates into two layers, take the ethyl acetate layer. Repeat the extraction one more time. Combine the ethyl acetate extracts, evaporate the solvent at about 50° under reduced pressure to dryness, and dissolve the residue in methanol equivalent to 1/5 of the initial volume of the USP *Lonicera japonica* Flower Dry Extract RS solution.

Sample solution: 100 mg of Japanese Honeysuckle Flower Dry Extract in 10 mL of methanol. Sonicate for 10 min, and filter. Evaporate the filtrate at about 50° under reduced pressure to dryness. Add 5 mL of water to dissolve the residue, and then add 10 mL of ethyl acetate and mix. After the solution separates into two layers, take the ethyl

acetate layer. Repeat the extraction one more time. Combine the ethyl acetate extracts, evaporate the solvent at about 50° under reduced pressure to dryness, and add 2 mL of methanol to dissolve the residue.

Chromatographic system

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 µm

Application volume: 5 µL, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Temperature: About 25°

Developing solvent system: The upper layer solution of a mixture of *n*-butyl acetate, formic acid, and water (7:5:5)

Developing distance: 6 cm

Derivatization reagent A: 10 mg/mL of 2-aminoethyl diphenylborinate in methanol

Derivatization reagent B: 50 mg/mL of polyethylene glycol 4000 in alcohol

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Treat the plate with *Derivatization reagent A* and allow to air-dry. Immediately, treat the plate with *Derivatization reagent B*, allow to air-dry, and examine under UV light at 366 nm.

System suitability requirements: Under UV light at 366 nm, in the lower-third section, *Standard solution B* exhibits one blue fluorescent band corresponding in R_f to the band due to chlorogenic acid and one yellow band above chlorogenic acid corresponding in R_f to the band due to luteolin-7-*O*-glucoside in *Standard solution A*. One faint blue fluorescent band is below the chlorogenic acid band and another yellow band, due to rutin, is below the faint blue fluorescent band. In the middle-third section, *Standard solution B* exhibits three blue fluorescent bands: the band with the highest R_f is due to 3,5-di-*O*-caffeoylquinic acid; the band with the middle R_f is due to 4,5-di-*O*-caffeoylquinic acid; and the band with the lowest R_f is due to 3,4-di-*O*-caffeoylquinic acid.

Acceptance criteria: Under UV light at 366 nm, in the lower-third section, the *Sample solution* exhibits one blue fluorescent band corresponding in R_f and color to the band of chlorogenic acid and one yellow band above chlorogenic acid corresponding in R_f and color to the band of luteolin-7-*O*-glucoside in *Standard solution A*. One faint blue fluorescent band is below the chlorogenic acid band and another yellow band, due to rutin, is below the faint blue fluorescent band corresponding in R_f and color to similar bands in *Standard solution B*. In the middle-third section, the *Sample solution* exhibits three blue fluorescent bands due to 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, and 3,4-di-*O*-caffeoylquinic acid corresponding in R_f and color to similar bands in *Standard solution B*.

• B. Caffeoylquinic Acids HPLC Profile

Analysis: Proceed as directed in the test for *Content of Caffeoylquinic Acids*.

Acceptance criteria: The *Sample solution* exhibits the most intense peak with a retention time corresponding to chlorogenic acid in *Standard solution A* and peaks at the retention

times corresponding to neochlorogenic acid, cryptochlorogenic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid in *Standard solution B*. The chlorogenic acid content is NMT 65% of the total caffeoylquinic acids.

- **C. Iridoids HPLC Profile**

Analysis: Proceed as directed in the test for *Content of Iridoids*.

Acceptance criteria: The *Sample solution* exhibits a peak with a retention time corresponding to secoxyloganin in *Standard solution A* and two additional iridoid peaks of sweroside and centauroside at retention times corresponding to the same iridoids in *Standard solution B*.

COMPOSITION

- **Content of Caffeoylquinic Acids**

Solution A: 0.1% Phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	86	14
8	81	19
14	81	19
34	69	31
35	10	90
39.5	10	90
40	86	14
48	86	14

[Note—Protect from light and proceed under low actinic light. The *Standard solutions* and the *Sample solution* are stable for 24 h at room temperature.]

Solvent: Methanol and water (7.5: 2.5)

Standard solution A: 0.30 mg/mL of USP Chlorogenic Acid RS in methanol

Standard solution B: 2.5 mg/mL of USP *Lonicera japonica* Flower Dry Extract RS in *Solvent*. Sonicate for 15 min, and pass through a membrane filter of 0.45- μ m or finer pore size.

Sample solution: Accurately transfer about 25 mg of Japanese Honeysuckle Flower Dry Extract into a suitable stoppered conical flask, and accurately add 10.0 mL of *Solvent*. Weigh the filled flask with a precision of ± 0.1 mg, stopper, and then sonicate for 30 min. After cooling to room temperature, adjust to the initial weight by adding *Solvent*. Pass through a membrane filter of 0.45- μ m or finer pore size, and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 327 nm

Column: 4.6-mm × 25-cm; 5- μ m packing *L1*

Column temperature: 15°

Flow rate: 0.7 mL/min

Injection volume: 2 μ L

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Resolution: NLT 1.5 between the chlorogenic acid and cryptochlorogenic acid peaks, and the 3,4-di-*O*-caffeoylquinic acid and 3,5-di-*O*-caffeoylquinic acid peaks, *Standard solution B*

Tailing factor: NMT 2.0 for the chlorogenic acid peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the chlorogenic acid peak in repeated injections, *Standard solution A*

Chromatographic similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram for caffeoylquinic acids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatograms of *Standard solution A* and *Standard solution B*, and the reference chromatogram for caffeoylquinic acids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used, identify the retention times of the peaks of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid in the *Sample solution*. [Note—See *Table 2* for relative retention times. These values are not monograph requirements. They may vary due to differences in the chromatographic conditions allowed by the system suitability requirements.]

Table 2

Analyte	Approximate Relative Retention Time	Conversion Factor
Neochlorogenic acid ^a	0.75	1.00
Chlorogenic acid ^b	1.00	1.00
Cryptochlorogenic acid ^c	1.05	1.00
3,4-Di- <i>O</i> -caffeoylquinic acid ^d	2.65	0.92
3,5-Di- <i>O</i> -caffeoylquinic acid ^e	2.88	0.77
4,5-Di- <i>O</i> -caffeoylquinic acid ^f	3.01	0.77

^a (1*R*,3*R*,4*S*,5*R*)-3-{[(*E*)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy}-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

^b (1*S*,3*R*,4*R*,5*R*)-3-{[(*E*)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy}-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

^c (1*S*,3*R*,4*S*,5*R*)-4-{[(*E*)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy}-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

^d (1*S*,3*R*,4*R*,5*R*)-3,4-Bis{[(*E*)-3-(3,4-dihydroxyphenyl)acryloyl]oxy}-1,5-dihydroxycyclohexane-1-carboxylic acid.

^e (1*S*,3*R*,4*S*,5*R*)-3,5-Bis{[(*E*)-3-(3,4-dihydroxyphenyl)acryloyl]oxy}-1,5-dihydroxycyclohexane-1-carboxylic acid.

^f (1*R*,3*R*,4*S*,5*R*)-3,4-Bis{[(*E*)-3-(3,4-dihydroxyphenyl)acryloyl]oxy}-1,5-dihydroxycyclohexane-1-carboxylic acid.

Separately calculate the percentages of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid in the portion of Japanese Honeysuckle Flower Dry Extract taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of chlorogenic acid from *Standard solution A*

C_S = concentration of USP Chlorogenic Acid RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Japanese Honeysuckle Flower Dry Extract taken to prepare the *Sample solution* (mg)

F = conversion factor for the analyte (see *Table 2*)

Calculate the content of caffeoylquinic acids as the sum of the percentages of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid.

Calculate the percentage of the labeled amount of caffeoylquinic acids in the portion of Dry Extract taken:

$$\text{Result} = (P/L) \times 100$$

P = content of caffeoylquinic acids as determined above (%)

L = labeled amount of caffeoylquinic acids (%)

Acceptance criteria: 90.0%–110.0% on the dried basis

- **Content of Iridoids**

Solution A, Solution B, Mobile phase, Standard solution B, and Sample solution:

Prepare as directed in the test for *Content of Caffeoylquinic Acids*.

Standard solution A: 0.05 mg/mL of USP Secoxyloganin RS in methanol

Chromatographic system: Proceed as directed in the test for *Content of Caffeoylquinic Acids* except for the *Detector*.

Detector: UV 240 nm

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Resolution: NLT 1.5 between the peak of secoxyloganin and the peak before it, *Standard solution B*

Tailing factor: NMT 2.0 for the secoxyloganin peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the secoxyloganin peak in repeated injections, *Standard solution A*

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram for iridoids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatograms of *Standard solution A* and *Standard solution B*, and the reference chromatogram for iridoids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used, identify the peaks of sweroside, secoxyloganin, and centauroside in the *Sample solution*. [Note—The approximate relative retention times for the peaks of sweroside, secoxyloganin, and centauroside are 0.85, 1.00, and 1.80, respectively.]

Separately calculate the percentages of secoxyloganin, sweroside, and centauroside in the portion of Japanese Honeysuckle Flower Dry Extract taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of secoxyloganin from *Standard solution A*

C_S = concentration of USP Secoxyloganin RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Japanese Honeysuckle Flower Dry Extract taken to prepare the *Sample solution* (mg)

F = conversion factor for the analyte (1.00 for secoxyloganin, 1.03 for sweroside, and 0.89 for centauroside)

Calculate the content of iridoids as the sum of the percentages of secoxyloganin, sweroside, and centauroside.

Calculate the percentage of the labeled amount of iridoids in the portion of Dry Extract taken:

$$\text{Result} = (P/L) \times 100$$

P = content of iridoids as determined above (%)

L = labeled amount of iridoids (%)

Acceptance criteria: 90.0%–110.0% on the dried basis

CONTAMINANTS

- **Articles of Botanical Origin** (561), *Pesticide Residue Analysis*: Meets the requirements
- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10^4 cfu/g, the total combined molds and yeasts count does not exceed 10^2 cfu/g, and the bile-tolerant Gram-negative bacteria does not exceed 10^2 cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

• Limit of Triterpenoid Saponins

Standard solution: 5.0 mg/mL of USP *Lonicera macranthoides* Flower Dry Extract RS in methanol. Sonicate for 10 min, and filter.

Sample solution: 50 mg of Japanese Honeysuckle Flower Dry Extract in 10 mL of methanol. Sonicate for 10 min, and filter.

Chromatographic system

(See HPTLC for Articles of Botanical Origin (203).)

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 μm

Application volume: 3 μL , as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Developing solvent system: The upper layer solution of a mixture of *n*-butanol, formic acid, and water (4:1:5)

Developing distance: 6 cm

Derivatization reagent: 10% Sulfuric acid in ethanol

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Treat the plate with *Derivatization reagent*, heat at 105° for 5 min, and examine immediately under UV light at 366 nm.

System suitability requirements: Under UV light at 366 nm, in the lower-third section, the *Standard solution* exhibits three clearly separated brown bands: the band with the highest R_f is due to dipsacocide B; the middle band is due to macranthoidin A; the band with the lowest R_f is due to macranthoidin B.

Acceptance criteria: Under UV light at 366 nm, the *Sample solution* does not exhibit any bands corresponding in R_f and color to the bands due to dipsacocide B, macranthoidin A, and macranthoidin B in the *Standard solution*.

- **Loss on Drying** (731)

Sample: 1 g of Japanese Honeysuckle Flower Dry Extract

Analysis: Dry the *Sample* at 105° for 2 h.

Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store in a cool place.
- **Labeling:** The label states the Latin binomial and part(s) of the plant from which the article was prepared. It meets other labeling requirements under *Botanical Extracts* (565).

- **USP Reference Standards** (11)

USP Chlorogenic Acid RS

USP *Lonicera japonica* Flower Dry Extract RS

USP *Lonicera macranthoides* Flower Dry Extract RS

USP Luteolin-7-*O*-glucoside RS

USP Rutin RS

USP Secoxyloganin RS

■ 2S (USP39)

BRIEFING

Japanese Honeysuckle Flower Powder. Because there is no existing *USP* monograph for this dietary supplement, a new monograph is being proposed. The liquid chromatographic procedures in the tests for *Content of Caffeoylquinic Acids* and *Content of Iridoids* are both conducted using the Thermo Scientific Synchronis C18 brand of *L1* column with 5- μ m packing and a UV detector at 327 and 240 nm, respectively. The typical retention times for neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid are 8.1, 10.8, 11.4, 28.7, 31.2, and 32.6 min, respectively. The typical retention times for sweroside, secoxyloganin, and centauroside are 12.9, 15.2, and 27.3 min, respectively.

(BDSHM: C. Ma.)

Correspondence Number—C159654

Comment deadline: November 30, 2015

Add the following:

- **Japanese Honeysuckle Flower Powder**

DEFINITION

Japanese Honeysuckle Flower Powder consists of the dried flower buds or dried flowers in the early opening stage of *Lonicera japonica* Thunb. (Fam. Caprifoliaceae) collected in early summer and reduced to a fine or very fine powder. It contains NLT 3.8% of caffeoylquinic acids, calculated as the sum of chlorogenic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid on the dried basis; and NLT 0.80% of iridoids, calculated as the sum of sweroside, secoxyloganin, and centauroside on the dried basis.

IDENTIFICATION

• A. HPTLC for Articles of Botanical Origin (203)

Standard solution A: 0.2 mg/mL of USP Chlorogenic Acid RS, 0.25 mg/mL of USP Rutin RS, and 0.2 mg/mL of USP Luteolin-7-*O*-glucoside RS in methanol

Standard solution B: 10 mg/mL of USP *Lonicera japonica* Flower Dry Extract RS in methanol. Sonicate for 10 min, and filter. Evaporate the filtrate at about 50° under reduced pressure to dryness. Add 5 mL of water to dissolve the residue, and then add 10 mL of ethyl acetate and mix. After the solution separates into two layers, take the ethyl acetate layer. Repeat the extraction one more time. Combine the ethyl acetate extracts, evaporate the solvent at about 50° under reduced pressure to dryness, and dissolve the residue in methanol equivalent to 1/5 of the initial volume of the USP *Lonicera japonica* Flower Dry Extract RS solution.

Sample solution: 500 mg of Japanese Honeysuckle Flower Powder in 10 mL of methanol. Sonicate for 10 min, and filter. Evaporate the filtrate at about 50° under reduced pressure to dryness. Add 5 mL of water to dissolve the residue, and then add 10 mL of ethyl acetate and mix. After the solution separates into two layers, take the ethyl acetate layer. Repeat the extraction one more time. Combine the ethyl acetate extracts, evaporate the solvent at about 50° under reduced pressure to dryness, and add 2 mL of methanol to dissolve the residue.

Chromatographic system

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 µm

Application volume: 5 µL, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Temperature: About 25°

Developing solvent system: The upper layer solution of a mixture of *n*-butyl acetate, formic acid, and water (7:5:5)

Developing distance: 6 cm

Derivatization reagent A: 10 mg/mL of 2-aminoethyl diphenylborinate in methanol

Derivatization reagent B: 50 mg/mL of polyethylene glycol 4000 in alcohol

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Treat the plate with *Derivatization reagent A* and allow to air-dry. Immediately, treat the plate with

Derivatization reagent B, allow to air-dry, and examine under UV light at 366 nm.

System suitability requirements: Under UV light at 366 nm, in the lower-third section, *Standard solution B* exhibits one blue fluorescent band corresponding in R_f to the band due to chlorogenic acid and one yellow band above chlorogenic acid corresponding in R_f to the band due to luteolin-7-*O*-glucoside in *Standard solution A*. One faint blue fluorescent band is below the chlorogenic acid band and another yellow band, due to rutin, is below the faint blue fluorescent band. In the middle-third section, *Standard solution B* exhibits three blue fluorescent bands: the band with the highest R_f is due to 3,5-di-*O*-caffeoylquinic acid; the band with the middle R_f is due to 4,5-di-*O*-caffeoylquinic acid; and the band with the lowest R_f is due to 3,4-di-*O*-caffeoylquinic acid.

Acceptance criteria: Under UV light at 366 nm, the *Sample solution* exhibits the most intense band corresponding in R_f and color to the band of chlorogenic acid and a yellow band above chlorogenic acid corresponding in R_f and color to the band due to luteolin-7-*O*-glucoside in *Standard solution A*. The *Sample solution* exhibits another yellow band in the lower-third section corresponding to rutin and two blue bands in the middle-third section due to 3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid corresponding in R_f and color to similar bands in *Standard solution B*.

- **B. Caffeoylquinic Acids HPLC Profile**

Analysis: Proceed as directed in the test for *Content of Caffeoylquinic Acids*.

Acceptance criteria: The *Sample solution* exhibits the most intense peak with a retention time corresponding to chlorogenic acid in *Standard solution A* and peaks at the retention times corresponding to 3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid in *Standard solution B*. It meets the content ratios in *Table 2*.

- **C. Iridoids HPLC Profile**

Analysis: Proceed as directed in the test for *Content of Iridoids*.

Acceptance criteria: The *Sample solution* exhibits a peak with a retention time corresponding to secoxyloganin in *Standard solution A* and two additional iridoid peaks of sweroside and centauroside at retention times corresponding to the same iridoids in *Standard solution B*.

COMPOSITION

- **Content of Caffeoylquinic Acids**

Solution A: 0.1% Phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	86	14
8	81	19
14	81	19
34	69	31
35	10	90
39.5	10	90
40	86	14
48	86	14

[Note—Protect from light and proceed under low actinic light. The *Standard solutions* and the *Sample solution* are stable for 24 h at room temperature.]

Solvent: Methanol and water (7.5: 2.5)

Standard solution A: 0.30 mg/mL of USP Chlorogenic Acid RS in methanol

Standard solution B: 2.5 mg/mL of USP *Lonicera japonica* Flower Dry Extract RS in *Solvent*. Sonicate for 15 min, and pass through a membrane filter of 0.45- μ m or finer pore size.

Sample solution: Accurately transfer about 100 mg of Japanese Honeysuckle Flower Powder into a suitable stoppered conical flask, and accurately add 10.0 mL of *Solvent*. Weigh the filled flask with a precision of ± 0.1 mg, stopper, and then sonicate for 30 min. After cooling to room temperature, adjust to the initial weight by adding *Solvent*. Pass through a membrane filter of 0.45- μ m or finer pore size, and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 327 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing *L1*

Column temperature: 15°

Flow rate: 0.7 mL/min

Injection volume: 2 μ L

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Resolution: NLT 1.5 between the chlorogenic acid and cryptochlorogenic acid peaks, and the 3,4-di-*O*-caffeoylquinic acid and 3,5-di-*O*-caffeoylquinic acid peaks, *Standard solution B*

Tailing factor: NMT 2.0 for the chlorogenic acid peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the chlorogenic acid peak in repeated injections, *Standard solution A*

Chromatographic similarity: The chromatogram of *Standard solution B* is similar to

the reference chromatogram for caffeoylquinic acids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatograms of *Standard solution A* and *Standard solution B*, and the reference chromatogram for caffeoylquinic acids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used, identify the retention times of the peaks of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid in the *Sample solution*. [Note—See *Table 2* for relative retention times. These values are not monograph requirements. They may vary due to differences in the chromatographic conditions allowed by the system suitability requirements.]

Table 2

Analyte	Approximate Relative Retention Time	Conversion Factor	Content Ratio
Neochlorogenic acid ^a	0.75	1.00	Minor peak ^b
Chlorogenic acid ^c	1.00	1.00	1.0
Cryptochlorogenic acid ^d	1.05	1.00	Minor peak ^b
3,4-Di- <i>O</i> -caffeoylquinic acid ^e	2.65	0.92	Minor peak ^b
3,5-Di- <i>O</i> -caffeoylquinic acid ^f	2.88	0.77	0.3–0.8
4,5-Di- <i>O</i> -caffeoylquinic acid ^g	3.01	0.77	0.04–0.2

^a (1*R*,3*R*,4*S*,5*R*)-3-{[(*E*)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy}-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

^b The peak area is lower than the peak area of 4,5-di-*O*-caffeoylquinic acid.

^c (1*S*,3*R*,4*R*,5*R*)-3-{[(*E*)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy}-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

^d (1*S*,3*R*,4*S*,5*R*)-4-{[(*E*)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy}-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

^e (1*S*,3*R*,4*R*,5*R*)-3,4-Bis{[(*E*)-3-(3,4-dihydroxyphenyl)acryloyl]oxy}-1,5-dihydroxycyclohexane-1-carboxylic acid.

^f (1*S*,3*R*,4*S*,5*R*)-3,5-Bis{[(*E*)-3-(3,4-dihydroxyphenyl)acryloyl]oxy}-1,5-dihydroxycyclohexane-1-carboxylic acid.

^g (1*R*,3*R*,4*S*,5*R*)-3,4-Bis{[(*E*)-3-(3,4-dihydroxyphenyl)acryloyl]oxy}-1,5-dihydroxycyclohexane-1-carboxylic acid.

Separately calculate the percentages of chlorogenic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid in the portion of Japanese Honeysuckle Flower Powder

taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of chlorogenic acid from *Standard solution A*

C_S = concentration of USP Chlorogenic Acid RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Japanese Honeysuckle Flower Powder taken to prepare the *Sample solution* (mg)

F = conversion factor for the analyte (see *Table 2*)

Calculate the content of caffeoylquinic acids as the sum of the percentages of chlorogenic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid.

Acceptance criteria: NLT 3.8% on the dried basis

- **Content of Iridoids**

Solution A, Solution B, Mobile phase, Standard solution B, and Sample solution:

Prepare as directed in the test for *Content of Caffeoylquinic Acids*.

Standard solution A: 0.05 mg/mL of USP Secoxyloganin RS in methanol

Chromatographic system: Proceed as directed in the test for *Content of Caffeoylquinic Acids* except for the *Detector*.

Detector: UV 240 nm

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Resolution: NLT 1.5 between the peak of secoxyloganin and the peak before it, *Standard solution B*

Tailing factor: NMT 2.0 for the secoxyloganin peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the secoxyloganin peak in repeated injections, *Standard solution A*

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram for iridoids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatograms of *Standard solution A* and *Standard solution B*, and the reference chromatogram for iridoids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used, identify the peaks of sweroside, secoxyloganin, and centauroside in the *Sample solution*. [Note—The approximate relative retention times for the peaks of sweroside, secoxyloganin, and centauroside are 0.85, 1.00, and 1.80, respectively.]

Separately calculate the percentages of secoxyloganin, sweroside, and centauroside in

the portion of Japanese Honeysuckle Flower Powder taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of secoxyloganin from *Standard solution A*

C_S = concentration of USP Secoxyloganin RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Japanese Honeysuckle Flower Powder taken to prepare the *Sample solution* (mg)

F = conversion factor for the analyte (1.00 for secoxyloganin, 1.03 for sweroside, and 0.89 for centauroside)

Calculate the content of iridoids as the sum of the percentages of secoxyloganin, sweroside, and centauroside.

Acceptance criteria: NLT 0.80% on the dried basis

CONTAMINANTS

- **Articles of Botanical Origin** <561>, *Limits of Elemental Impurities*: Meets the requirements
- **Articles of Botanical Origin** <561>, *Pesticide Residue Analysis*: Meets the requirements
- **Microbial Enumeration Tests** <2021>: The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria does not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** <2022>, *Test Procedures, Test for Absence of Salmonella species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

• Botanical Characteristics

Macroscopic: Yellowish-white to yellowish-green powder, darkening over time to a golden color

Microscopic: Glandular hairs are numerous. The heads of glandular hairs are multicellular, subround or slightly oblate, usually 30–70 μm in diameter, exceptionally up to 110 μm . The stalks of glandular hairs are unicellular or multicellular with up to five cells, usually 20–70 μm long, exceptionally up to 700 μm . Non-glandular hairs occur in two types: one with thick walls, unicellular, 45–900 μm long, 15–40 μm in diameter, with fine verrucae on the surface, some have corneous spirals; another type with thin walls, slender, curved, or shrunken, with fine verrucae on the surface. Clusters of calcium oxalate are usually 6–45 μm in diameter. Pollen grains are spherical, with three germinal pores, 60–90 μm in diameter.

• Limit of Triterpenoid Saponins

Standard solution: 5.0 mg/mL of USP *Lonicera macranthoides* Flower Dry Extract RS in methanol. Sonicate for 10 min, and filter.

Sample solution: 500 mg of Japanese Honeysuckle Flower Powder in 10 mL of methanol. Sonicate for 10 min, and filter.

Chromatographic system

(See *HPTLC for Articles of Botanical Origin* (203).)

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 μm

Application volume: 3 μL , as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Developing solvent system: The upper layer solution of a mixture of *n*-butanol, formic acid, and water (4:1:5)

Developing distance: 6 cm

Derivatization reagent: 10% Sulfuric acid in ethanol

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Treat the plate with *Derivatization reagent*, heat at 105° for 5 min, and examine immediately under UV light at 366 nm.

System suitability requirements: Under UV light at 366 nm, in the lower-third section, the *Standard solution* exhibits three clearly separated brown bands: the band with the highest R_f is due to dipsacoside B; the middle band is due to macranthoidin A; the band with the lowest R_f is due to macranthoidin B.

Acceptance criteria: Under UV light at 366 nm, the *Sample solution* does not exhibit any bands corresponding in R_f and color to the bands due to dipsacoside B, macranthoidin A, and macranthoidin B in the *Standard solution*.

- **Articles of Botanical Origin** (561), *Methods of Analysis, Alcohol-Soluble Extractives, Method 1*: NLT 30.0%
- **Articles of Botanical Origin** (561), *Methods of Analysis, Water-Soluble Extractives, Method 2*: NLT 35.0%
- **Loss on Drying** (731)
Sample: 2 g of Japanese Honeysuckle Flower Powder
Analysis: Dry the *Sample* at 105° for 2 h.
Acceptance criteria: NMT 12.0%
- **Articles of Botanical Origin** (561), *Methods of Analysis, Total Ash*
Sample: 4 g of Japanese Honeysuckle Flower Powder
Acceptance criteria: NMT 10.0%
- **Articles of Botanical Origin** (561), *Methods of Analysis, Acid-Insoluble Ash*: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store in a cool place.
- **Labeling:** The label states the Latin binomial and part(s) of the plant from which the article

was derived.

- **USP Reference Standards** <11>

USP Chlorogenic Acid RS
 USP *Lonicera japonica* Flower Dry Extract RS
 USP *Lonicera macranthoides* Flower Dry Extract RS
 USP Luteolin-7-*O*-glucoside RS
 USP Rutin RS
 USP Secoxyloganin RS

■ 2S (USP39)

BRIEFING

Olive Leaf. Because there is currently no existing *USP* monograph for this dietary supplement, a new monograph is being proposed. The liquid chromatographic procedure in the test for *Content of Oleuropein* is performed on the Phenomenex Luna C18(2) brand of L1 column with 5- μ m packing. The typical retention time of oleuropein is 18 min.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C160782

Comment deadline: November 30, 2015

Add the following:

- **Olive Leaf**

DEFINITION

Olive Leaf consists of the dried leaf of *Olea europaea* L. (Fam. Oleaceae). It contains NLT 6.0% of oleuropein ($C_{25}H_{32}O_{13}$), calculated on the dried basis.

IDENTIFICATION

- **A. HPTLC for Articles of Botanical Origin** <203>

Flavonoids

Standard solution A: 1 mg/mL of USP Rutin RS and 2 mg/mL each of USP Verbascoside RS, USP Oleuropein RS, and USP Oleanolic Acid RS in methanol

Standard solution B: 25 mg/mL of USP Olive Leaf Dry Extract RS in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: Suspend about 500 mg of Olive Leaf, finely powdered, in 5 mL of methanol, and sonicate for 10 min. Centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 μ m (HPTLC plate)¹

Application volume: 2 μ L each of *Standard solution A*, *Standard solution B*, and *Sample solution* as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient temperature, not to exceed 30°

Developing solvent system: Ethyl formate, formic acid, toluene, and water (60:8:3:6)

Developing distance: 6 cm

Derivatization reagent A: 5 mg/mL of 2-aminoethyl diphenylborinate in ethyl acetate

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber and dry in air. Heat at 100° for 3 min. While the plate is still warm, treat with *Derivatization reagent A*, and examine under long-wave UV light (365 nm). [Note—Immediately after examination, use the same developed and derivatized plate for *Identification test B*.]

System suitability: Under long-wave UV (365 nm), the derivatized chromatogram of *Standard solution B* displays, in its lower third, three yellowish bands: the first close to the application line; the second located just above, or coincident with, the rutin band in *Standard solution A*; the third, due to luteolin-7-*O*-glucoside positioned above the light-blue band corresponding to verbascoside in *Standard solution A*. There is a bluish-green band just above the band due to rutin. A faint light-blue band may appear just below the verbascoside band. The bands due to verbascoside and luteolin-7-*O*-glucoside are clearly separated. Above the yellow band due to luteolin-7-*O*-glucoside, there is a faint blue zone and a faint yellow band.

Acceptance criteria: Under long-wave UV light (365 nm), the chromatogram of the *Sample solution* displays the bands similar in position and color to those seen in *Standard solution B*. Additional faint bands may be observed.

● B. HPTLC for Articles of Botanical Origin (203)

Secoiridoids and Triterpenoids

[Note—Apply *Derivatization reagent B* to the plate used earlier in *Identification test A*. Perform the second derivatization within 20 min of the first one.]

Chromatographic system

Derivatization reagent B: 85 mL of ice-cold methanol combined with 10 mL of glacial acetic acid, 5 mL of sulfuric acid, and 0.5 mL of *p*-anisaldehyde

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Treat the plate with *Derivatization reagent B*, dry in air, heat for 3 min at 100°, and immediately examine under white light.

System suitability: Under white light, the derivatized chromatogram of the *Standard solution B* exhibits, in its lower third, a number of faint grayish-brown bands. In the middle of the chromatogram, an intense gray-brown band corresponding to oleuropein in *Standard solution A* appears. An intense violet band, corresponding to the oleanolic acid band in *Standard solution A*, is seen near the solvent front, and a weaker blue band due to maslinic acid just below it.

Acceptance criteria: Under white light, the chromatogram of the *Sample solution* displays the bands similar in position and color to those seen in *Standard solution B*. Additional bands may be observed.

COMPOSITION**• Content of Oleuropein**

Mobile phase: 0.1% Phosphoric acid in water and acetonitrile (80:20)

Standard solution: 0.2 mg/mL of USP Oleuropein RS in *Mobile phase*

Sample solution: Transfer about 1.0 g of Olive Leaf, finely powdered and accurately weighed, to a 50-mL centrifuge tube, add 15 mL of *Mobile phase*, cap tightly, and sonicate for 20 min. Centrifuge, and transfer the supernatant to a 50-mL volumetric flask. Repeat with two additional 15-mL aliquots of *Mobile phase*, and combine all supernatants into the same volumetric flask. Dilute with *Mobile phase* to volume, and mix well. Dilute the resulting solution 10-fold with *Mobile phase*. Pass through a PTFE filter of 0.45- μ m or finer pore size, discarding the initial few mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: HPLC

Detector: UV 230 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 25°

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for the oleuropein peak

Relative standard deviation: NMT 2.0% determined for the oleuropein peak in replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Using the chromatogram of the *Standard solution*, identify the retention time of the peak corresponding to oleuropein in the *Sample solution*.

Calculate the percentage of oleuropein (C₂₅H₃₂O₁₃) in the portion of Olive Leaf taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times D \times 100$$

r_U = peak area of oleuropein from the *Sample solution*

r_S = peak area of oleuropein from the *Standard solution*

C_S = concentration of USP Oleuropein RS in the *Standard solution* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Olive Leaf taken to prepare the *Sample solution* (mg)

D = dilution factor, 10

Acceptance criteria: NLT 6.0% on the dried basis

CONTAMINANTS

- **Articles of Botanical Origin** <561>, *Limits of Elemental Impurities*: Meets the requirements
- **Articles of Botanical Origin** <561>, *Pesticide Residue Analysis*: Meets the requirements
- **Microbial Enumeration Tests** <2021>: The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^5 cfu/g, and the bile-tolerant Gram-negative bacterial count does not exceed 10^2 cfu/g.
- **Absence of Specified Microorganisms** <2022>, *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

- **Botanical Characteristics**

Macroscopic: The leaf is simple, sessile, thick, coriaceous, lanceolate to obovate, 3–8 cm long, 0.5–2 cm broad; base cuneate; apex apiculate to mucronulate. The margins are entire and folded abaxially. Nervation of the leaf is reticular. The upper surface is grayish-green to dark-green, glabrous and shiny; the lower surface silvery (or reddish in some species), and tomentous (densely pilous), densely covered with a layer of peltate scales but without filamentous hairs.

Microscopic: The leaf has iso-bilateral structure. Both the adaxial epidermis and the abaxial epidermis are simple, with thick cuticle. The anticlinal walls of both adaxial and abaxial epidermal cells are straight. The adaxial epidermis has sporadic and the abaxial epidermis numerous densely distributed, nonglandular, very large scutiform trichomes. The leaves are hypostomatic and their numerous stomata possess highly cutinized guard cells. The mesophyll is constituted of three layers of condensed long palisade cells located under the adaxial epidermis and one or two layers of short palisade cells on the abaxial epidermis. The central part of the leaf contains tiny cells of spongy parenchyma with large intercellulars. Present in the mesophyll are filiform sclereids, which are long, fiber-like, and sometimes branched. Microscopically, the olive leaf can be clearly identified by fragments of epidermis with long filiform sclereids and characteristic very large scutiform trichomes. Presence of fragments of epidermis with crypts may indicate adulteration with oleander leaves, while presence of numerous anomocytic stomata and large cluster crystals suggests adulteration with pittosporum.

- **Articles of Botanical Origin** <561>, *Foreign Organic Matter*: NMT 2.0%
- **Loss on Drying** <731>
 - Sample:** 1.0 g of Olive Leaf, finely powdered
 - Analysis:** Dry the *Sample* at 105° for 2 h.
 - Acceptance criteria:** NMT 10.0%
- **Articles of Botanical Origin** <561>, *Total Ash*
 - Sample:** 1.0 g of Olive Leaf, finely powdered
 - Acceptance criteria:** NMT 9.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.

- **Labeling:** The label states the official name of the article and the corresponding Latin binominal.
- **USP Reference Standards** <11>
 - USP Oleanolic Acid RS
 - USP Oleuropein RS
 - USP Olive Leaf Dry Extract RS
 - USP Rutin RS
 - USP Verbascoside RS

■ 2S (USP39)

¹ Suitable commercially available plates are HPTLC Silica Gel 60 F₂₅₄ from EMD Millipore (e.g., Part No. 1.05642.0001).

BRIEFING

Olive Leaf Dry Extract. Because there is currently no *USP* monograph for this dietary supplement product, a new monograph is being proposed. The liquid chromatographic procedure in the test for *Content of Oleuropein* is performed on the Phenomenex Luna C18(2) brand of L1 column with 5- μ m packing. The typical retention time of oleuropein is 18 min.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C149313

Comment deadline: November 30, 2015

Add the following:

- **Olive Leaf Dry Extract**

DEFINITION

Olive Leaf Dry Extract is prepared from the dried leaf of *Olea europaea* L. (Fam. Oleaceae) by extraction with hydroalcoholic mixtures, ethyl acetate, or other suitable solvents. It contains NLT 90% and NMT 110% of the labeled amount of oleuropein (C₂₅H₃₂O₁₃), calculated on the dried basis. The ratio of starting plant material to extract is between 5:1 and 60:1. It may contain suitable added substances.

IDENTIFICATION

- **A. HPTLC for Articles of Botanical Origin** <203>

Flavonoids

Standard solution A: 1 mg/mL of USP Rutin RS and 2 mg/mL each of USP Verbascoside RS, USP Oleuropein RS, and USP Oleanolic Acid RS in methanol

Standard solution B: 25 mg/mL of USP Olive Leaf Dry Extract RS in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: Suspend about 125 mg of Olive Leaf Dry Extract in 5 mL of methanol, and sonicate for 10 min. Centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 μ m (HPTLC

plate)¹

Application volume: 2 μ L each of *Standard solution A*, *Standard solution B*, and *Sample solution* as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient temperature, not to exceed 30°

Developing solvent system: Ethyl formate, formic acid, toluene, and water (60:8:3:6)

Developing distance: 6 cm

Derivatization reagent A: 5 mg/mL of 2-aminoethyl diphenylborinate in ethyl acetate

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber and dry in air. Heat at 100° for 3 min. While the plate is still warm, treat with *Derivatization reagent A*, and examine under long-wave UV light (365 nm).

[Note—Immediately after examination, use the same developed and derivatized plate for *Identification test B*.]

System suitability: Under long-wave UV (365 nm), the derivatized chromatogram of *Standard solution B* displays, in its lower third, three yellowish bands: the first close to the application line; the second located just above, or coincident with, the rutin band in *Standard solution A*; the third, due to luteolin-7-*O*-glucoside positioned above the light-blue band corresponding to verbascoside in *Standard solution A*. There is a bluish-green band just above the band due to rutin. A faint light-blue band may appear just below the verbascoside band. The bands due to verbascoside and luteolin-7-*O*-glucoside are clearly separated. Above the yellow band due to luteolin-7-*O*-glucoside, there is a faint blue zone and a faint yellow band.

Acceptance criteria: Under long-wave UV light (365 nm), the chromatogram of the *Sample solution* displays the bands similar in position and color to those seen in *Standard solution B*. Additional faint bands may be observed.

• B. HPTLC for Articles of Botanical Origin (203)

Secoiridoids and Triterpenoids

[Note—Apply *Derivatization reagent B* to the plate used earlier in *Identification test A*. Perform the second derivatization within 20 min of the first one.]

Chromatographic system

Derivatization reagent B: 85 mL of ice-cold methanol combined with 10 mL of glacial acetic acid, 5 mL of sulfuric acid, and 0.5 mL of *p*-anisaldehyde

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Treat the plate with *Derivatization reagent B*, dry in air, heat for 3 min at 100°, and immediately examine under white light.

System suitability: Under white light, the chromatogram of the *Standard solution B* exhibits, in its lower third, a number of faint grayish-brown bands. In the middle of the chromatogram, an intense gray-brown band corresponding to oleuropein in *Standard solution A* appears. An intense violet band, corresponding to the oleanolic acid band in

Standard solution A, is seen near the solvent front, and a weaker blue band due to maslinic acid just below it.

Acceptance criteria: Under white light, the chromatogram of the *Sample solution* displays the bands similar in position and color to those seen in *Standard solution B*; however, the bands due to oleanolic and maslinic acids may be faint or absent. Additional bands may be observed.

COMPOSITION

• Content of Oleuropein

Mobile phase: 0.1% Phosphoric acid in water and acetonitrile (80:20)

Standard solution: 0.2 mg/mL of USP Oleuropein RS in *Mobile phase*

Sample solution: Accurately weigh the amount of Olive Leaf Dry Extract calculated to contain 20 mg of oleuropein, into a 50-mL volumetric flask, add 25 mL of *Mobile phase*, and sonicate for 5 min. Dilute with *Mobile phase* to volume, and mix well. Pass through a PTFE filter of 0.45- μ m or finer pore size, discarding the initial few mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: HPLC

Detector: UV 230 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 25°

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for the oleuropein peak

Relative standard deviation: NMT 2.0% determined for the oleuropein peak in replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Using the chromatogram of the *Standard solution*, identify the retention time of the peak corresponding to oleuropein in the *Sample solution*.

Calculate the percentage of oleuropein (C₂₅H₃₂O₁₃) in the portion of Olive Leaf Dry Extract taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak area of oleuropein from the *Sample solution*

r_S = peak area of oleuropein from the *Standard solution*

C_S = concentration of USP Oleuropein RS in the *Standard solution* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Olive Leaf Dry Extract taken to prepare the *Sample solution* (mg)

Calculate the percentage of the labeled amount of oleuropein ($C_{25}H_{32}O_{13}$) in the portion of Olive Leaf Dry Extract taken:

$$\text{Result} = (P/L) \times 100$$

P = oleuropein content as determined above

L = labeled amount of oleuropein

Acceptance criteria: 90.0%–110.0% on the dried basis

CONTAMINANTS

- **Articles of Botanical Origin** (561), *Pesticide Residue Analysis*: Meets the requirements
- **Botanical Extracts** (565), *Residual Solvents*: Meets the requirements
- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

- **Loss on Drying** (731)
 - Sample:** 1 g of Olive Leaf Dry Extract
 - Analysis:** Dry the *Sample* at 105° for 2 h.
 - Acceptance criteria:** NMT 8.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling:** The label states the official name of the article and the corresponding Latin binominal. The label also indicates the content of oleuropein, the extraction solvent used in preparation, and the ratio of the starting crude plant material to Dry Extract. It meets the labeling requirements of *Botanical Extracts* (565).
- **USP Reference Standards** (11)
 - USP Oleanolic Acid RS
 - USP Oleuropein RS
 - USP Olive Leaf Dry Extract RS
 - USP Rutin RS
 - USP Verbascoside RS

■ 2S (USP39)

¹ Suitable commercially available plates are HPTLC Silica Gel 60 F₂₅₄ from EMD Millipore (e.g., Part No. 1.05642.0001).

BRIEFING

Olive Leaf Powder. Because there is currently no *USP* monograph for this dietary

supplement product, a new monograph is being proposed. The liquid chromatographic procedure in the test for *Content of Oleuropein* is performed on the Phenomenex Luna C18(2) brand of L1 column with 5- μ m packing. The typical retention time of oleuropein is 18 min.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C160783

Comment deadline: November 30, 2015

Add the following:

▪ **Olive Leaf Powder**

DEFINITION

Olive Leaf Powder consists of the dried leaf of *Olea europaea* L. (Fam. Oleaceae) reduced to fine or very fine powder. It contains NLT 6.0% of oleuropein ($C_{25}H_{32}O_{13}$), calculated on the dried basis.

IDENTIFICATION

• **A. HPTLC for Articles of Botanical Origin** <203>

Flavonoids

Standard solution A: 1 mg/mL of USP Rutin RS and 2 mg/mL each of USP Verbascoside RS, USP Oleuropein RS, and USP Oleanolic Acid RS in methanol

Standard solution B: 25 mg/mL of USP Olive Leaf Dry Extract RS in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: Suspend about 500 mg of Olive Leaf Powder in 5 mL of methanol, and sonicate for 10 min. Centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 μ m (HPTLC plate)¹

Application volume: 2 μ L each of *Standard solution A*, *Standard solution B*, and *Sample solution* as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient temperature, not to exceed 30°

Developing solvent system: Ethyl formate, formic acid, toluene, and water (60:8:3:6)

Developing distance: 6 cm

Derivatization reagent A: 5 mg/mL of 2-aminoethyl diphenylborinate in ethyl acetate

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber and dry in air. Heat at 100° for 3 min. While the plate is still warm, treat with *Derivatization reagent A*, and examine under long-wave UV light (365 nm).

[Note—Immediately after examination, use the same developed and derivatized plate for *Identification test B*.]

System suitability: Under long-wave UV (365 nm), the derivatized chromatogram of *Standard solution B* displays, in its lower third, three yellowish bands: the first close to the application line; the second located just above, or coincident with, the rutin band in *Standard solution A*; the third, due to luteolin-7-*O*-glucoside positioned above the light-blue band corresponding to verbascoside in *Standard solution A*. There is a bluish-green band just above the band due to rutin. A faint light-blue band may appear just below the verbascoside band. The bands due to verbascoside and luteolin-7-*O*-glucoside are clearly separated. Above the yellow band due to luteolin-7-*O*-glucoside, there is a faint blue zone and a faint yellow band.

Acceptance criteria: Under long-wave UV light (365 nm), the chromatogram of the *Sample solution* displays the bands similar in position and color to those seen in *Standard solution B*. Additional faint bands may be observed.

- **B. HPTLC for Articles of Botanical Origin** (203)

- **Secoiridoids and Triterpenoids**

[Note—Apply *Derivatization reagent B* to the plate used earlier in *Identification test A*. Perform the second derivatization within 20 min of the first one.]

- **Chromatographic system**

- **Derivatization reagent B:** 85 mL of ice-cold methanol combined with 10 mL of glacial acetic acid, 5 mL of sulfuric acid, and 0.5 mL of *p*-anisaldehyde

- **Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

- Treat the plate with *Derivatization reagent B*, dry in air, heat for 3 min at 100°, and immediately examine under white light.

- **System suitability:** Under white light, the derivatized chromatogram of *Standard solution B* exhibits, in its lower third, a number of faint grayish-brown bands. In the middle of the chromatogram, an intense gray-brown band, corresponding to oleuropein in *Standard solution A*, appears. An intense violet band, corresponding to the oleanolic acid band in *Standard solution A*, is seen near the solvent front, and a weaker blue band due to maslinic acid just below it.

- **Acceptance criteria:** Under white light, the chromatogram of the *Sample solution* displays the bands similar in position and color to those seen in *Standard solution B*. Additional bands may be observed.

COMPOSITION

- **Content of Oleuropein**

- **Mobile phase:** 0.1% Phosphoric acid in water and acetonitrile (80:20)

- **Standard solution:** 0.2 mg/mL of USP Oleuropein RS in *Mobile phase*

- **Sample solution:** Transfer about 1.0 g of Olive Leaf Powder, accurately weighed, to a 50-mL centrifuge tube, add 15 mL of *Mobile phase*, cap tightly, and sonicate for 20 min. Centrifuge, and transfer the supernatant to a 50-mL volumetric flask. Repeat with two additional 15-mL aliquots of *Mobile phase*, and combine all supernatants into the same volumetric flask. Dilute with *Mobile phase* to volume, and mix well. Dilute the resulting solution 10-fold with *Mobile phase*. Pass through a PTFE filter of 0.45- μ m or finer pore size, discarding the initial few mL of the filtrate.

- **Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: HPLC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 25°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for the oleuropein peak

Relative standard deviation: NMT 2.0% determined for the oleuropein peak in replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Using the chromatogram of the *Standard solution*, identify the retention time of the peak corresponding to oleuropein in the *Sample solution*.

Calculate the percentage of oleuropein (C₂₅H₃₂O₁₃) in the portion of Olive Leaf Powder taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times D \times 100$$

r_U = peak area of oleuropein from the *Sample solution*

r_S = peak area of oleuropein from the *Standard solution*

C_S = concentration of USP Oleuropein RS in the *Standard solution* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Olive Leaf Powder taken to prepare the *Sample solution* (mg)

D = dilution factor, 10

Acceptance criteria: NLT 6.0% on the dried basis

CONTAMINANTS

- **Articles of Botanical Origin** (561), *Limits of Elemental Impurities*: Meets the requirements
- **Articles of Botanical Origin** (561), *Pesticide Residue Analysis*: Meets the requirements
- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10⁵ cfu/g, the total combined molds and yeasts count does not exceed 10⁵ cfu/g, and the bile-tolerant Gram-negative bacterial count does not exceed 10² cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

- **Botanical Characteristics**

Macroscopic: The powder is yellowish-green.

Microscopic: The powder shows fragments of abaxial epidermis with small anomocytic stomata and adaxial epidermis with thick-walled polygonal cells, palisade composed of three layers of cells and spongy parenchyma composed of small cells. Fragments of the lamina show thick cuticle. Long filiform sclereids and very large characteristic scutiform trichomes are abundant. Presence of fragments of epidermis with crypts may indicate adulteration with oleander leaves, while presence of numerous anomocytic stomata and large cluster crystals suggests adulteration with pittosporum.

- **Loss on Drying** (731)

Sample: 1.0 g of Olive Leaf Powder

Analysis: Dry the *Sample* at 105° for 2 h.

Acceptance criteria: NMT 10.0%

- **Articles of Botanical Origin** (561), *Total Ash*

Sample: 1.0 g of Olive Leaf Powder

Acceptance criteria: NMT 9.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
 - **Labeling:** The label states the official name of the article and the corresponding Latin binominal.
 - **USP Reference Standards** (11)
 - USP Oleanolic Acid RS
 - USP Oleuropein RS
 - USP Olive Leaf Dry Extract RS
 - USP Rutin RS
 - USP Verbascoside RS
- 2S (USP39)

¹ Suitable commercially available plates are HPTLC Silica Gel 60 F₂₅₄ from EMD Millipore (e.g., Part No. 1.05642.0001).

BRIEFING

Red Clover Aerial Parts Isoflavone Aglycones Dry Extract. Because there is no existing *USP* monograph for this dietary supplement, a new monograph is being proposed. The article defines an extract where isoflavones exist predominantly as aglycones. The chromatographic tests in this monograph are analogous to those in the *Powdered Red Clover Extract* monograph proposed elsewhere in this issue of *Pharmacopeial Forum*, although no hydrolysis is required in the test for *Content of Isoflavones*. The liquid chromatographic procedure in the test for *Content of Isoflavones* is performed on the Waters Sunfire C18 brand of L1 end-capped column with 5- μ m packing. The typical retention times for daidzein, genistein, formononetin, and biochanin A are 7.2, 9.7, 11.5, and 13.1 min, respectively.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C149439

Comment deadline: November 30, 2015

Add the following:**■ Red Clover Aerial Parts Isoflavone Aglycones Dry Extract****DEFINITION**

Red Clover Aerial Parts Isoflavone Aglycones Dry Extract is prepared from dried aerial parts of *Trifolium pratense* L. (Fam. Fabaceae) by extraction with hydroalcoholic mixtures or other suitable solvents. It contains NLT 36.0% and NMT 44.0% of isoflavones, predominantly as aglycones, calculated on the dried basis as the sum of daidzein, genistein, formononetin, and biochanin A. It contains NMT 1.0% of daidzein and NMT 1.0% of genistein, both on the dried basis. The ratio of 5,7-dihydroxyisoflavones to 7-hydroxyisoflavones is between 0.9 and 1.7. It may contain suitable excipients.

IDENTIFICATION**• A. HPTLC for Articles of Botanical Origin <203>****Presence of biochanin A and formononetin**

Solvent: Methanol and water (7:3)

Standard solution A: 1 mg/mL each of USP Biochanin A RS and USP Formononetin RS in methanol

Standard solution B: 10 mg/mL of USP Red Clover Aerial Parts Isoflavone Aglycones Dry Extract RS in *Solvent*. Shake to disperse, heat on a water bath at 60°–80° for 10 min, centrifuge, and use the supernatant.

Sample solution: 10 mg/mL of Dry Extract in *Solvent*. Shake to disperse, heat on a water bath at 60°–80° for 10 min, centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 µm (HPTLC plate)¹

Application volume: 2 µL each of *Standard solution A*, *Standard solution B*, and *Sample solution*, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Temperature: Ambient, not to exceed 30°

Developing solvent system: Ethyl acetate, toluene, and formic acid (30:70:1)

Developing distance: 6 cm

Derivatization reagent: 5 mg/mL of 2-aminoethyl diphenylborinate in ethyl acetate

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* and dry in air. Develop in a saturated chamber. Remove the plate from the chamber, heat at 100° for 5 min, derivatize while warm with the

Derivatization reagent, dry in air, and examine under UV light at 365 nm.

System suitability: *Standard solution A* exhibits a bluish band at about one-third of the chromatogram due to formononetin, and a greenish band due to biochanin A at about the middle of the chromatogram. *Standard solution B* exhibits prominent bands of similar color and position to those of formononetin and biochanin A in *Standard solution A*. A red band appears below the formononetin band in *Standard solution B*.

Acceptance criteria: The *Sample solution* exhibits the following prominent bands similar in position and color to those in *Standard solution B*: two greenish bands at about the middle of the chromatogram (a distinction from white clover, soy, and alfalfa), one of them corresponding to biochanin A in *Standard solution A*; a bluish band at about one-third of the chromatogram, corresponding to the formononetin band in *Standard solution B*; and a red band below that due to formononetin.

● B. HPLC

Analysis: Proceed as directed in the test for *Content of Isoflavones*.

Using the values obtained in the test for *Content of Isoflavones*, calculate the ratio of 5,7-dihydroxyisoflavones to 7-hydroxyisoflavones:

$$\text{Result} = (B + G)/(D + F)$$

B = percentage of biochanin A

G = percentage of genistein

D = percentage of daidzein

F = percentage of formononetin

Acceptance criteria: The *Sample solution* exhibits peaks for daidzein, genistein, formononetin, and biochanin A at retention times corresponding to those in *Standard solution A* and *Standard solution B*. The ratio of 5,7-dihydroxyisoflavones to 7-hydroxyisoflavones is between 0.9 and 1.7.

COMPOSITION

● Content of Isoflavones

Solution A: Acetonitrile and water (1:3) containing 0.05% trifluoroacetic acid

Solution B: Acetonitrile containing 0.05% trifluoroacetic acid

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
2.5	87	13
7.5	80	20
7.8	73	27
8.0	55	45
11.0	50	50
13.0	40	60
15.0	26	74
16.0	0	100
18.1	100	0
23.0	100	0

Diluent: Alcohol and water (1:1)

Standard solution A: Prepare a 0.10–0.15 mg/mL solution of USP Red Clover Aerial Parts Isoflavone Aglycones Dry Extract RS in *Diluent*, sonicate briefly, and filter through a membrane of 0.45- μ m or finer pore size.

Standard solution B: 0.1 mg/mL of USP Formononetin RS, 0.02 mg/mL of USP Genistein RS, 0.02 mg/mL of USP Daidzein RS, and 0.1 mg/mL of USP Biochanin A RS in a mixture of *n*-propanol and water (1:1)

Sample solution: Accurately weigh a quantity of Dry Extract, equivalent to 12 mg of the labeled content of isoflavones, and transfer to a 100-mL volumetric flask. Add 50 mL of *Diluent*, sonicate to disperse, and dilute with *Diluent* to volume. Before injection, pass through a filter of 0.45- μ m or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; end-capped 5- μ m packing *L1*

Column temperature: 45°

Flow rate: 1.0 mL/min

Injection volume: 10 μ L

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Tailing factor: NMT 2.0 for the formononetin peak, *Standard solution B*

Relative standard deviation: NMT 2.0%, determined from the formononetin peak in replicate injections, *Standard solution B*

Chromatogram similarity: The chromatogram of *Standard solution A* is similar to the reference chromatogram provided with the lot of USP Red Clover Aerial Parts

Isoflavone Aglycones Dry Extract RS being used.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Identify the peaks corresponding to daidzein, genistein, formononetin, and biochanin A in the *Sample solution* by comparison with the chromatograms obtained from *Standard solution A*, *Standard solution B*, and the reference chromatogram supplied.

Calculate the percentage of each isoflavone in the portion of Dry Extract taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak area of a relevant isoflavone from the *Sample solution*

r_S = peak area of a relevant isoflavone from *Standard solution B*

C_S = concentration of a relevant isoflavone in *Standard solution B* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Dry Extract taken to prepare the *Sample solution* (mg)

Acceptance criteria: 36.0%–44.0% of isoflavones as the sum of daidzein, genistein, formononetin, and biochanin A, calculated on the dried basis; NMT 1.0% of daidzein and NMT 1.0% of genistein, both on the dried basis

CONTAMINANTS

- **Articles of Botanical Origin** (561), *Pesticide Residue Analysis*: Meets the requirements
- **Botanical Extracts** (565), *Preparations, General Pharmacopeial Requirements, Residual Solvents*: Meets the requirements
- **Microbial Enumeration Tests** (2021): The total aerobic microbial count is NMT 10^4 cfu/g, and the total combined molds and yeasts count is NMT 10^3 cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species and Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

- **Loss on Drying** (731)

Sample: 1.0 g of Red Clover Aerial Parts Isoflavone Aglycones Dry Extract

Analysis: Dry the *Sample* at 105° for 2 h.

Acceptance criteria: NMT 3.0%

- **Residue on Ignition** (281)

Analysis: Ignite 1.0 g of Red Clover Aerial Parts Isoflavone Aglycones Dry Extract.

Acceptance criteria: NMT 4.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, protected from moisture, in a cool place.
- **Labeling:** The label states the official name of the article and the corresponding Latin binominal. The label also indicates the content of isoflavones, their status as aglycones,

the extraction solvent used for preparation, and the ratio of the starting crude plant material to the Dry Extract. It meets the requirements of *Botanical Extracts* (565), *Preparations*, *General Pharmacopeial Requirements*, *Labeling*.

- **USP Reference Standards** (11)

USP Biochanin A RS

USP Daidzein RS

USP Formononetin RS

USP Genistein RS

USP Red Clover Aerial Parts Isoflavone Aglycones Dry Extract RS

- 2S (USP39)

¹ Suitable commercially available plates are HPTLC Silica Gel 60 F₂₅₄ from EMD Millipore (e.g., Part No. 1.05642.0001).

BRIEFING

Powdered Red Clover Extract, *USP 38* page 6191. It is proposed to make the following revisions to the monograph.

1. In the *Content of Isoflavones* test, the proposed revisions will streamline the preparation of *Standard solution A* and the *Sample solution*, correct the calculation, and add a calculation for percent label claim.
2. The test for enterobacterial count is removed as it is no longer required in *Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements* (2023).

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C160606

Comment deadline: November 30, 2015

Powdered Red Clover Extract

DEFINITION

Powdered Red Clover Extract is prepared from Red Clover by extraction with hydroalcoholic mixtures or other suitable solvents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of isoflavones, calculated on the dried basis as the sum of daidzein, genistein, formononetin, and biochanin A. It may contain suitable added substances.

IDENTIFICATION

Change to read:

- **A.**

- **HPTLC for Articles of Botanical Origin** (203) ■ 2S (USP39)

Presence of biochanin A and formononetin

Solvent A: Methanol and water (7:3)

Solvent B: Methanol and water (6:4)

Standard solution A: 0.5 mg/mL of USP Biochanin A RS in methanol

Standard solution B: 0.5 mg/mL of USP Formononetin RS in methanol

Standard solution C: 10 mg/mL of USP Powdered Red Clover Extract RS in *Solvent A*. Shake to disperse, heat on a water bath at 60°–80° for 10 min, cool, centrifuge, and use the supernatant.

Sample solution: 10 mg/mL of Powdered Extract in *Solvent A*. Shake to disperse, heat on a water bath at 60°–80° for 10 min, cool, centrifuge, and use the supernatant. [Note—Reserve a portion of the supernatant for *Identification* test *B*.]

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

■ 2S (USP39)

Adsorbent: Chromatographic silica gel with an average particle size of 5 µm (HPTLC plates)

Application volume: 4 µL each of *Standard solution A*, *Standard solution B*, and the *Sample solution*, and 3 µL of *Standard solution C*, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

■ **Temperature:** Ambient, not to exceed 30° ■ 2S (USP39)

Developing solvent system: Ethyl acetate, toluene, and formic acid (30:70:1)

Developing distance: 6 cm

Derivatization reagent: 5 mg/mL of 2-aminoethyl diphenylborinate in ethyl acetate

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber. Remove the plate from the chamber, heat at 100° for 5 min, treat while still warm with *Derivatization reagent*, dry in air, and examine under UV light at 365 nm.

System suitability: *Standard solution C* exhibits, at about the middle of the chromatogram, a greenish band corresponding to biochanin A in *Standard solution A*, and a bluish band, at about one-third of the chromatogram, corresponding to formononetin in *Standard solution B*. Below the band due to formononetin, *Standard solution C* exhibits a red band.

Acceptance criteria: The *Sample solution* exhibits the following bands similar in position and color to the corresponding bands in the chromatogram of *Standard solution C*: two greenish bands at about the middle of the chromatogram (a distinction from white clover, soy, and alfalfa), one of these greenish bands corresponds to the band due to biochanin A in *Standard solution A*; a bluish band at about one-third of the chromatogram, corresponding to the formononetin band in *Standard solution B*; below the formononetin band, the *Sample solution* exhibits a red band.

Change to read:• **B.**■ **HPTLC for Articles of Botanical Origin** (203) ■ 2S (USP39)**Presence of flavone glycosides****Solvent A:** Methanol and water (7:3)**Solvent B:** Methanol and water (6:4)**Standard solution A:** 0.1 mg/mL of USP Hyperoside RS in methanol**Standard solution B:** 25 mg/mL of USP Powdered Red Clover Extract RS in *Solvent A*. Shake to disperse, heat on a water bath at 60°–80° for 10 min, cool, centrifuge, and use the supernatant.**Sample solution:** Use the solution prepared in *Identification* test A.**Chromatographic system**~~(See *Chromatography* (621), *Thin-Layer Chromatography*.)~~

■ 2S (USP39)

Adsorbent: Chromatographic silica gel with an average particle size of 5 µm (HPTLC plates)**Application volume:** 4 µL of *Standard solution A*, 8 µL of *Standard solution B*, and 2 µL of the *Sample solution*, as 8-mm bands**Relative humidity:** Condition the plate to a relative humidity of about 33% using a suitable device.■ **Temperature:** Ambient, not to exceed 30° ■ 2S (USP39)**Developing solvent system:** Ethyl acetate, formic acid, glacial acetic acid, and water (100:11:11:27)**Developing distance:** 6 cm**Derivatization reagent:** 5 mg/mL of 2-aminoethyl diphenylborinate in ethyl acetate**Analysis****Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber. Remove the plate from the chamber, heat at 100° for 5 min, treat while still warm with *Derivatization reagent*, dry in air, and examine under UV light at 365 nm.

System suitability: *Standard solution B* exhibits, at about the middle of the chromatogram, two yellow bands. The lower band corresponds in color and R_f to hyperoside in *Standard solution A*. The upper yellow band is due to isoquercitrin. The solution also exhibits two green bands or a broad green band, above the yellow bands, and a blue band in the upper third section of the chromatogram. The yellow bands due to hyperoside and isoquercitrin are clearly separated.**Acceptance criteria:** The *Sample solution* exhibits the following bands similar in position and color to the corresponding bands in *Standard solution B*: a yellow band in about the middle of the chromatogram corresponding to hyperoside in *Standard solution A*; another

yellow band, at an R_F slightly higher than that of hyperoside; two green bands or a broad green band above the yellow bands; and a blue band in the upper third section of the chromatogram.

- **C. HPLC**

Analysis: Proceed as directed in the test for *Content of Isoflavones*.

Using the values obtained in the test for *Content of Isoflavones*, calculate the ratio of 5,7-dihydroxyisoflavones to 7-hydroxyisoflavones:

$$\text{Result} = (B + G)/(D + F)$$

B = percentage of biochanin A

G = percentage of genistein

D = percentage of daidzein

F = percentage of formononetin

Acceptance criteria: The *Sample solution* exhibits peaks for daidzein, genistein, formononetin, and biochanin A at retention times that correspond to those in *Standard solution A*. The ratio of 5,7-dihydroxyisoflavones to 7-hydroxyisoflavones is between 0.1 and 10.0.

COMPOSITION

Change to read:

- **Content of Isoflavones**

Solution A: Acetonitrile and water (1:3) containing 0.05% trifluoroacetic acid

Solution B: Acetonitrile containing 0.05% trifluoroacetic acid

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
2.5	87	13
7.5	80	20
7.8	73	27
8.0	55	45
11.0	50	50
13.0	40	60
15.0	26	74
16.0	0	100
18.1	100	0
23.0	100	0

Solvent: Alcohol and water (1:1)

Standard stock solution A: ~~Transfer a quantity of USP Powdered Red Clover Extract RS,~~

equivalent to 30 mg of the labeled content of isoflavones, to a 250-mL volumetric flask. Add 15 mL of dehydrated alcohol, sonicate until dissolved, and dilute with *Solvent* to volume.

■ **2S (USP39)**

Standard solution A: Evaporate 50 mL of *Standard stock solution A* to dryness under vacuum. Add 15 mL of 2-N hydrochloric acid, and heat on a water bath for 30 min. Quantitatively transfer the resulting solution, with the aid of 15 mL of alcohol, to a 50-mL volumetric flask, and dilute with *Solvent* to volume.

■ Suspend 10–15 mg of USP Powdered Red Clover Extract RS in 15 mL of 2 N hydrochloric acid, sonicate to disperse, and heat on a water bath for 30 min. Add 15 mL of alcohol and mix well. ■ **2S (USP39)**

Centrifuge or filter through a membrane of 0.45- μ m or finer pore size.

Standard solution B: 0.1 mg/mL of USP Formononetin RS, 0.02 mg/mL of USP Genistein RS, 0.02 mg/mL of USP Daidzein RS, and 0.1 mg/mL of USP Biochanin A RS in a mixture of *n*-propanol and water (1:1)Sonicate, and filter through a membrane of 0.45- μ m or finer pore size.

■ **2S (USP39)**

Sample stock solution: Transfer a quantity of Powdered Extract, equivalent to 30 mg of the labeled content of isoflavones, to a 250-mL volumetric flask. Add 15 mL of dehydrated alcohol, sonicate until dissolved, and dilute with *Solvent* to volume.

■ **2S (USP39)**

Sample solution: Evaporate 50.0 mL of the *Sample stock solution* to dryness under vacuum.

■ Accurately transfer a quantity of Powdered Extract, equivalent to 6 mg of the labeled content of isoflavones, to a 50-mL volumetric flask. ■ **2S (USP39)**

Add 15 mL of 2 N hydrochloric acid,

■ sonicate to disperse, ■ **2S (USP39)**

and heat on a water bath for 30 min. Quantitatively transfer the resulting solution with the aid of 15 mL of alcohol to a 50-mL volumetric flask

■ Allow to cool, add 15 mL of alcohol ■ **2S (USP39)**

, and dilute with *Solvent* to volume. Centrifuge or pass through a filter of 0.45- μ m or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; end-capped 5- μ m packing *L1*

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Tailing factor: NMT 2.0 for the formononetin peak, *Standard solution B*

Relative standard deviation: NMT 2.0%, determined from the formononetin peak in replicate injections, *Standard solution B*

Chromatogram similarity: The chromatogram of *Standard solution A* is similar to the reference chromatogram provided with the lot of USP Powdered Red Clover Extract RS being used.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Identify the peaks corresponding to daidzein, genistein, formononetin, and biochanin A in the *Sample solution* by comparison with the chromatogram from *Standard solution A* and the reference chromatogram. Measure the areas of the analyte peaks.

Calculate the percentage of each isoflavone in the portion of Powdered Extract taken:

$$\text{Result} = (r_U/r_S) \times C_S \times 100$$

r_U = peak area of a relevant isoflavone in the *Sample solution*

r_S = peak area of a corresponding isoflavone in the *Standard solution B*

C_S = concentration of a relevant isoflavone in *Standard solution B* (mg/mL)

■ Calculate the percentage of each isoflavone in the portion of Powdered Extract taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak area of a relevant isoflavone from the *Sample solution*

r_S = peak area of a corresponding isoflavone from *Standard solution B*

C_S = concentration of a relevant isoflavone in *Standard solution B* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Powdered Extract used to prepare the *Sample solution* (mg)

Calculate the percentage of the labeled amount of isoflavones in the portion of Powdered Extract taken:

$$\text{Result} = (\Sigma P_i/L) \times 100$$

ΣP_i = total combined content of isoflavones as determined above

L = labeled amount of isoflavones ■ 2S (USP39)

Acceptance criteria: 90.0%–110.0% of the labeled amount of isoflavones as the sum of daidzein, genistein, formononetin, and biochanin A, on the dried basis

CONTAMINANTS

- **Elemental Impurities—Procedures** (233)

Acceptance criteria

Arsenic: NMT 1.0 µg/g

Cadmium: NMT 0.5 µg/g

Lead: NMT 5.0 µg/g

Mercury: NMT 1.0 µg/g

- **Botanical Extracts** (565), *Preparations, General Pharmacopeial Requirements, Pesticide Residues*: Meets the requirements
- **Botanical Extracts** (565), *Preparations, General Pharmacopeial Requirements, Residual Solvents*: Meets the requirements

Change to read:

- **Microbial Enumeration Tests** (2021): The total aerobic microbial count does not exceed 10^4 cfu/g and the total combined molds and yeasts count does not exceed 10^3 cfu/g, ~~and the enterobacterial count is NMT 10^3 cfu/g~~
 - **2S (USP39)**
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

- **Loss on Drying** (731)
 - Sample:** 1 g
 - Analysis:** Dry the *Sample* at 105° for 2 h.
 - Acceptance criteria:** NMT 5.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, in a cool place.
- **Labeling:** The label states the Latin binomial and, following the official name, the part of the plant from which the article was prepared. The label also indicates the content of isoflavones, the extracting solvent used for preparation, and the ratio of the starting crude plant material to Powdered Extract. It meets the requirements in *Botanical Extracts* (565), *Preparations, General Pharmacopeial Requirements, Labeling*.
- **USP Reference Standards** (11)
 - USP Biochanin A RS
 - USP Daidzein RS
 - USP Formononetin RS
 - USP Genistein RS
 - USP Hyperoside RS
 - USP Powdered Red Clover Extract RS

BRIEFING

Powdered St. John's Wort Extract, *USP 38* page 6211. It is proposed to update the monograph *Definition* to expressly permit the use of suitable added substances.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C162959

Comment deadline: November 30, 2015

Powdered St. John's Wort Extract

DEFINITION

Change to read:

Powdered St. John's Wort Extract is prepared from comminuted St. John's Wort extracted with 80% methanol or other suitable solvents. It contains NLT 90.0% and NMT 110.0% of the labeled combined total of hypericin ($C_{30}H_{16}O_8$) and pseudohypericin ($C_{30}H_{16}O_9$) and NLT 90.0% and NMT 110.0% of hyperforin ($C_{35}H_{52}O_4$), on the dried-

■ the anhydrous ■ 2S (USP39) basis.

■ It may contain suitable added substances. ■ 2S (USP39)

IDENTIFICATION

Change to read:

- **A. HPTLC for Articles of Botanical Origin** (203)

Standard solution A: 0.5 mg/mL of USP Rutin RS in *methanol*

Standard solution B: 0.5 mg/mL of USP Hyperoside RS in *methanol*

Standard solution C: 50 mg/mL of USP Powdered St. John's Wort Extract RS in *methanol*.
Sonicate for 20 min, centrifuge, and use the clear supernatant.

Sample solution: 50 mg/mL of Powdered St. John's Wort Extract in *methanol*, sonicate for 20 min, centrifuge, and use the clear supernatant.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

■ ■ 2S (USP39)

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 μ m (HPTLC plates)

Application volume: 2 μ L, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient, not to exceed 30°

Developing solvent system: *Ethyl acetate, glacial acetic acid, formic acid, water, and methylene chloride* (10: 1.0: 1.0: 1.1: 2.5)

Developing distance: 6 cm

Derivatization reagent A: 5 mg/mL of 2-aminoethyl diphenylborinate in *ethyl acetate*

Derivatization reagent B: 10 mg/mL of polyethylene glycol 400 in *methylene chloride*

Analysis

Samples: *Standard solution A, Standard solution B, Standard solution C, and Sample solution*

Apply the *Samples* as bands, and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Heat the plate at 105° for 3 min, treat while still warm with *Derivatization reagent A*, and dry in air. Then treat with *Derivatization reagent B*, dry in air, and examine under UV light at 366 nm.

System suitability: *Standard solution C* exhibits, in the lower third section, two yellowish-orange fluorescent bands corresponding to rutin and hyperoside in *Standard solution A* and *Standard solution B*, respectively; a blue fluorescent band directly below the hyperoside band corresponding to chlorogenic acid; and two red fluorescent bands due to pseudohypericin (lower R_F) and hypericin in the upper third section of the chromatogram. The bands due to pseudohypericin and hypericin are clearly separated.

Acceptance criteria: The *Sample solution* exhibits the following: two yellowish-orange fluorescent bands at R_F corresponding to rutin and hyperoside in *Standard solution A*, *Standard solution B*, and *Standard solution C*; a blue fluorescent band directly below the hyperoside band corresponding to chlorogenic acid in *Standard solution C*; two red fluorescent bands at R_F corresponding to pseudohypericin and hypericin in *Standard solution C*; and two to three yellowish-orange fluorescent bands in the middle third section of the chromatogram corresponding to similar bands in *Standard solution C*.

• B. Thin-Layer Chromatography

Presence of hyperforin

Standard solution: 50 mg/mL of USP Powdered St. John's Wort Extract RS in *methanol*. Sonicate for 20 min, and use the clear supernatant.

Sample solution: 50 mg/mL of Powdered St. John's Wort Extract in *methanol*. Sonicate for 20 min, and use the clear supernatant.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.50-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Developing solvent system: *Hexane* and *ethyl acetate* (4:1)

Derivatization reagent: 0.38 g of *ceric ammonium sulfate* and 3.8 g of *ammonium molybdate* in 100 mL of 2 N *sulfuric acid*.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatograms in a saturated chamber until the solvent front has moved NLT 18 cm, and dry the plate in a current of air. Treat the plate with *Derivatization reagent*, heat at 140° for 15 min, and examine under white light.

Acceptance criteria: The blue zone due to hyperforin at an R_F value of about 0.54 in the *Sample solution* corresponds in color and position to that in the *Standard solution*.

COMPOSITION

Change to read:

• Content of Hypericin and Pseudohypericin

[Note—Conduct all sample preparations with minimal exposure to subdued light, and use low-actinic glassware.]

Solvent: *Methanol* and *acetone* (1:1)

Solution A: *Phosphoric acid* and *water* (3:997)

Solution B: *Acetonitrile*

Solution C: *Methanol*

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	100	0	0
10	85	15	0
30	70	20	10
40	10	75	15
55	5	80	15
56	100	0	0
66	100	0	0

Standard solution A: 2.5 µg/mL of USP Oxybenzone RS in *Solvent*

Standard solution B: 1 mg/mL of USP Powdered St. John's Wort Extract RS in *Solvent*

Sample solution: 1 mg/mL of Powdered St. John's Wort Extract in a mixture of *methanol* and *water* (9:1). Sonicate to dissolve, pass through a PTFE filter of 0.45-µm or finer pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm and Vis 588 nm

Columns

Guard: Packing *L1*

Analytical: 4.6-mm × 25-cm; packing *L1*

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL. [Note—First equilibrate the system with 100% *Solution A*.]

System suitability

Samples: *Standard solution A* (record the peak responses at 270 nm) and *Standard solution B* (record the peak responses at 270 and 588 nm)

Suitability requirements

Chromatogram similarity: The chromatograms of *Standard solution B* are similar to the respective reference chromatograms provided with the lot of USP Powdered St. John's Wort Extract RS being used.

Column efficiency: NLT 100,000 theoretical plates for oxybenzone, *Standard solution A*

Tailing factor: NMT 1.5 for oxybenzone, *Standard solution A*

Relative standard deviation: NMT 2.0% in replicate injections, *Standard solution A*

Analysis

Samples: *Standard solution A* and *Sample solution*

Measure the areas of the relevant peaks in the *Sample solution* at 270 nm. Calculate the percentage of the labeled amount of hypericin ($C_{30}H_{16}O_8$) and pseudohypericin ($C_{30}H_{16}O_9$) in the portion of Powdered St. John's Wort Extract taken:

$$\text{Result} = (\Sigma r_{Uj}/F_i) \times (1/r_S) \times (C_S/C_U) \times 100$$

$\Sigma r_{Uj}/F_i$ = sum of the peak areas of hypericin and pseudohypericin from the *Sample solution* divided by their respective response factors relative to oxybenzone; 1.30 for hypericin and 1.24 for pseudohypericin

r_S = peak area of oxybenzone from *Standard solution A*

C_S = concentration of USP Oxybenzone RS in *Standard solution A* (mg/mL)

C_U = nominal concentration of the total hypericins content in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% on the dried-

■ the anhydrous ■ 2S (USP39)

basis

Change to read:

• Content of Hyperforin

Analysis: Using the chromatograms obtained in the test for *Content of Hypericin and Pseudohypericin*, calculate the percentage of hyperforin ($C_{35}H_{52}O_4$) in the portion of Powdered Extract taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 1/F \times 100$$

r_U = peak area of hyperforin from the *Sample solution*

r_S = peak area of oxybenzone from *Standard solution A*

C_S = concentration of USP Oxybenzone RS in *Standard solution A* (mg/mL)

C_U = nominal concentration of hyperforin in the *Sample solution* (mg/mL)

F = relative response factor for hyperforin relative to oxybenzone, 0.46

Acceptance criteria: 90.0%–110.0% on the dried-

■ the anhydrous ■ 2S (USP39)

basis

CONTAMINANTS

Delete the following:

• **Heavy Metals** (231), *Method II*: NMT 50 µg/g • (Official 1-Dec-2015)

• **Articles of Botanical Origin** (561), *Pesticide Residue Analysis*: Meets the requirements

Add the following:

- ● **Botanical Extracts** (565), *Preparations, General Pharmacopeial Requirements, Residual Solvents*: Meets the requirements ■ 2S (USP39)
- **Microbial Enumeration Tests** (2021): The total bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species and Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

- **Water Determination** (921), *Method I*: NMT 5%
- **Articles of Botanical Origin** (561), *Methods of Analysis, Total Ash*: NMT 7.0%

Add the following:

- ● **Botanical Extracts** (565), *Preparations, General Pharmacopeial Requirements, Residue on Evaporation*: Meets the requirements ■ 2S (USP39)

Delete the following:

- ● ~~**Other Requirements**: It meets the requirements in *Botanical Extracts* (565), *Residue on Evaporation and Residual Solvents*. ■ 2S (USP39)~~

ADDITIONAL REQUIREMENTS

- **Packaging and Storage**: Preserve in tight containers, protected from moisture and light.
- **Labeling**: The label states the Latin binomial and, following the official name, the part of the plant from which the article was prepared. The label also indicates the content of hypericin, pseudohypericin, and hyperforin; the extracting solvent or solvent mixture used for preparation; and the ratio of the starting crude plant material to Powdered Extract. The label bears a statement indicating that "Rare cases of allergic reactions and photosensitivity have been reported with the use of St. John's Wort. St. John's Wort interacts with numerous medications. Check with your healthcare provider before using."
- **USP Reference Standards** (11)
 - USP Hyperoside RS
 - USP Oxybenzone RS
 - USP Rutin RS
 - USP Powdered St. John's Wort Extract RS

BRIEFING

Schizochytrium Oil, page 7915 of the *Second Supplement to USP 38*. On the basis of comments received regarding the additional types of schizochytrium oils that currently exist on the market, the following changes are proposed to be implemented as a flexible monograph:

1. The *Definition* section is revised by removing the acceptance criteria for DHA and adding EPA, DPA and others as the minor polyunsaturated fatty acid components in the article.
2. *Identification test A* is revised by adding *Standard solution 2a* and *Standard solution 2b* to the *Analysis* section, and additional acceptance criteria are added.
3. The table in the *Identification test A* is changed to *Table 1* and is revised by adding new

columns titled *Acceptance criteria II, III, and IV* for articles labeled as Type IA, Type II, or Type III, respectively.

4. A labeling requirement is added to the *Labeling* section to indicate whether the article meets the acceptance criteria for Type IA, Type II, or Type III.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(NBDS: H. Dinh.)

Correspondence Number—C136489

Comment deadline: November 30, 2015

Schizochytrium Oil

DEFINITION

Change to read:

Schizochytrium Oil is obtained by fermentation and extraction of algae of the genus *Schizochytrium* and contains ~~NLT 30.0% (w/w) of docosahexaenoic acid (DHA, C₂₂H₃₂O₂) (C22:6 n-3), as the main polyunsaturated fatty acid~~

■ docosahexaenoic acid (DHA, C₂₂H₃₂O₂) (C22:6 n-3) as the major polyunsaturated fatty acid component. It may contain eicosapentaenoic acid (EPA, C₂₀H₃₀O₂) (C20:5 n-3), docosapentaenoic acid (DPA, C₂₂H₃₄O₂) (C22:5 n-6), arachidonic acid (C₂₀H₃₂O₂) (C20:4 n-6), and dihomo gamma linolenic acid (C₂₀H₃₄O₂) (C20:3 n-6) as the minor polyunsaturated fatty acid components. DHA content may be standardized with other appropriate oils.

■ 2S (USP39)

Suitable antioxidants in appropriate concentrations may be added.

IDENTIFICATION

Change to read:

- **A. Long Chain Unsaturated Fatty Acid Profile:** Proceed as directed in *Fats and Fixed Oils* (401), *Omega-3 Fatty Acids Determination and Profile*.

Analysis

Samples: ~~Standard Solution 2~~

■ *Standard Solution 2a, Standard Solution 2b, 2S (USP39)* and *Test Solution 1*

Calculate the area percentage for dihomo gamma linolenic acid, arachidonic acid, and docosapentaenoic acid as methyl ester in *Test Solution 1*:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual fatty acid as methyl ester

r_T = sum of the responses of all the peaks, except the solvent and butylated hydroxytoluene peaks

Acceptance criteria: The retention times of the peaks of the docosahexaenoic acid methyl

ester and the eicosapentaenoic acid methyl ester of *Test Solution 1* correspond to those of *Standard Solution 2*

■ *Standard Solution 2a* and *Standard Solution 2b*, respectively, ■ 2S (USP39)

as obtained in the test for *Content of DHA and EPA*. The area percentages for the methyl esters of dihomogamma linolenic acid, arachidonic acid, and docosapentaenoic acid from the chromatogram of *Test Solution 1* meet *Acceptance criteria I, II, III, or IV* in the table below:

■ *Table 1*.

Fatty Acid	Relative Retention Time	Shorthand Notation	Lower Limit (Area %)	Upper Limit (Area %)
Dihomogammalinolenic acid	0.71	20:3 n-6	1.7	2.8
Arachidonic acid	0.73	20:4 n-6	0.6	1.3
Eicosapentaenoic acid (EPA)	0.79	20:5 n-3	1.3	3.9
Docosapentaenoic acid (DPA n-6)	0.94	22:5 n-6	10.5	16.5
Docosahexaenoic acid (DHA)	1.00	22:6 n-3	30.0	40.0

Table 1

Fatty Acid	Relative Retention Time	Shorthand Notation	Acceptance criteria I Limit (Area%)	Acceptance criteria II (For articles labeled as Type IA) Limit (Area%)	Acceptance criteria III (For articles labeled as Type II) Limit (Area%)	Acceptance criteria IV (For articles labeled as Type III) Limit (Area%)
Dihomogamma linolenic acid	0.71	20:3 n-6	NMT 2.8	NMT 1.0	NMT 1.0	NMT 1.0
Arachidonic acid	0.73	20:4 n-6	NMT 1.3	NMT 3.5	NMT 3.5	NMT 3.5
Eicosapentaenoic acid (EPA)	0.79	20:5 n-3	NMT 3.9 ^a	NMT 3.5 ^a	NLT 10.0 ^a	NMT 20.0 ^a
Docosapentaenoic acid (DPA)	0.94	22:5 n-6	NMT 16.5	NMT 25.0	NMT 3.5	NMT 6.0
Docosahexaenoic acid (DHA)	1.00	22:6 n-3	NLT 30.0 ^a	NLT 35.0 ^a	NLT 20.0 ^a	NLT 35.0 ^a

^a Limit is expressed as w/w%.

■ 2S (USP39)

COMPOSITION

Change to read:

● **Content of DHA**

■ **and EPA** ■ 2S (USP39)

Analysis: Proceed as directed in *Fats and Fixed Oils* (401), *Omega-3 Fatty Acids Determination and Profile*.

■ Use *Analysis* (for triglycerides). ■ 2S (USP39)

Acceptance criteria: NLT 30.0% (w/w) docosahexaenoic acid (DHA)

■ Meets the acceptance criteria for DHA and EPA in *Table 1* ■ 2S (USP39)

IMPURITIES

Change to read:

■ 2S (USP38)

● **Limit of Arsenic**

[Note—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of arsenic as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N *nitric acid* for 30 min and by rinsing with deionized water.]

Solution A: Transfer 1 g of ultrapure palladium metal to a Teflon beaker. Add 20 mL of water and 10 mL of *nitric acid*, and warm on a hot plate to dissolve. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution B: Transfer 1 g of ultrapure magnesium nitrate to a Teflon beaker. Add 40 mL of water and 1 mL of *nitric acid*, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A*, *Solution B*, and 2% *nitric acid* (3:2:5). A volume of 5 μL provides 0.015 mg of palladium and 0.01 mg of *magnesium nitrate*.

Blank: *Nitric acid* and water (1:19)

Standard stock solution: Transfer 10.0 mL of *Standard Arsenic Solution*, prepared as directed in *Arsenic* (211), to a 100-mL volumetric flask. Add 40 mL of water and 5 mL of *nitric acid*, and dilute with water to volume. This solution contains 0.10 $\mu\text{g}/\text{mL}$ of arsenic.

Standard solutions: Dilute the *Standard stock solution* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 $\mu\text{g}/\text{mL}$ of arsenic.

Sample solution: For preparation of the *Sample solution*, use a microwave oven with a magnetron frequency of 2455 MHz and a selectable output power of 0–950 W in 1% increments, equipped with advanced composite vessels with 100-mL polytef liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes. [**Caution**—Wear proper eye protection and protective clothing and gloves.] Transfer approximately 500 mg of Schizochytrium Oil, weighed to the nearest 0.1 mg, to a Teflon digestion vessel liner. Prepare samples in duplicate. Add 15 mL of *nitric acid*, and swirl gently. Cover the vessels with lids, leaving the vent fitting off. Predigest overnight under a hood. Place the rupture membrane in the vent fitting, and tighten the lid. Place all vessels on the microwave oven turntable. Connect the vent tubes to the vent trap, and connect the pressure-sensing line to the appropriate vessel. Initiate a two-stage digestion procedure by heating the microwave at 15% power for 15 min, followed by 25% power for 45 min. Remove the turntable of vessels from the oven, and allow the vessels to cool to room temperature. [Note—A cool water bath may be used to speed the cooling process.] Vent the vessels when they reach room temperature. Remove the lids, and slowly add 2 mL of *hydrogen peroxide* to each. Allow the reactions to subside, and seal the vessels. Return the vessels on the turntable to the microwave oven, and heat for an additional 15 min at 30% power. Remove the vessels from the oven, and allow them to cool to room temperature. Transfer the cooled digests to 25-mL volumetric flasks, and dilute with water to volume.

Analysis: Program the graphite furnace as follows. Dry at 115°, using a 1-s ramp, a 65-s hold, and an argon flow of 300 mL/min; char the sample at 1000°, using a 1-s ramp, a 20-s hold, and an airflow of 300 mL/min; cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min; atomize at 2400°, using a 0-s ramp and a 5-s hold with the argon flow stopped; and clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for arsenic. Determine the peak area at the arsenic emission line at 193.7 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of arsenic, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, *C*, in µg/mL, of arsenic in each mL of the *Sample solution* by interpolation from the regression line.

Calculate the content of arsenic in the portion of Schizochytrium Oil taken:

$$\text{Result} = (C/W) \times 25$$

C = concentration as obtained above

W = weight of Schizochytrium Oil taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

Change to read:

• **Limit of Lead**

[Note—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N *nitric acid* for 30 min and by rinsing with deionized water.]

Solution A: 10 g of ultrapure monobasic ammonium phosphate in 1 mL of *nitric acid* and 40 mL of water to dissolve the phosphate. Dilute with deionized water to 100 mL.

Solution B: Transfer 1 g of ultrapure magnesium nitrate to a Teflon beaker. Add 40 mL of water and 1 mL of *nitric acid*, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A*, *Solution B*, and 2% *nitric acid* (2:1:2). A volume of 5 µL provides 0.2 mg of phosphate plus 0.01 mg of *magnesium nitrate*.

Blank: *Nitric acid* and water (1:19)

Standard stock solution: Transfer 10.0 mL of **lead nitrate stock solution TS_{2S} (USP38)** to a 100-mL volumetric flask, add 40 mL of water and 5 mL of *nitric acid*, and dilute with water to volume. Transfer 1.0 mL of this solution to a second 100-mL volumetric flask, add 50 mL of water and 1 mL of *nitric acid*, and dilute with water to volume. This solution contains 0.10 µg/mL of lead.

Standard solutions: Dilute the *Standard stock solution* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of lead.

Sample solution: Prepare as directed for *Sample solution* in the test for *Limit of Arsenic*.

Analysis: Program the graphite furnace as follows. Dry at 120°, using a 1-s ramp, a 55-s hold, and an argon flow of 300 mL/min; char the sample at 850°, using a 1-s ramp, a 30-s hold, and an airflow of 300 mL/min; cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min; atomize at 2100°, using a 0-s ramp and a 5-s hold with the argon flow stopped; and clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for lead. Determine the peak area at the lead emission line at 283.3 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of lead, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, *C*, in µg/mL, of lead in each mL of the *Sample solution* by interpolation from the regression line.

Calculate the content of lead in the portion of Schizochytrium Oil taken:

$$\text{Result} = (C/W) \times 25$$

C = concentration, as obtained above

W = weight of Schizochytrium Oil taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

• **Limit of Cadmium**

[Note—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of cadmium as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N *nitric acid* for 30 min and by rinsing with deionized water.]

Solution A: 10 g of ultrapure monobasic ammonium phosphate in 40 mL of water and 1 mL of *nitric acid* to dissolve the phosphate. Dilute with deionized water to 100 mL.

Solution B: Transfer 1 g of ultrapure magnesium nitrate to a Teflon beaker. Add 40 mL of water and 1 mL of *nitric acid*, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A*, *Solution B*, and 2% *nitric acid* to volume (2:1:2). A volume of 5 µL provides 0.2 mg of phosphate and 0.01 mg of *magnesium nitrate*.

Blank: *Nitric acid* and water (1:19)

Standard stock solution A: 0.1372 mg/mL of *cadmium nitrate*

Standard stock solution B: *Standard stock solution A*, *nitric acid*, and water (2:1:97). This solution contains 0.10 µg/mL of cadmium. [Note—Before makeup to final volume, dissolve in a portion of water and *nitric acid*.]

Standard solutions: Dilute *Standard stock solution B* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of cadmium.

Sample solution: Prepare as directed for *Sample solution* in the test for *Limit of Arsenic*.

Analysis: Program the graphite furnace as follows. Dry at 120°, using a 1-s ramp, a 55-s hold, and an argon flow of 300 mL/min; char the sample at 850°, using a 1-s ramp, a 30-s hold, and an airflow of 300 mL/min; cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min; atomize at 2400°, using a 0-s ramp and a 5-s hold with the argon flow stopped; and clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for cadmium. Determine the peak area at the cadmium emission line at 228.8 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of cadmium, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, *C*, in µg/mL, of cadmium in each mL of the *Sample solution* by interpolation from the regression line.

Calculate the content of cadmium in the portion of Schizochytrium Oil taken:

$$\text{Result} = (C/W) \times 25$$

C = concentration, as obtained above

W = weight of Schizochytrium Oil taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

- **Limit of Mercury**

Sample solution: Prepare as directed for the *Sample solution* in the test for *Limit of Arsenic*, combining the two duplicate cooled digests into 1.0 mL of *Potassium Permanganate Solution* (see *Mercury* (261), *Method IIa* and *Method IIb*, *Reagents*).

Analysis: Proceed as directed for *Mercury* (261), *Method IIa* and *Method IIb*, except use a *Standard Mercury Solution* with the equivalent of 0.1 µg/mL of mercury.

Acceptance criteria: NMT 0.1 µg/g

SPECIFIC TESTS

- **Fats and Fixed Oils** (401), *Anisidine Value*: NMT 20.0
- **Fats and Fixed Oils** (401), *Acid Value*: The free fatty acids in 10 g require NMT 1.42 mL of 0.1 N sodium hydroxide for neutralization.
- **Fats and Fixed Oils** (401), *Peroxide Value*: NMT 5.0
- **Fats and Fixed Oils** (401), *Total Oxidation Value (TOTOX)*: NMT 26, calculated:

$$\text{Result} = (2 \times PV) + AV$$

PV= peroxide value

AV= anisidine value

- **Fats and Fixed Oils** (401), *Unsaponifiable Matter*: NMT 4.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

Change to read:

- **Labeling:** The label states the content of docosahexaenoic acid in mg/g
 - and eicosapentaenoic acid in mg/g if its content is NLT 10%. ■2S (USP39)
 It also states the name and concentration of any added antioxidant.
 - Articles intended to meet the acceptance criteria II, III, or IV are labeled as Type 1A, Type II, or Type III, respectively. ■2S (USP39)
- **USP Reference Standards** <11>
 - USP Docosahexaenoic Acid Ethyl Ester RS
 - USP Eicosapentaenoic Acid Ethyl Ester RS
 - USP Methyl Tricosanoate RS

BRIEFING

Turmeric, page 7926 of the *Second Supplement to USP 38*. The monograph proposes a new HPTLC procedure as *Identification* test A, which discontinues the use of chloroform and permits differentiation from two common confounders, *Curcuma zanthorrhiza* Roxb. and *Curcuma aromatica* Salisb.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C162474

Comment deadline: November 30, 2015

Turmeric**DEFINITION****Change to read:**

Turmeric is the dried rhizome of *Curcuma longa* L., also known as *C. domestica* Val. (Fam. Zingiberaceae). It is commonly known as Curcuma, Curcum, Haridra, and Indian Saffron. It contains NLT 3.0% of curcuminoids, calculated on the dried

- anhydrous ■2S (USP39)
- basis.

IDENTIFICATION**Delete the following:**

- **A. Thin-Layer Chromatography**

Standard solution: 0.2 mg/mL of USP Curcuminoids RS in acetone

Sample solution: Pulverize about 5 g of Turmeric. Transfer about 0.2 g of the pulverized sample to a test tube, add 3 mL of acetone, sonicate for 30 min, and centrifuge. Use the supernatant.

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, typically 20 cm long (TLC plates)

Application volume: 10 µL, as bands

Developing solvent system: Chloroform, methanol, and formic acid (96:4:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands (see *Chromatography* (621)). Use a saturated chamber. Develop the chromatograms until the solvent front has moved up about three-fourths of the length of the plate. Remove the plate from the chamber, dry, and examine under white light and under UV light at 365 nm.

Acceptance criteria: The *Sample solution* chromatogram shows yellowish-brown bands due to bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin at R_f values of about 0.4, 0.6, and 0.7, respectively, corresponding in position and color to those obtained with the *Standard solution*. ■ 2S (USP39)

Add the following:

■ ● A. HPTLC for Articles of Botanical Origin <203>

Standard solution: 1 mg/mL of USP Curcuminoids RS in *methanol*

Sample solution: Suspend about 200 mg of Turmeric, finely powdered, in 3 mL of *methanol*, and sonicate for 10 min. Centrifuge or filter, and use the supernatant or the filtrate.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 μm (HPTLC plate).¹

■ 2S (USP39)

Application volume: 2 μL each of the *Standard solution* and the *Sample solution* as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient, not to exceed 30°

Developing solvent system: *Toluene* and *glacial acetic acid* (4:1)

Developing distance: 6 cm

Derivatization reagent: 85 mL of ice-cold *methanol* combined with 10 mL of *glacial acetic acid*, 5 mL of *sulfuric acid*, and 0.5 mL of *p-anisaldehyde*

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber and dry in air. Treat with *Derivatization reagent*, heat at 100° for 3 min, and examine under long-wave UV light (365 nm) and under white light.

System suitability: Under long-wave UV light (365 nm), the derivatized chromatogram of the *Standard solution* exhibits, in its lower half, three bands, in the order of increasing R_f : an orange band due to bisdesmethoxycurcumin, an orange band due to desmethoxycurcumin, and the red band due to curcumin. Under white light, the two lower bands appear orange, while the topmost band is reddish-pink.

Acceptance criteria: Under long-wave UV light (365 nm), the derivatized chromatogram of the *Sample solution* displays two orange bands and one red band, similar in position and

color to those observed in the *Standard solution*. At the bottom part of the upper half of the plate, two purple bands are seen. Under white light, two orange bands and a darker red band are seen coincident with the bands due to bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin in the *Standard solution*, in the order of increasing R_f . In the upper half of the plate, the lower of the two bands appears purple, while the upper band is brown. No bands appear in the topmost quarter of the plate, which are characteristic of *Curcuma zanthorrhiza* Roxb. and *Curcuma aromatica* Salisb. These confounders, and occasional adulterants, of Turmeric also lack the lower orange band corresponding to bisdesmethoxycurcumin. Additional weak bands may be observed in the *Sample solution* under either illumination conditions. ■2S (USP39)

Delete the following:

- ● ~~B.~~ The retention times of the peaks for curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin of the *Sample solution* chromatogram correspond to those of the *Standard solution* for the appropriate USP Reference Standard, as obtained in the test for *Content of Curcuminoids*. ■2S (USP39)

Add the following:

- ● **B. HPLC**

Analysis: Proceed as directed in the test for *Content of Curcuminoids*.

Acceptance criteria: The retention times of the peaks for curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin of the *Sample solution* correspond to those of *Standard solution A* and *Standard solution B*. ■2S (USP39)

COMPOSITION

Change to read:

- **Content of Curcuminoids**

Mobile phase: *Tetrahydrofuran* and 1 mg/mL of *citric acid* in water (4:6)

[~~Note—Sonication may be necessary to dissolve the USP Reference Standard in each *Standard solution*; all solutions should be passed through a filter of 0.45- μ m pore size before injection. USP Curcumin RS, USP Desmethoxycurcumin RS, and USP Bisdesmethoxycurcumin RS can also be prepared in one standard solution containing the final concentration specified below for each.]~~

■ ■2S (USP39)

Standard solution A: 40 μ g/mL of USP Curcuminoids RS in *Mobile phase*

~~**Standard solution B:** 40 μ g/mL of USP Curcumin RS in *Mobile phase*~~

~~**Standard solution C:** 10 μ g/mL of USP Desmethoxycurcumin RS in *Mobile phase*~~

~~**Standard solution D:** 2 μ g/mL of USP Bisdesmethoxycurcumin RS in *Mobile phase*~~

■ **Standard solution B:** A composite solution containing 40 μ g/mL of USP Curcumin RS, 10 μ g/mL of USP Desmethoxycurcumin RS, and 2.0 μ g/mL of USP Bisdesmethoxycurcumin RS in *Mobile phase*. Use sonication if necessary. Before injection, pass through a filter of 0.45- μ m pore size, and discard the initial 10 mL of the filtrate. ■2S (USP39)

Sample stock solution: Pulverize about 5.0 g of Turmeric. Transfer about 0.5 g of the pulverized sample to a 50-mL volumetric flask, add 30 mL of *acetone*, and sonicate for 30

min. Dilute with *acetone* to volume, mix, and centrifuge.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

■ Before injection, pass through a filter of 0.45- μ m pore size, and discard the initial 10 mL of the filtrate. ■ 2S (USP39)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV

■ Vis ■ 2S (USP39)
420 nm

Column: 4.6-mm \times 20-cm; 5- μ m packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

System suitability

Samples: *Standard solution A*

■ and *Standard solution B* ■ 2S (USP39)

[Note—The relative retention times for the curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin peaks are 1.0, 1.2, and 1.4, respectively.]

Suitability requirements

Chromatogram similarity: The chromatogram of *Standard solution A* is similar to the reference chromatogram provided with USP Curcuminoids RS.

Resolution: NLT 2.0 between curcumin and desmethoxycurcumin peaks and desmethoxycurcumin and bisdesmethoxycurcumin peaks,

■ *Standard solution B* ■ 2S (USP39)

Tailing factor: NMT 1.5 for bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin peaks,

■ *Standard solution B* ■ 2S (USP39)

Relative standard deviation: NMT 2.0% for the desmethoxycurcumin peak, in replicate injections,

■ *Standard solution B* ■ 2S (USP39)

Analysis

Samples: ~~*Standard solution A,*~~

■ ■ 2S (USP39)

~~*Standard solution B, Standard solution C, Standard solution D*~~

■ ■ 2S (USP39)

and *Sample solution*

Calculate the percentages of curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin in the portion of Turmeric taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times D \times 100$$

r_U = ~~peak response for curcumin, desmethoxycurcumin, or bisdesmethoxycurcumin~~
 ■ peak area of the relevant analyte ■ 2S (USP39)
 from the *Sample solution*

r_S = ~~peak response for curcumin, desmethoxycurcumin, or bisdesmethoxycurcumin from the appropriate *Standard solution*~~
 ■ peak area of the relevant analyte from *Standard solution B* ■ 2S (USP39)

C_S = concentration of the ~~appropriate *Standard solution*~~
 ■ the relevant analyte in *Standard solution B* ■ 2S (USP39)
 (mg/mL)

V = volume of the *Sample stock solution* (mL)

W = weight of Turmeric used to prepare the *Sample stock solution* (mg)

D = dilution factor to obtain the *Sample solution* from the *Sample stock solution*, 10

Acceptance criteria: NLT ■ 3.0% ■ 2S (USP38) as the sum of curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin on the ~~dried~~
 ■ anhydrous ■ 2S (USP39)
 basis

CONTAMINANTS

Delete the following:

- **Heavy Metals**, ~~Method III (231):~~ NMT 20 ppm ■ 2S (USP38)

Add the following:

- **Articles of Botanical Origin** (561), *Limits of Elemental Impurities*: Meets the requirements ■ 2S (USP38)
- **Articles of Botanical Origin** (561), *Pesticide Residue Analysis*: Meets the requirements
- **Articles of Botanical Origin** (561), *Test for Aflatoxins*: Meets the requirements
- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacterial count does not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

● Botanical Characteristics

Macroscopic: Turmeric occurs as ovate, oblong, or pear-shaped primary rhizomes, also known as bulb or round turmeric, about 3 cm in diameter and 4–5 cm long, and showing transverse annular leaf scars, and as cylindrical, sometimes short-branched secondary rhizomes, also known as finger or long turmeric, about 1 cm in diameter and 2–7 cm long, and showing scars of lateral branches. The cured and dried turmeric of commerce is bright yellow to dull yellow in appearance, with a rough or polished surface and a characteristic aromatic odor. The texture is hard and uneasily broken, and the fracture is smooth and finely granular. Internally it is orange-yellow to orange, showing a cortex separated from a central cylinder by a distinct endodermis.

Microscopic: Transverse section of rhizome shows a row of thin-walled, flattened

epidermal cells; a few layers of thin-walled, brick-shaped parenchyma cells of the cork; a broad cortex consisting of multiple layers of thin-walled parenchyma cells showing scattered vascular bundles; a thin layer of oblong cells of the endodermis; pericycle consisting of one to two rows of parenchyma cells; and a pith consisting of parenchyma cells showing scattered vascular bundles, most of them forming discontinuous rings near the endodermis and fewer inward. The vascular bundles are of the collateral type; the vessels have mainly spiral thickening, and a few have reticulate and annular thickening. Scattered throughout the parenchyma of the pith and cortex are oleoresin cells containing oil and scattered particles of an orange-yellow pigment, and prisms of calcium oxalate, which are usually obscured due to the bright yellow color of the pigment content. The parenchyma cells are full of starch granules, 15–30 µm in size, and flat or disk-shaped. Bast fibers are absent.

- **Articles of Botanical Origin** <561>, *Methods of Analysis, Volatile Oil Determination*: NLT 3.0 mL/100 g
- **Articles of Botanical Origin** <561>, *Methods of Analysis, Foreign Organic Matter*: NMT 2.0%
- **Articles of Botanical Origin** <561>, *Methods of Analysis, Alcohol-Soluble Extractives, Method 2*: NLT 100 mg/g
- **Articles of Botanical Origin** <561>, *Methods of Analysis, Water-Soluble Extractives, Method 2*: NLT 9.0%

Change to read:

- **Water Determination** <921>, *Method Ia*: ■ NMT ■_{2S} (USP38) 10%
- **Articles of Botanical Origin** <561>, *Methods of Analysis, Total Ash*: NMT 7.0%
- **Articles of Botanical Origin** <561>, *Methods of Analysis, Acid-Insoluble Ash*: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage**: Preserve in well-closed containers. Protect from light and moisture, and store at room temperature.
- **Labeling**: The label states the Latin binomial and, following the official name, the part of the plant contained in the article.
- **USP Reference Standards** <11>
 - USP Bidesmethoxycurcumin RS
 - USP Curcumin RS
 - USP Curcuminoids RS
 - USP Desmethoxycurcumin RS

¹ Suitable commercially available plates are HPTLC Silica Gel 60 F₂₅₄ from EMD Millipore (e.g., Part No. 1.05642.0001).

BRIEFING

Powdered Turmeric, page 7928 of the *Second Supplement to USP 38*. The monograph proposes a new HPTLC procedure as *Identification* test A, which discontinues the use of chloroform and permits differentiation from two common confounders, *Curcuma zanthorrhiza* Roxb. and *Curcuma aromatica* Salisb.

Additionally, minor editorial changes have been made to update the monograph to current *USP*

style.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C162475

Comment deadline: November 30, 2015

Powdered Turmeric

DEFINITION

Change to read:

Powdered Turmeric is Turmeric reduced to a fine or very fine powder. ■ It contains NLT 3.0% of curcuminoids, calculated on the dried

■ anhydrous ■ 2S (USP39)

■ 2S (USP38) basis.

IDENTIFICATION

Delete the following:

■ ● **A. Thin-Layer Chromatography**

Standard solution: 0.2 mg/mL of USP Curcuminoids RS in acetone

Sample solution: Transfer about 0.2 g of Powdered Turmeric to a test tube, add 3 mL of acetone, sonicate for 30 min, and centrifuge. Use the supernatant.

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, typically 20 cm long (TLC plates)

Application volume: 10 µL, as bands

Developing solvent system: Chloroform, methanol, and formic acid (96:4:1)

Analysis

Samples: *Standard solution and Sample solution*

Apply the *Samples* as bands (see *Chromatography* (621)). Use a saturated chamber.

Develop the chromatograms until the solvent front has moved up about three-fourths of the length of the plate. Remove the plate from the chamber, dry, and examine under white light and under UV light at 365 nm.

Acceptance criteria: The *Sample solution* chromatogram shows yellowish-brown bands due to bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin at R_f values of about 0.4, 0.6, and 0.7, respectively, corresponding in position and color to those obtained with the *Standard solution*. ■ 2S (USP39)

Add the following:

■ ● **A. HPTLC for Articles of Botanical Origin <203>**

Standard solution: 1 mg/mL of USP Curcuminoids RS in *methanol*

Sample solution: Suspend about 200 mg of Powdered Turmeric in 3 mL of *methanol*, and sonicate for 10 min. Centrifuge or filter, and use the supernatant or the filtrate.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 μm (HPTLC plate).¹

■ ■2S (USP39)

Application volume: 2 μL each of the *Standard solution* and the *Sample solution* as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient, not to exceed 30°

Developing solvent system: *Toluene* and *glacial acetic acid* (4:1)

Developing distance: 6 cm

Derivatization reagent: 85 mL of ice-cold *methanol* combined with 10 mL of *glacial acetic acid*, 5 mL of *sulfuric acid*, and 0.5 mL of *p-anisaldehyde*

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber and dry in air. Treat with *Derivatization reagent*, heat at 100° for 3 min, and examine under long-wave UV light (365 nm) and under white light.

System suitability: Under long-wave UV light (365 nm), the derivatized chromatogram of the *Standard solution* exhibits, in its lower half, three bands, in the order of increasing R_f : an orange band due to bisdesmethoxycurcumin, an orange band due to desmethoxycurcumin, and the red band due to curcumin. Under white light, the two lower bands appear orange, while the topmost band is reddish-pink.

Acceptance criteria: Under long-wave UV light (365 nm), the derivatized chromatogram of the *Sample solution* displays two orange bands and one red band, similar in position and color to those observed in the *Standard solution*. At the bottom part of the upper half of the plate, two purple bands are seen. Under white light, two orange bands and a darker red band are seen coincident with the bands due to bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin in the *Standard solution*, in the order of increasing R_f . In the upper half of the plate, the lower of the two bands appears purple, while the upper band is brown. No bands appear in the topmost quarter of the plate, which are characteristic of *Curcuma zanthorrhiza* Roxb. and *Curcuma aromatica* Salisb. These confounders, and occasional adulterants, of Powdered Turmeric also lack the lower orange band corresponding to bisdesmethoxycurcumin. Additional weak bands may be observed in the *Sample solution* under either illumination conditions. ■2S (USP39)

Delete the following:

- ● ~~B.~~ The retention times of the peaks for curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin of the *Sample solution* chromatogram correspond to those of the *Standard solution* for the appropriate USP Reference Standard, as obtained in the test for *Content of Curcuminoids*. ■2S (USP39)

Add the following:

- ● B. HPLC

Analysis: Proceed as directed in the test for *Content of Curcuminoids*.

Acceptance criteria: The retention times of the peaks for curcumin, desmethoxycurcumin,

and bisdesmethoxycurcumin of the *Sample solution* correspond to those of *Standard solution A* and *Standard solution B*. ■2S (USP39)

COMPOSITION

Change to read:

● **Content of Curcuminoids**

Mobile phase: *Tetrahydrofuran* and 1 mg/mL of *citric acid* in water (4:6)

[~~Note—Sonication may be necessary to dissolve the USP Reference Standard in each *Standard solution*; all solutions should be passed through a filter of 0.45- μ m pore size before injection. USP Curcumin RS, USP Desmethoxycurcumin RS, and USP Bisdesmethoxycurcumin RS can also be prepared in one standard solution containing the final concentration specified below for each.]~~

■ ■2S (USP39)

Standard solution A: 40 μ g/mL of USP Curcuminoids RS in *Mobile phase*

~~**Standard solution B:** 40 μ g/mL of USP Curcumin RS in *Mobile phase*~~

~~**Standard solution C:** 10 μ g/mL of USP Desmethoxycurcumin RS in *Mobile phase*~~

~~**Standard solution D:** 2 μ g/mL of USP Bisdesmethoxycurcumin RS in *Mobile phase*~~

■ **Standard solution B:** A composite solution containing 40 μ g/mL of USP Curcumin RS, 10 μ g/mL of USP Desmethoxycurcumin RS, and 2.0 μ g/mL of USP Bisdesmethoxycurcumin RS in *Mobile phase*. Use sonication if necessary. Before injection, pass through a filter of 0.45- μ m pore size, and discard the initial 10 mL of the filtrate. ■2S (USP39)

Sample stock solution: Transfer about 0.5 g of Powdered Turmeric, accurately weighed, to a 50-mL volumetric flask, add 30 mL of *acetone*, and sonicate for 30 min. Dilute with *acetone* to volume, mix, and centrifuge.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

■ Before injection, pass through a filter of 0.45- μ m pore size, and discard the initial 10 mL of the filtrate. ■2S (USP39)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV

■ Vis ■2S (USP39)

420 nm

Column: 4.6-mm \times 20-cm; 5- μ m packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

System suitability

Samples: *Standard solution A*

■ and *Standard solution B* ■2S (USP39)

[~~Note—The relative retention times for the curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin peaks are 1.0, 1.2, and 1.4, respectively.]~~

Suitability requirements

Chromatogram similarity: The chromatogram of *Standard solution A* is similar to the reference chromatogram provided with USP Curcuminoids RS.

Resolution: NLT 2.0 between curcumin and desmethoxycurcumin peaks and desmethoxycurcumin and bisdesmethoxycurcumin peaks,

■ *Standard solution B* ■ 2S (USP39)

Tailing factor: NMT 1.5 for bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin peaks,

■ *Standard solution B* ■ 2S (USP39)

Relative standard deviation: NMT 2.0% for the desmethoxycurcumin peak, in replicate injections,

■ *Standard solution B* ■ 2S (USP39)

Analysis

Samples: ~~*Standard solution A,*~~

■ ■ 2S (USP39)

~~*Standard solution B, Standard solution C, Standard solution D*~~

■ ■ 2S (USP39)

and *Sample solution*

Calculate the percentages of curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin in the portion of Powdered Turmeric taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times D \times 100$$

r_U = ~~peak response for curcumin, desmethoxycurcumin, or bisdesmethoxycurcumin~~

■ peak area of the relevant analyte ■ 2S (USP39)
from the *Sample solution*

r_S = ~~peak response for curcumin, desmethoxycurcumin, or bisdesmethoxycurcumin from the appropriate *Standard solution*~~

■ peak area of the relevant analyte from *Standard solution B* ■ 2S (USP39)

C_S = concentration of the ~~appropriate *Standard solution*~~

■ relevant analyte in *Standard solution B* ■ 2S (USP39)
(mg/mL)

V = volume of the *Sample stock solution* (mL)

W = weight of Powdered Turmeric used to prepare the *Sample stock solution* (mg)

D = dilution factor to obtain the *Sample solution* from the *Sample stock solution*, 10

Acceptance criteria: NLT ■ 3.0% ■ 2S (USP38) as the sum of curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin

■ on the anhydrous basis ■ 2S (USP39)

CONTAMINANTS**Delete the following:**

■ • ~~Heavy Metals, Method III (231): NMT 20 ppm~~ ■ 2S (USP38)

Add the following:

USP Desmethoxycurcumin RS

¹ Suitable commercially available plates are HPTLC Silica Gel 60 F₂₅₄ from EMD Millipore (e.g., Part No. 1.05642.0001).

BRIEFING

Powdered Turmeric Extract, *USP 38* page 6241. It is proposed to make the following revisions:

1. Implement a new HPTLC procedure as *Identification* test *A*, which discontinues the use of chloroform and permits differentiation from two common confounders, *Curcuma zanthorrhiza* Roxb. and *Curcuma aromatica* Salisb.
2. Update the formula in the test for *Content of Curcuminoids* for clarity.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BDSHM: A. Bzhelyansky.)

Correspondence Number—C162476

Comment deadline: November 30, 2015

Powdered Turmeric Extract

DEFINITION

Powdered Turmeric Extract is prepared from the pulverized rhizomes of *Curcuma longa* L. (Fam. Zingiberaceae), using acetone, methanol, or other suitable solvents. It contains NLT 20% of total curcuminoids, calculated on the dried basis. It may contain other added substances.

IDENTIFICATION

Delete the following:

■ ● **A. Thin-Layer Chromatography**

Standard solution: 0.2 mg/mL of USP Curcuminoids RS in acetone

Sample solution: 10 mg/mL of Powdered Turmeric Extract in acetone

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, typically 20 cm long (TLC plates)

Application volume: 10 μ L, as bands

Developing solvent system: Chloroform, methanol, and formic acid (96:4:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the samples as bands to a suitable thin-layer chromatographic plate (see *Chromatography (621), Thin-Layer Chromatography*). Use a saturated chamber. Develop the chromatograms until the solvent front has moved up about three-fourths of the length of the plate. Remove the plate from the chamber, dry, and examine in daylight and under UV light at 365 nm.

Acceptance criteria: The ~~Sample solution~~ chromatogram shows yellowish-brown bands due to bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin at R_f values of about 0.4, 0.6, and 0.7, respectively, corresponding in position and color to those obtained from the ~~Standard solution~~. ■ 2S (USP39)

Add the following:

■ ● **A. HPTLC for Articles of Botanical Origin <203>**

Standard solution: 1 mg/mL of USP Curcuminoids RS in *methanol*

Sample solution: 50 mg/mL of Powdered Turmeric Extract in *methanol*. Sonicate to disperse, centrifuge or filter, and use the supernatant or the filtrate.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 μm (HPTLC plate).¹

■ ■ 2S (USP39)

Application volume: 2 μL each of the *Standard solution* and the *Sample solution* as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient, not to exceed 30°

Developing solvent system: *Toluene* and *glacial acetic acid* (4:1)

Developing distance: 6 cm

Derivatization reagent: 85 mL of ice-cold *methanol* combined with 10 mL of *glacial acetic acid*, 5 mL of *sulfuric acid*, and 0.5 mL of *p-anisaldehyde*

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber and dry in air. Treat with *Derivatization reagent*, heat at 100° for 3 min, and examine under long-wave UV light (365 nm) and under white light.

System suitability: Under long-wave UV light (365 nm), the derivatized chromatogram of the *Standard solution* exhibits, in its lower half, three bands, in the order of increasing R_f : an orange band due to bisdesmethoxycurcumin, an orange band due to desmethoxycurcumin, and the red band due to curcumin. Under white light, the two lower bands appear orange, while the topmost band is reddish-pink.

Acceptance criteria: Under long-wave UV light (365 nm), the derivatized chromatogram of the *Sample solution* displays two orange bands and one red band, similar in position and color to those observed in the *Standard solution*. At the bottom part of the upper half of the plate, two purple bands are seen. Under white light, two orange bands and a darker red band are seen coincident with the bands due to bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin in the *Standard solution*, in the order of increasing R_f . In the upper half of the plate, the lower of the two bands appears purple, while the upper band is brown. No bands appear in the topmost quarter of the plate, which are characteristic of *Curcuma zanthorrhiza* Roxb. and *Curcuma aromatica* Salisb. These confounders, and occasional adulterants, of Powdered Turmeric Extract also lack the lower orange band corresponding to bisdesmethoxycurcumin. Additional weak bands may

be observed in the *Sample solution* under either illumination conditions. ■ 2S (USP39)

Delete the following:

- ● ~~B.~~ The retention times of the peaks for curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin of the *Sample solution* chromatogram correspond to those of the *Standard solution* for the appropriate USP Reference Standard, as obtained in the test for *Content of Curcuminoids*. ■ 2S (USP39)

Add the following:

- ● **B. HPLC**

Analysis: Proceed as directed in the test for *Content of Curcuminoids*.

Acceptance criteria: The retention times of the peaks for curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin of the *Sample solution* correspond to those of *Standard solution A* and *Standard solution B*. ■ 2S (USP39)

COMPOSITION

Change to read:

- **Content of Curcuminoids**

Mobile phase: *Tetrahydrofuran* and 1 mg/mL of *citric acid* in water (4:6)

[~~Note—Sonication may be necessary to dissolve the USP Reference Standard in each *Standard solution*; all solutions should be passed through a filter of 0.45- μ m pore size before injection. USP Curcumin RS, USP Desmethoxycurcumin RS, and USP Bisdesmethoxycurcumin RS can also be prepared in one standard solution containing the final concentration specified below for each.]~~

■ ■ 2S (USP39)

Standard solution A: 40 μ g/mL of USP Curcuminoids RS in *Mobile phase*

~~**Standard solution B:** 40 μ g/mL of USP Curcumin RS in *Mobile phase*~~

~~**Standard solution C:** 10 μ g/mL of USP Desmethoxycurcumin RS in *Mobile phase*~~

~~**Standard solution D:** 2 μ g/mL of USP Bisdesmethoxycurcumin RS in *Mobile phase*~~

■ **Standard solution B:** A composite solution containing 40 μ g/mL of USP Curcumin RS, 10 μ g/mL of USP Desmethoxycurcumin RS, and 2.0 μ g/mL of USP Bisdesmethoxycurcumin RS in *Mobile phase*. Use sonication if necessary. Before injection, pass through a filter of 0.45- μ m pore size, and discard the initial 10 mL of the filtrate. ■ 2S (USP39)

~~**Sample solution:** Transfer about 100 mg of Powdered Turmeric Extract, accurately weighed, to a 50-mL volumetric flask, add 30 mL of acetone, and sonicate for 30 min. Dilute with acetone to volume and centrifuge. Transfer 5 mL to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.~~

■ **Sample stock solution:** Transfer about 100 mg of Powdered Turmeric Extract, accurately weighed, to a 50-mL volumetric flask, add 30 mL of *acetone*, and sonicate for 30 min. Dilute with *acetone* to volume, mix, and centrifuge.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Before injection, pass through a filter of 0.45- μ m pore size, and discard the initial 10 mL of the filtrate. ■ 2S (USP39)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV

■ Vis_{2S} (USP39)

420 nm

Column: 4.6-mm × 20-cm; 5-μm packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 μL

System suitability

Samples: *Standard solution A*

■ and *Standard solution B*_{2S} (USP39)

[Note—The relative retention times for the curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin peaks are 1.0, 1.2, and 1.4, respectively.]

Suitability requirements

Chromatogram similarity: The chromatogram of *Standard solution A* is similar to the reference chromatogram provided with USP Curcuminoids RS.

Resolution: NLT 2.0, between curcumin and desmethoxycurcumin peaks and desmethoxycurcumin and bisdesmethoxycurcumin peaks,

■ *Standard solution B*_{2S} (USP39)

Tailing factor: NMT 1.5 for bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin peaks,

■ *Standard solution B*_{2S} (USP39)

Relative standard deviation: NMT 2.0% for the desmethoxycurcumin peak, in replicate injections,

■ *Standard solution B*_{2S} (USP39)

Analysis

Samples: ~~*Standard solution A*~~,

■ ~~*2S*~~ (USP39)

~~*Standard solution B*~~, ~~*Standard solution C*~~, ~~*Standard solution D*~~

■ ~~*2S*~~ (USP39)

and *Sample solution*

Calculate the percentage of curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin in the portion of Powdered Turmeric Extract taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = ~~peak response for curcumin, desmethoxycurcumin, or bisdesmethoxycurcumin from the *Sample solution*~~

r_S = ~~peak response for curcumin, desmethoxycurcumin, or bisdesmethoxycurcumin from the appropriate *Standard solution*~~

C_S = ~~concentration of the appropriate *Standard solution* (mg/mL)~~

C_U = ~~concentration of Powdered Turmeric Extract in the *Sample solution* (mg/mL)~~

■ Calculate the percentages of curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin

in the portion of Powdered Turmeric Extract taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times D \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of the relevant analyte from *Standard solution B*

C_S = concentration of the relevant analyte in *Standard solution B* (mg/mL)

V = volume of the *Sample stock solution* (mL)

W = weight of Powdered Turmeric Extract used to prepare the *Sample stock solution* (mg)

D = dilution factor to obtain the *Sample solution* from the *Sample stock solution*,
10² (USP39)

Add the percentages due to curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin.

Acceptance criteria: NLT 20% on the dried basis

CONTAMINANTS

Delete the following:

- ~~Heavy Metals, Method III (231): NMT 20 ppm~~ (Official 1-Dec-2015)
- **Articles of Botanical Origin** (561), *Pesticide Residue Analysis*: Meets the requirements
- **Botanical Extracts** (565), *Preparations, Residual Solvents*: Meets the requirements
- **Articles of Botanical Origin** (561), *Test for Aflatoxins*: Meets the requirements
- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10⁴ cfu/g, and the total combined molds and yeasts count does not exceed 10³ cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS

- **Loss on Drying** (731)
 - Sample:** 1.0 g of Powdered Turmeric Extract
 - Analysis:** Dry the *Sample* at 105° for 2 h.
 - Acceptance criteria:** NMT 7.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Protect from light and moisture, and store at controlled room temperature.
- **Labeling:** The label states the Latin binomial and, following the official name, the part of the plant from which the article was prepared. It meets other labeling requirements in *Botanical Extracts* (565).
- **USP Reference Standards** (11)
 - USP Bisdesmethoxycurcumin RS
 - USP Curcumin RS
 - USP Curcuminoids RS

USP Desmethoxycurcumin RS

¹ Suitable commercially available plates are HPTLC Silica Gel 60 F₂₅₄ from EMD Millipore (e.g., Part No. 1.05642.0001).

BRIEFING

Benzalkonium Chloride Solution, *NF 33* page 6540. As part of the USP monograph modernization effort, it is proposed to replace the test procedure for *Alcohol Content* with a GC method with improved accuracy and precision, as well as reliability. The proposed procedure is based on a GC method of analysis performed with the Restek Rxi-624Sil MS brand of G43 column. The typical retention times for alcohol, 2-propanol, and tertiary butyl alcohol are 1.8, 2.15, and 2.4 min, respectively.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC1: H. Wang.)

Correspondence Number—C159907

Comment deadline: November 30, 2015

Benzalkonium Chloride Solution

DEFINITION

Benzalkonium Chloride Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of benzalkonium chloride in a solution having a concentration of 1.0% or more; and NLT 93.0% and NMT 107.0% of the labeled amount of benzalkonium chloride in a solution having a concentration of less than 1.0%. It may contain a suitable coloring agent and may contain NMT 10% of alcohol.

[**Caution**—Mixing Benzalkonium Chloride Solution with ordinary soaps and anionic detergents may decrease or destroy the bacteriostatic activity of the Solution.]

IDENTIFICATION

• **A.**

Analysis: To 2 mL of a solution having an equivalent of 10 mg/mL of benzalkonium chloride add 1 mL of 2 N nitric acid.

Acceptance criteria: A white precipitate is formed and is dissolved after adding 5 mL of *alcohol*.

• **B. Identification Tests—General** (191), *Chloride*: A solution of it in a mixture of equal volumes of water and *alcohol* meets the requirements.

• **C.**

Analysis: Dissolve the residue, obtained by evaporating on a steam bath a volume of Solution equivalent to 200 mg of benzalkonium chloride, in 1 mL of *sulfuric acid*. Add 100 mg of *sodium nitrate*, and heat on a steam bath for 5 min. Cool, dilute with water to 10 mL, add 500 mg of zinc dust, and warm for 5 min on a steam bath. To 2 mL of the clear supernatant add 1 mL of sodium nitrite solution (1 in 20), cool in ice water, then add 3 mL of a solution of 500 mg of *2-naphthol* in 10 mL of 6 N *ammonium hydroxide*.

Acceptance criteria: An orange-red color is produced.

• **D. Chromatographic Identity**

Analysis: Proceed as directed in the test for *Ratio of Alkyl Components*.

Acceptance criteria: The retention times of the major peaks for benzalkonium chloride of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY

• **Ratio of Alkyl Components**

Solution A: Adjust a 0.1 M solution of *sodium acetate* with *glacial acetic acid* to a pH of 5.0.

Mobile phase: *Acetonitrile* and *Solution A* (9:11). *Acetonitrile* and *Solution A* may be adjusted from (2:3) to (3:2) to meet system suitability requirements.

Standard solution: 4 mg/mL of benzalkonium chloride prepared from USP Benzalkonium Chloride RS and water

Sample solution: Transfer a volume of *Solution*, equivalent to 400 mg of benzalkonium chloride, to a 100-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing *L10* or 4.6-mm × 25-cm; 10-μm packing *L10*

Flow rate: 2 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

[Note—See *Table 1*. Relative retention times are provided for information only, and the Standard should be used to ensure appropriate peak identification.]

Table 1

Name	Relative Retention Time
C ₁₀ homolog	0.9
C ₁₂ homolog	1.0
C ₁₄ homolog	1.3
C ₁₆ homolog	1.7

Suitability requirements

Resolution: NLT 1.5 between the C₁₂ and C₁₄ homologs

Relative standard deviation: NMT 2.0% for the C₁₂ homolog

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the homolog peaks by comparison of the retention times from the *Sample solution* with those of the *Standard solution*.

Calculate the percentage of each quaternary ammonium homolog in the portion of Solution taken:

$$\text{Result} = \frac{r_U \times M_r}{\sum_i (r_U \times M_r)} \times 100$$

r_U = peak area of each homolog from the *Sample solution*

M_r = molecular weight of each homolog. The molecular weights of the C₁₀, C₁₂, C₁₄, and C₁₆ homologs are 312, 340, 368, and 396, respectively.

Acceptance criteria: On the solid basis, the content of the *n*-C₁₂H₂₅ homolog is NLT 40.0%, and the content of the *n*-C₁₄H₂₉ homolog is NLT 20.0% of the total alkylbenzyltrimethylammonium chloride content. The amount of the *n*-C₁₂H₂₅ and *n*-C₁₄H₂₉ homolog components together is NLT 70.0% of the total alkylbenzyltrimethylammonium chloride content.

- **Total Alkylbenzyltrimethylammonium Chlorides**

Sample solution: Evaporate or dilute with water to 30 mL a volume of Solution equivalent to 500 mg of benzalkonium chloride.

Analysis: Transfer the *Sample solution*, with the aid of a minimum quantity of water, to a glass-stoppered, 250-mL conical separator. Transfer 25 mL of *methylene chloride*. Add 10 mL of 0.1 N sodium hydroxide, and 10.0 mL of freshly prepared potassium iodide solution (1 in 20), insert the stopper in the separator, shake, allow the layers to separate, and discard the methylene chloride layer. Wash the aqueous layer with three 10-mL portions of *methylene chloride*, and discard the washings. Transfer the aqueous layer to a glass-stoppered, 250-mL conical flask, and rinse the separator with three 5-mL portions of water, adding the washings to the flask. Add 40 mL of cold *hydrochloric acid* to the flask, mix, and titrate with 0.05 M potassium iodate VS until the solution becomes light brown in color. Add 5 mL of *methylene chloride*, insert the stopper into the flask, and shake vigorously. Continue the titration, dropwise, with shaking after each addition, until the methylene chloride layer no longer changes color and the aqueous layer is clear yellow. Record the titrant volume, V_T , in mL. Perform a blank determination, using 20 mL of water as the sample, and record the titrant volume, V_B , in mL. [Note— $V_B > V_T$.] The difference between the two titrations represents the amount of potassium iodate equivalent to the weight of benzalkonium chloride in the sample. Each mL of 0.05 M potassium iodate is equivalent to $x/10$ mg of benzalkonium chloride, where x represents the average molecular weight of the sample, derived by summing, for all homologs, the products:

$$\text{Result } (x) = \sum_i [(r_U/r_T) \times M_r]$$

r_U = peak area of each homolog from the test for *Ratio of Alkyl Components*

r_T = sum of all the peak areas of the homologs from the test for *Ratio of Alkyl Components*

M_r = molecular weight of each homolog. The molecular weights of the C₁₀, C₁₂, C₁₄, and C₁₆ homologs are 312, 340, 368, and 396, respectively.

Acceptance criteria

For labeled concentrations of NLT 1.0%: 95.0%–105.0%

For labeled concentrations less than 1.0%: 93.0%–107.0%

OTHER COMPONENTS

Change to read:

- **Alcohol Content** (if added)

Internal standard solution: ~~0.06 mL/mL of tertiary butyl alcohol in water~~

Alcohol stock solution: ~~0.03 mL/mL of alcohol (C₂H₅OH) in water~~

Standard solutions: ~~Introduce 5, 10, and 20 mL, respectively, of Alcohol stock solution into three separate identical 50 mL volumetric flasks. To each flask add a 5 mL portion of the Internal standard solution. Dilute with water to volume, and mix thoroughly. The Standard solutions contain 0.003, 0.006, and 0.012 mL/mL of alcohol (C₂H₅OH), respectively.~~

Sample solution: ~~Weigh an appropriate amount of Solution into a 50 mL volumetric flask, and pipet 5 mL of the Internal standard solution into the flask. Dilute with water to volume, and mix thoroughly to obtain a solution containing 0.003–0.012 mL/mL of alcohol (C₂H₅OH).~~

Chromatographic system

~~(See Chromatography (621), System Suitability.)~~

Mode: ~~GC~~

Detector: ~~Flame ionization~~

Column: ~~0.25-mm × 30-m glass or quartz capillary; 1.4-μm layer of phase G43~~

Temperatures

Detector: ~~250°~~

Injection port: ~~250°~~

Column: ~~See Table 2.~~

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	7
40	30	250	±5

Run time: 29 min

Carrier gas: Helium or nitrogen

Flow rate: See Table 3.

Table 3

Initial Flow (mL/min)	Flow Ramp (mL/min ²)	Final Flow (mL/min)	Hold Time at Final Flow (min)
±	—	±	8
±	±0	3	2±

Injection volume: 1 µL

Injection type: Split 75:1

System suitability

Sample: Standard solution containing 0.006 mL/mL of alcohol (C₂H₅OH)

[Note—The relative retention times for alcohol and tertiary butyl alcohol are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between alcohol and tertiary butyl alcohol

Relative standard deviation: NMT 10%

Analysis

Samples: Standard solutions and Sample solution

Plot the peak response ratios of the alcohol to tertiary butyl alcohol in the Standard solutions versus the content, in mL/mL, of alcohol, and draw the straight line best fitting the plotted points. From the graph obtained, determine the content, *C*, in mL/mL, of alcohol (C₂H₅OH) in the Sample solution.

Calculate the percentage of alcohol (C₂H₅OH) in the portion of Solution (v/v) taken:

$$\text{Result} = V \times (C \times D / W) \times 100$$

V = volume of the Sample solution, 50 mL

D = density of the Benzalkonium Chloride Solution (g/mL)

W = weight of Benzalkonium Chloride Solution taken to prepare the Sample solution (g)

▪ **Diluent:** 2-Propanol and water (8:2)

Internal standard solution: 0.005 g/mL of tertiary butyl alcohol in water

Alcohol stock solution: 0.015 g/mL of alcohol (C₂H₅OH) in water

Standard solutions: Introduce 1, 2, and 4 mL, respectively, of Alcohol stock solution into

three separate and identical 25-mL volumetric flasks. To each flask add a 5-mL portion of the *Internal standard solution*. Dilute with *Diluent* to volume, and mix thoroughly. The *Standard solutions* contain 0.0006, 0.0012, and 0.0024 g/mL of alcohol (C₂H₅OH), respectively.

Sample solution: Weigh an appropriate amount of Solution into a 25-mL volumetric flask, and pipet 5 mL of the *Internal standard solution* into the flask. Dilute with *Diluent* to volume, and mix thoroughly to obtain a solution containing 0.0006–0.0024 g/mL of alcohol (C₂H₅OH).

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 15-m glass or quartz capillary; 1.4-μm layer of phase G43

Temperatures

Injection port: 250°

Detector: 320°

Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	—	50	4
50	10	70	0
70	50	300	4

Run time: 14.6 min

Carrier gas: Helium

Flow rate: See *Table 3*.

Table 3

Initial Flow (mL/min)	Flow Ramp (mL/min ²)	Final Flow (mL/min)	Hold Time at Final Flow (min)
1.4	—	1.4	6
1.4	5	3	8.6

Injection volume: 0.5 μL

Injection type: Split, 75:1

Inlet liner: 4-mm liner with deactivated glass wool

System suitability

Sample: *Standard solution* containing 0.0012 g/mL of alcohol (C₂H₅OH)

[Note—The relative retention times for alcohol, 2-propanol, and tertiary butyl alcohol are 0.75, 0.90, and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 2.0 between 2-propanol and tertiary butyl alcohol**Relative standard deviation:** NMT 2% for the peak response ratio of alcohol to the internal standard**Analysis****Samples:** *Standard solutions* and *Sample solution*

Plot the peak response ratios of alcohol to tertiary butyl alcohol in the *Standard solutions* versus the content, in g/mL, of alcohol, and draw the straight line best fitting the plotted points. From the graph obtained, determine the content, *C*, in g/mL, of alcohol (C₂H₅OH) in the *Sample solution*.

Calculate the percentage of alcohol (C₂H₅OH) in the portion of Solution (v/v) taken:

$$\text{Result} = (V \times C \times D_S) / (W \times D_A) \times 100$$

V = volume of the *Sample solution*, 25 mL*D_S* = density of the Solution (g/mL)*W* = weight of Solution taken to prepare the *Sample solution* (g)*D_A* = density of alcohol (g/mL)

■ 2S (NF34)

Acceptance criteria: If present, 95.0%–105.0% of the labeled amount of alcohol (C₂H₅OH)**IMPURITIES**● **Limit of Amines and Amine Salts****Sample:** A quantity of Solution equivalent to 5.0 g of benzalkonium chloride

Analysis: Dissolve the *Sample* by heating carefully (e.g., on top of a steam bath with water as the steam source) in 20 mL of a mixture of *methanol* and 1 N hydrochloric acid VS (97:3). [Note—The mixed solution, however, must not reach the boiling point.] Add 100 mL of *isopropyl alcohol*, and pass a stream of nitrogen slowly through the solution. Gradually add 12.0 mL of 0.1 N tetrabutylammonium hydroxide VS while recording the potentiometric titration curve.

Acceptance criteria: If the curve shows two inflection points, the volume of titrant added between the two points is NMT 5.0 mL, corresponding to NMT 0.1 mmol/g of amines and amine salts. If the curve shows no point of inflection, the substance being examined does not comply with the test. If the curve shows one point of inflection, repeat the test, but add 3.0 mL of a 25.0 mg/mL solution of *dimethyldecylamine* in *isopropyl alcohol* before the titration. If after the addition of 12.0 mL of the titrant, the titration curve shows only one point of inflection, the substance being examined does not comply with the test.

● **Limit of Benzyl Alcohol, Benzaldehyde, and (Chloromethyl)benzene**

[Note—Prepare the solutions immediately before use.]

Solution A: Dissolve 1.09 g of *sodium 1-hexanesulfonate* and 6.9 g of *monobasic sodium phosphate* in water in a 1000-mL volumetric flask, adjust with *phosphoric acid* to a pH of 3.5, and dilute with water to volume.

Solution B: *Methanol*

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	80	20
10	80	20
14	50	50
35	50	50
36	20	80
55	20	80
56	80	20
65	80	20

Standard solution A: 0.25 mg/mL of USP Benzyl Alcohol RS in methanol

Standard solution B: 0.075 mg/mL of USP Benzaldehyde RS in methanol

Standard solution C: 0.025 mg/mL of USP Benzyl Alcohol RS in methanol, prepared from *Standard solution A* and methanol

Sample solution: Determine the density of the Solution. Dilute a quantity of the Solution equivalent to 2.5 g of benzalkonium chloride with methanol to 50.0 mL. This solution contains 50 mg/mL of benzalkonium chloride.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 210 nm for benzyl alcohol and (chloromethyl)benzene; UV 257 nm for benzaldehyde

Column: 4.6-mm × 15-cm; 5-µm packing *L1*

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

[Note—See *Table 5* for relative retention times.]

Table 5

Name	Relative Retention Time
Benzyl alcohol	1.0
Benzaldehyde	1.3
(Chloromethyl)benzene	2.4

Suitability requirements

Relative standard deviation: NMT 5.0% for benzyl alcohol, *Standard solution A*

Signal-to-noise ratio: NLT 10 for the principal peak, *Standard solution C*

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Calculate the content of (chloromethyl)benzene by multiplying the peak area of (chloromethyl)benzene by 1.3. [Note—The correction factor is used to adjust for baseline shift.]

Acceptance criteria

Benzyl alcohol: The response of the benzyl alcohol peak from the *Sample solution* is NMT that of the benzyl alcohol peak from *Standard solution A*, corresponding to NMT 0.5%.

Benzaldehyde: The response of the benzaldehyde peak from the *Sample solution* is NMT that of the benzaldehyde peak from *Standard solution B*, corresponding to NMT 0.15%.

(Chloromethyl)benzene: The response of the (chloromethyl)benzene peak from the *Sample solution* is NMT 0.1 times that of the principal peak from *Standard solution A*, corresponding to NMT 0.05%.

SPECIFIC TESTS

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): A solution containing less than 5.0% of benzalkonium chloride meets the requirements of the test for absence of *Pseudomonas aeruginosa*.
- **Acidity or Alkalinity**

Sample solution: Evaporate or dilute with *carbon dioxide-free water* to prepare a 50-mL solution of 10 mg/mL of benzalkonium chloride in water.

Analysis: To the *Sample solution* add 0.1 mL of *bromocresol purple TS*.

Acceptance criteria: NMT 0.5 mL of 0.1 N hydrochloric acid or 0.1 N sodium hydroxide is required to change the color of the indicator.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and prevent contact with metals.
- **Labeling:** Label it to indicate the concentration of benzalkonium chloride, and to indicate the name and quantity of the coloring agent added. The labeling also indicates the concentration of alcohol added.
- **USP Reference Standards** (11)
 - USP Benzaldehyde RS
 - USP Benzalkonium Chloride RS
 - USP Benzyl Alcohol RS

BRIEFING

Calcium Propionate, *NF 33* page 6560; **Potassium Benzoate**, *NF 33* page 6835; **Sodium Cetostearyl Sulfate**, *NF 33* page 6868. In preparation for the omission of the flame tests from

Identification Tests—General (191), proposed in PF 41(2) [Mar.–Apr. 2015], the reference to (191) in the *Identification* test is deleted and a complete description of the flame test is included in each monograph. Manufacturers are encouraged to submit a replacement wet chemistry test or an instrumental procedure for the Expert Committee's consideration.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

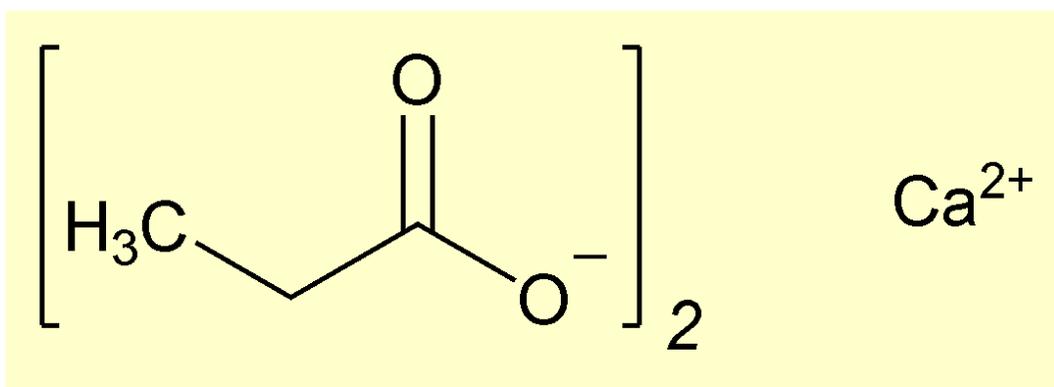
(EXC1: G. Holloway.)

Correspondence Number—C162764

Comment deadline: November 30, 2015

Calcium Propionate

Change to read:



▲NF33

$C_6H_{10}CaO_4$ 186.22
[4075-81-4].

DEFINITION

Calcium Propionate contains NLT 98.0% and NMT 100.5% of calcium propionate ($C_6H_{10}CaO_4$), calculated on the anhydrous basis.

IDENTIFICATION

Delete the following:

- ● **A. Identification Tests—General, Calcium** (191): A solution (1 in 20) meets the requirements of the flame test. ■2S (NF34)

Add the following:

- ● **A.** A solution of Calcium Propionate (1 in 20) imparts a transient yellowish red color to a nonluminous flame. ■2S (NF34)

ASSAY

- **Procedure**

Sample solution: 4 mg/mL of Calcium Propionate

Analysis: While stirring, preferably with a magnetic stirrer, add 30 mL of 0.05 M edetate disodium VS from a 50-mL buret to 100 mL of the *Sample solution*. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium VS is equivalent to 9.311 mg of calcium propionate ($C_6H_{10}CaO_4$).

Acceptance criteria: 98.0%–100.5% on the anhydrous basis

IMPURITIES

• Limit of Fluoride

Buffer: 294.1 mg/mL of sodium citrate dihydrate

Standard solution: 2.21 mg/mL of USP Sodium Fluoride RS. [Note—Store solution in a plastic bottle.] On the day of use, transfer 5.0 mL of the resulting solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains 5 µg of fluoride ion.

Electrode system: Use a fluoride-specific, ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ± 0.2 mV (see *pH* (791)).

Standard response line: Transfer 1.0, 2.0, 3.0, 5.0, 10.0, and 15.0 mL of the *Standard solution* to separate 250-mL plastic beakers. Add 50 mL of water, 5 mL of 1 N hydrochloric acid, 10 mL of *Buffer*, and 10 mL of 0.2 M edetate disodium to each beaker, and mix. Transfer each solution to separate 100-mL volumetric flasks, dilute with water to volume, and mix. Transfer a 50-mL portion of each solution to separate 125-mL plastic beakers, and read the potential, in mV, of each solution using the electrode system. Plot the calibration curve versus potential, in mV, on two-cycle semilogarithmic paper with µg of fluoride per 100 mL of solution on the logarithmic scale.¹

Sample: 1.0 g

Analysis: Transfer the *Sample* to a 150-mL glass beaker, add 10 mL of water, and, while stirring continuously, slowly add 20 mL of 1 N hydrochloric acid to dissolve the *Sample*. Boil rapidly for 1 min. Transfer to a 250-mL plastic beaker, and cool rapidly in ice water. Add 15 mL of *Buffer* and 10 mL of 0.2 M edetate disodium, and mix. Adjust the pH to 5.5 ± 0.1 with 1 N hydrochloric acid or 1 N sodium hydroxide, if necessary. Transfer to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer a 50-mL portion of this solution to a 125-mL plastic beaker, and record the potential using the electrode system. Determine the concentration of fluoride, in µg/mL, in the *Sample*.

Acceptance criteria: NMT 30 µg/g

Change to read:

• Limit of Lead

▲▲NF33

25% Sulfuric acid solution: Cautiously add 100 mL of sulfuric acid to 300 mL of water with constant stirring while cooling in an ice bath.

▲ **Standard solutions:**▲NF33 On the day of use, transfer 5.0, 10.0, and 20.0 mL of ▲ standard lead solution TS▲NF33 to three separate 100-mL volumetric flasks, add 10 mL of 3 N hydrochloric acid to each, and dilute with water to volume corresponding to 0.5-,

1.0-, and 2.0- $\mu\text{g}/\text{mL}$ standards.

Sample blank: Add 5 mL of 25% *Sulfuric acid solution* into an evaporating dish. Within a hood, place the dish on a steam bath to evaporate most of the water. Place the dish on a burner, and slowly pre-ash the sample by expelling most of the sulfuric acid. Place the dish in a muffle furnace that has been set at 525°, and ash the sample until the residue appears free from carbon. Cool, and cautiously wash down the inside of the evaporation dish with water. Add 5 mL of 1 N *hydrochloric acid*. Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 N *hydrochloric acid* and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer the solution quantitatively to a 10-mL volumetric flask, dilute to volume, and mix.

Sample solution: Place 10 g of Calcium Propionate, to the nearest 0.1 mg, into an evaporating dish. Add a sufficient amount of 25% *Sulfuric acid solution*, and distribute the sulfuric acid uniformly through the sample. Proceed as directed under *Sample blank* beginning with "Within a hood..."

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

Mode: Atomic absorption

Analytical wavelength: 283.3 nm

Lamp: Lead electrodeless discharge

Flame: Air-acetylene

Slit width: 0.7 nm

Instrument blank: Water

Standard curve

Samples: ▲ *Standard solutions* ▲NF33 and *Sample blank*

Plot: Corrected absorbance values versus their corresponding concentration ($\mu\text{g}/\text{mL}$).

[Note—Determine corrected absorbance values by subtracting the absorbance of the *Sample blank* from the absorbance of the ▲ *Standard solutions*. ▲NF33]

Analysis

Samples: *Sample blank* and *Sample solution*

[Note—Determine corrected absorbance values by subtracting the absorbance of the *Sample blank* from the absorbance of the *Sample solution*.]

From the *Standard curve*, determine the lead concentration in the *Sample solution*. Calculate the lead content in the portion of Calcium Propionate taken:

$$\text{Result} = (C_S \times V) / W$$

C_S = concentration of lead from the *Standard curve* ($\mu\text{g}/\text{mL}$)

V = final volume of the sample (mL)

W = weight of the sample taken (g)

Acceptance criteria: NMT 2 $\mu\text{g}/\text{g}$

- **Magnesium** (as MgO)

Magnesium standard solution: Dissolve 50.0 mg of *magnesium* metal in 1 mL of

hydrochloric acid in a 1000-mL volumetric flask, dilute with water to volume, and mix.

Sample solution: Place 400.0 mg of Calcium Propionate, 5 mL of 2.7 N *hydrochloric acid*, and about 10 mL of water in a small beaker, and dissolve the Calcium Propionate by heating on a hot plate.

Analysis: Evaporate the *Sample solution* to a volume of about 2 mL, and cool. Transfer the residual liquid to a 100-mL volumetric flask, dilute with water to volume, and mix. Dilute 7.5 mL of this solution with water to 20 mL, add 2 mL of 1 N *sodium hydroxide* and 0.05 mL of a 1:1000 solution of *thiazole yellow*, and mix. Allow to stand for 10 min, and shake. Any color produced does not exceed that produced by 1.0 mL of *Magnesium standard solution* in the same volume as that of a control containing 2.5 mL of the *Sample solution* (corresponding to 10 mg of Calcium Propionate) and the quantities of the reagents used in the test.

Acceptance criteria: The solution passes the test (about 0.4%).

SPECIFIC TESTS

• Water-Insoluble Substances

Sample: 10 g

Analysis: Dissolve the *Sample* in 100 mL of hot water, pass through a tared filtering crucible, wash the insoluble residue with hot water, and dry at 105° to constant weight.

Acceptance criteria: NMT 0.2%

- **pH** (791): 7.5–10.5, in a solution (1 in 10)
- **Water Determination** (921), *Method I*: NMT 5.0%
- **Loss on Drying** (731)

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 4%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store in tightly closed containers. No storage requirements specified.
- **USP Reference Standards** (11)
USP Sodium Fluoride RS

¹ A suitable source for semilogarithmic paper can be found at <http://statland.org/GraphPaper/gpaper.html>.

BRIEFING

Deoxycholic Acid, *NF 33* page 6636. See the *Briefing* under *Desoxycholic Acid* appearing elsewhere in this issue of *PF*. This monograph is proposed for inclusion in the *Second Supplement* to *USP 39—NF 34* with an official date of December 1, 2016. Use of the name Deoxycholic Acid will be permitted as of December 1, 2016, however its use will not be mandatory until December 1, 2021. The 60-month extension will provide the time deemed necessary for labeling changes to be made for the article and the numerous preparations in which it is an ingredient, as well as for practitioners, consumers, and regulatory agencies to become familiar with the new terminology. Because of the use of deoxycholic acid as an active pharmaceutical ingredient (API) in an FDA approved drug, it is proposed to change the

monograph designation from *NF* to *USP* and to include new tests and acceptance criteria to reflect the use of deoxycholic acid as an API. This monograph will be listed in the *NF* section as a cross reference to the *USP* general monographs section to reflect its use as both an active and inactive ingredient. The HPLC method in the *Assay* and *Organic Impurities* tests is based on analysis performed with the Phenomenex Pursuit 3 C18 brand of L1 column. The retention times for cholic acid; 3 α ,12 β -dihydroxy-5 β -cholan-24-oic acid; 3 α ,12 α -dihydroxy-5 β -chol-9(11)-en-24-oic acid; deoxycholic acid; and ethyl 3 α ,12 α -dihydroxy-5 β -cholan-24-oate are about 7.3, 9.0, 11.3, 13.0, and 20.9 min, respectively.

(EXC1: G. Holloway.)

Correspondence Number—C158480

Comment deadline: November 30, 2015

Change to read:

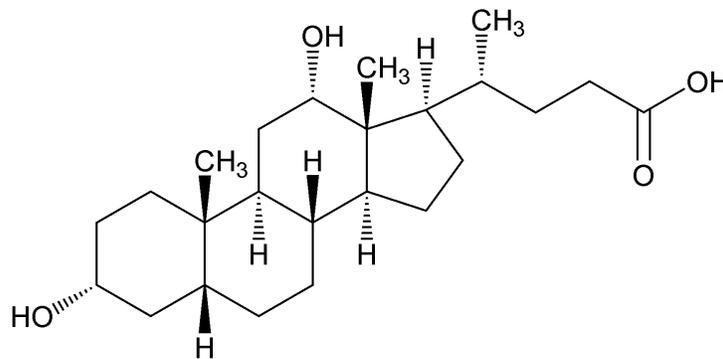
Desoxycholic

■ **Deoxycholic** ■_{2S} (NF34)

Acid

(Title for this monograph—to become official December 1, 2021)

(Prior to December 1, 2021, the current practice of labeling the article of commerce with the name Desoxycholic Acid may be continued. Use of the name Deoxycholic Acid will be permitted as of December 1, 2016; however, the use of this name will not be mandatory until December 1, 2021. The 60-month extension will provide the time needed by manufacturers and users to make necessary changes.)



$C_{24}H_{40}O_4$ 392.57

Desoxycholic acid;

3 α ,12 α -Dihydroxycholanic acid [83-44-3].

DEFINITION

Change to read:

Desoxycholic

■ **Deoxycholic** ■_{2S} (NF34)

Acid contains ~~NLT 98.0% and NMT 102.0%~~

■ **NLT 97.0% and NMT 103.0%** ■_{2S} (NF34)

of desoxycholic

- deoxycholic ■ 2S (NF34)
acid (C₂₄H₄₀O₄), calculated on the dried
- anhydrous ■ 2S (NF34)
basis.
- Deoxycholic Acid can be of animal or synthetic origin. ■ 2S (NF34)

IDENTIFICATION

Delete the following:

- • ~~A.~~

~~**Analysis:** Add 2 drops of benzaldehyde and 3 drops of 75% sulfuric acid to about 10 mg of Desoxycholic Acid, heat at 50° for 5 min, and then add 10 mL of glacial acetic acid.~~

~~**Acceptance criteria:** A green color appears (cholic acid produces a brown color). ■ 2S (NF34)~~

Add the following:

- • **A. Infrared Absorption** (197A) ■ 2S (NF34)

Add the following:

- • **B.** The retention time of the deoxycholic acid peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (NF34)

ASSAY

Change to read:

- **Procedure**

~~**Sample:** 500 mg~~

~~**Analysis:** Transfer the *Sample* into a 250 mL Erlenmeyer flask, and add 20 mL of water and 40 mL of alcohol. Cover the flask with a watch glass, heat the mixture gently on a steam bath until the *Sample* is dissolved, and allow the mixture to cool to room temperature. Add a few drops of phenolphthalein TS to the solution, and titrate with 0.1 N sodium hydroxide to a pink endpoint that persists for 15 s. Each mL of 0.1 N sodium hydroxide is equivalent to 39.26 mg of desoxycholic acid (C₂₄H₄₀O₄).~~

~~**Acceptance criteria:** 98.0%–102.0% on the dried basis~~

▪ **Diluent:** Methanol and water (80:20)

Solution A: 0.1% Formic acid in water

Solution B: 0.1% Formic acid in acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	75.0	25.0
2.0	55.0	45.0
14.0	42.0	58.0
24.0	0.0	100.0
35.0	0.0	100.0

Standard solution: 0.01 mg/mL of USP Deoxycholic Acid RS in *Diluent*

Sample solution: 0.01 mg/mL of Deoxycholic Acid in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Charged aerosol

Column: 4.6-mm × 15-cm; 3-μm packing *L1*

Flow rate: 1.0 mL/min

Autosampler temperature: 5°

Injection volume: 25 μL

Run time: 35 min

System suitability

Sample: *Standard solution*

[Note—The retention time of deoxycholic acid is about 13.0 min.]

Suitability requirements

Relative standard deviation: NMT 3.0% for six injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of deoxycholic acid (C₂₄H₄₀O₄) in the portion of Deoxycholic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak area of deoxycholic acid from the *Sample solution*

r_S = peak area of deoxycholic acid from the *Standard solution*

C_S = concentration of the USP Deoxycholic Acid RS in the *Standard solution*

C_U = concentration of Deoxycholic Acid in the *Sample solution*

P = labeled purity of USP Deoxycholic Acid RS expressed as a percentage

Acceptance criteria: 97.0%–103.0% on the anhydrous basis ■2S (NF34)

IMPURITIES

- **Residue on Ignition** (281)

Sample: 1.0 g

Acceptance criteria: NMT 0.2%

Change to read:

● **Limit of Lead**

[Note—

■ This test and *Acceptance criteria* are only applicable to Deoxycholic Acid of animal origin. ■ 2S (NF34)

Select reagents having as low a lead content as practicable, and store all solutions in high-density polyethylene containers. Rinse all plastic and glassware thoroughly with warm, 50% nitric acid followed by water.]

Standard solutions: [Note—Prepare these solutions on the day of use.] Transfer 10.0 and 50.0 mL of ▲ *standard lead solution TS* ▲NF33 into two separate 100-mL volumetric flasks, add 10 mL of 3 N *hydrochloric acid* to each, and dilute with water to volume. The third standard, 10.0 µg/mL, is taken directly from ▲ *standard lead solution TS*. ▲NF33

Sample solution: Transfer 10.0 g of ~~Desoxycholic~~

■ Deoxycholic ■ 2S (NF34)

Acid, weighed to the nearest 0.1 mg, into an evaporating dish. Add 5 mL of 25% sulfuric acid (made by adding 25 mL of *sulfuric acid* to 75 mL of water), and distribute the 25% sulfuric acid solution uniformly. Within a hood, place the dish on a steam bath to evaporate most of the water. Place the dish on a burner, and slowly pre-ash the remaining *Sample solution* by expelling most of the sulfuric acid. Place the dish in a muffle furnace that has been set at 525°, and ash the contents of the dish until the residue appears free from carbon. Cool, and cautiously wash down the inside of the evaporation dish with water. Add 5 mL of 1 N *hydrochloric acid*. Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 N *hydrochloric acid* and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer to a 10-mL volumetric flask, dilute with water to volume, and mix.

Sample blank: Prepare by ashing 5 mL of 25% sulfuric acid solution. Cool, and cautiously wash down the inside of the evaporation dish with water. Add 5 mL of 1 N *hydrochloric acid*. Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 N *hydrochloric acid* and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer to a 10-mL volumetric flask, dilute with water to volume, and mix.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption

Analytical wavelength: 283.3 nm

Lamp: Lead electrodeless discharge

Flame: Air-acetylene

Slit width: 0.7 nm

Instrument blank: Water

Standard curve

Samples: *Standard solutions* and *Sample blank*

Plot: Corrected absorbance values versus their corresponding concentration (µg/mL).

[Note—Determine corrected absorbance values by subtracting the absorbance of the *Sample blank* from the absorbance of the *Standard solutions*.]

Analysis

Samples: *Sample solution* and *Sample blank*

[Note—Determine corrected absorbance values by subtracting the absorbance of the *Sample blank* from the absorbance of the *Sample solution*.]

From the *Standard curve*, determine the lead concentration in the *Sample solution*.

Calculate the lead content in the portion of ~~Desoxycholic~~

■ Deoxycholic ■ 2S (NF34)

Acid taken:

$$\text{Result} = (C_S \times V) / W$$

C_S = concentration of lead from the *Standard curve* ($\mu\text{g/mL}$)

V = final volume of the sample (mL)

W = weight of the sample taken (g)

Acceptance criteria: NMT 4 $\mu\text{g/g}$

Add the following:

■ • **Heavy Metals** (231), *Method II*: NMT 20 $\mu\text{g/g}$

[Note—This test and *Acceptance criteria* are only applicable to Deoxycholic Acid of synthetic origin.]

■ 2S (NF34)

Add the following:

■ • **Organic Impurities**

Diluent, Mobile phase, Standard solution, and Chromatographic system: Proceed as described in the procedure for *Assay*.

Sensitivity solution: 0.5 $\mu\text{g/mL}$ of USP Deoxycholic Acid RS in *Diluent*

Sample stock solution: 1.0 mg/mL of Deoxycholic Acid in *Diluent*

Sample solution: 0.01 mg/mL of Deoxycholic Acid in *Diluent* prepared from the *Sample stock solution*

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[Note—The retention time of deoxycholic acid is about 13.0 min. The relative retention times for cholic acid and deoxycholic acid are 0.56 and 1, respectively. The relative retention times for other impurities are listed in *Table 2*.]

Suitability requirements

Relative standard deviation: NMT 3.0% for six injections, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Sample stock solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Deoxycholic Acid taken:

$$\text{Result} = [r_U / (r_S \times 100 + r_T)] \times 100$$

r_U = peak area of individual impurity from the *Sample stock solution*

r_S = peak area of deoxycholic acid from the *Sample solution*

r_T = sum of peak areas of all impurities from the *Sample stock solution*

Acceptance criteria

Deoxycholic Acid of animal origin

Cholic acid: NMT 1.0%

Total impurities: NMT 2.0%

Deoxycholic Acid of synthetic origin: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria NMT (%)
3 α , 12 β -Dihydroxy-5 β -cholan-24-oic acid	0.69	0.15
3 α , 12 α -Dihydroxy-5 β -chol-9(11)-en-24-oic acid	0.87	0.15
Ethyl 3 α , 12 α -dihydroxy-5 β -cholan-24-oate	1.61	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

■ 2S (NF34)

SPECIFIC TESTS

Change to read:

- **Optical Rotation** (781S), *Specific Rotation*

Sample solution: 1% (w/v) solution in *ethanol*

Acceptance criteria NLT +55°

■ **Deoxycholic Acid of animal origin:** NLT +55°

Deoxycholic Acid of synthetic origin: +50° to +60° ■ 2S (NF34)

Delete the following:

- • ~~**Melting Range or Temperature** (741), *Class I:* 172°–175° ■ 2S (NF34)~~

Delete the following:

- • ~~**Loss on Drying** (731)~~

~~**Analysis:** Dry at 140° under vacuum of NMT 5 mm of mercury for 4 h.~~

~~**Acceptance criteria:** NMT 1% ■ 2S (NF34)~~

Add the following:

- • **Water Determination** (921), *Method Ic:* NMT 1.0% ■ 2S (NF34)

Add the following:

- • **Clarity of Solution**

[Note—If intended for use in preparing parenteral dosage forms, it meets the requirements of the *Clarity of Solution* test.]

Hydrazine solution: 10 mg/mL of *hydrazine sulfate*. Allow to stand 4–6 h before use.

Methenamine solution: Transfer 2.5 g of *methenamine* to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

Primary opalescent suspension: To the flask containing *Methenamine solution* add 25.0 mL of the *Hydrazine solution*, mix, and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Opalescence standard: Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, and dilute with water to volume. Use this suspension within 24 h after preparation.

Reference suspension: Transfer 5.0 mL of the *Opalescence standard* to a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: 5 mg/mL of Deoxycholic Acid in 0.1 N *sodium hydroxide*

Analysis: Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer a portion of the *Reference suspension* to a separate matching test tube. Compare the *Sample solution* and the *Reference suspension* in diffused daylight, viewing vertically against a black background (see *Nephelometry, Turbidimetry, and Visual Comparison* (855)) for 5 min after preparation of the *Reference suspension*.

Acceptance criteria: The *Sample solution* is not more opalescent than the *Reference suspension*. ■2S (NF34)

Add the following:

■ ● **Color of Solution**

[Note—If intended for use in preparing parenteral dosage forms, it meets the requirements of the *Color of Solution* test.]

Diluent: 27.5 mL of *hydrochloric acid* in 1000 mL of water

Standard stock solutions: Prepare two solutions, A and B, containing, respectively, the following parts of *ferric chloride CS*, *cobaltous chloride CS*, *cupric sulfate CS*, and *Diluent*.

Standard stock solution A: 2.4: 0.6: 0: 7.0

Standard stock solution B: 2.4: 1.0: 0.4: 6.2

Standard solutions

[Note—Prepare and use these solutions immediately.]

Standard solution A: Transfer 2.5 mL of *Standard stock solution A* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Standard solution B: Transfer 2.5 mL of *Standard stock solution B* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Sample solution: 5 mg/mL of Deoxycholic Acid in 0.1 N *sodium hydroxide*

Analysis: Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm, to

obtain a depth of 40 mm. Similarly transfer portions of *Standard solution A* and *Standard solution B* to separate matching test tubes. Compare the color of the *Sample solution* with that of the *Standard solution A* and *Standard solution B* in diffused daylight, viewing vertically against a white background (see *Nephelometry, Turbidimetry, and Visual Comparison* (855)).

Acceptance criteria: The *Sample solution* is not more intensely colored than *Standard solution A* and *Standard solution B*. ■ 2S (NF34)

Add the following:

- ● **Bacterial Endotoxins Test** (85): If labeled for use in preparing parenteral dosage forms, it meets the requirements of NMT 240 Endotoxin Units/g. ■ 2S (NF34)

Add the following:

- ● **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): If labeled for use in preparing parenteral dosage forms, the total aerobic microbial count does not exceed 100 cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g. ■ 2S (NF34)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. No storage requirements specified.

Add the following:

- ● **Labeling:** Label it to indicate whether Deoxycholic Acid is derived from an animal or synthetic source. Deoxycholic Acid intended for use in preparing parenteral dosage forms is so labeled. Deoxycholic Acid of animal origin is labeled for excipient use only. ■ 2S (NF34)

Add the following:

- ● **USP Reference Standards** (11)
 - USP Deoxycholic Acid RS
 - USP Endotoxin RS
- 2S (NF34)

BRIEFING

Desoxycholic Acid, NF 33 page 6636. Because of the use of desoxycholic acid as an active pharmaceutical ingredient (API) in an FDA approved drug, it is proposed to change the monograph designation from *NF* to *USP* and to update the monograph tests to reflect the use of desoxycholic acid as an API. This updated monograph will be listed in the *NF* section as a cross reference to the *USP* general monographs section to reflect its use as both an active and inactive ingredient. The proposed revisions are listed as follows:

1. The title "Desoxycholic Acid" is proposed to be changed to "Deoxycholic Acid" to reflect the nomenclature used in the *USAN* and in the FDA Inactive Ingredient Database. This revised monograph is proposed for inclusion in the *Second Supplement to USP 39–NF 34*, with an official date of December 1, 2016. Use of the name "Deoxycholic Acid" will be permitted as of December 1, 2016; however, its use will not be mandatory until December 1, 2021. The 60-month extension will provide the time deemed necessary for labeling changes to be made for the article and the numerous preparations in which it is an ingredient, as well as for practitioners, consumers, and regulatory agencies to

- become familiar with the new title.
2. In the *Definition*, revise the acceptance criteria from NLT 98.0% and NMT 102.0% to NMT 97.0% and NMT 103.0%. The acceptance criteria range in the *Assay* is widened to account for the performance of the proposed HPLC method with charged aerosol detection (CAD).
 3. In the *Definition*, add a statement that Desoxycholic Acid can be of animal or synthetic origin.
 4. Replace the current *Identification* test *A* based on the wet chemistry procedure with an identification test based on *Infrared Absorption* (197A). This test requires the use of USP Deoxycholic Acid RS.
 5. Add *Identification* test *B* based on the identification by retention time as obtained in the *Assay*.
 6. Replace the existing titrimetric method employed in the *Assay* with an HPLC-CAD method. This method is based on analyses performed with the Phenomenex Pursuit 3 C18 brand of L1 column. The retention times for cholic acid; 3 α , 12 β -dihydroxy-5 β -cholan-24-oic acid; 3 α , 12 α -dihydroxy-5 β -chol-9(11)-en-24-oic acid; desoxycholic acid; and ethyl 3 α , 12 α -dihydroxy-5 β -cholan-24-oate are about 7.3, 9.0, 11.3, 13.0 and 20.9 min, respectively.
 7. Add a test for *Organic Impurities* that employs the same HPLC-CAD method as in the *Assay*.
 8. Add a *Note* to the test for *Limit of Lead* stating that it is applicable only to Desoxycholic Acid of animal origin.
 9. Add a *Heavy Metals* (231) test with a statement that it is only applicable to Desoxycholic acid of synthetic origin.
 10. In the *Optical Rotation* test, assign the *Acceptance criteria* of NLT +55° to Desoxycholic Acid of animal origin, and add an *Acceptance criteria* of +50° to +60° for Desoxycholic Acid of synthetic origin.
 11. In the *Specific Tests*, delete the test for *Melting Range or Temperature* (741)
 12. In the *Specific Tests*, replace *Loss on Drying* with *Water Determination, Method Ic*, and change the *Acceptance criteria* from NMT 1% to NMT 1.0%. Replace "on the dried basis" with "on the anhydrous basis" in the *Definition* and *Assay* acceptance criteria.
 13. In the *Specific Tests*, add tests for *Clarity of Solution*, *Color of Solution*, *Bacterial Endotoxins Test* (85), *Microbial Enumeration Tests* (61), and *Tests for Specified Microorganisms* (62) for desoxycholic acid intended for use in parenteral dosage forms.
 14. Add the *Labeling* section to indicate the origin of Desoxycholic Acid, the use in parenteral dosage forms, and to label Desoxycholic Acid of animal origin for excipient use only.
 15. Add the *USP Reference Standards* section containing USP Deoxycholic Acid RS and USP Endotoxin RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC1: G. Holloway.)

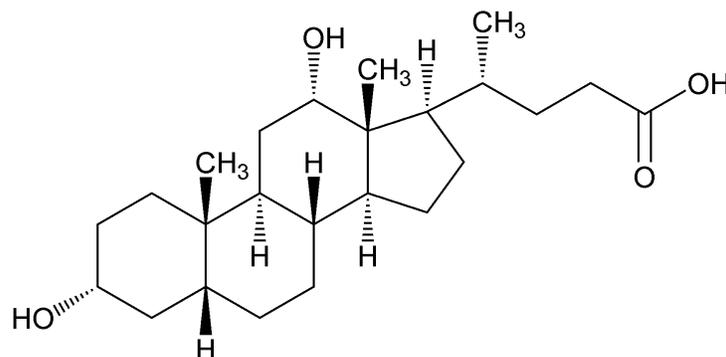
Correspondence Number—C158480

Comment deadline: November 30, 2015

Desoxycholic Acid

(Title for this monograph—not to change until December 1, 2021)

(Prior to December 1, 2021, the current practice of labeling the article of commerce with the name Desoxycholic Acid may be continued. Use of the name Deoxycholic Acid will be permitted as of December 1, 2016; however, the use of this name will not be mandatory until December 1, 2021. The 60-month extension will provide the time needed by manufacturers and users to make necessary changes.)



$C_{24}H_{40}O_4$ 392.57

Desoxycholic acid;

3 α ,12 α -Dihydroxycholan-20-oic acid [83-44-3].

DEFINITION

Change to read:

Desoxycholic Acid contains NLT 98.0% and NMT 102.0%

■ NLT 97.0% and NMT 103.0% ■ 2S (NF34)

of desoxycholic acid ($C_{24}H_{40}O_4$), calculated on the dried

■ anhydrous ■ 2S (NF34)

basis.

■ Desoxycholic Acid can be of animal or synthetic origin. ■ 2S (NF34)

IDENTIFICATION

Delete the following:

■ ● ~~A.~~

~~**Analysis:** Add 2 drops of benzaldehyde and 3 drops of 75% sulfuric acid to about 10 mg of Desoxycholic Acid, heat at 50° for 5 min, and then add 10 mL of glacial acetic acid.~~

~~**Acceptance criteria:** A green color appears (cholic acid produces a brown color). ■ 2S (NF34)~~

Add the following:

■ ● **A. Infrared Absorption** (197A) ■ 2S (NF34)

Add the following:

■ ● **B.** The retention time of the desoxycholic acid peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■ 2S (NF34)

ASSAY**Change to read:**● **Procedure****Sample:** 500 mg

Analysis: Transfer the *Sample* into a 250 mL Erlenmeyer flask, and add 20 mL of water and 40 mL of alcohol. Cover the flask with a watch glass, heat the mixture gently on a steam bath until the *Sample* is dissolved, and allow the mixture to cool to room temperature. Add a few drops of phenolphthalein TS to the solution, and titrate with 0.1 N sodium hydroxide to a pink endpoint that persists for 15 s. Each mL of 0.1 N sodium hydroxide is equivalent to 39.26 mg of desoxycholic acid (C₂₄H₄₀O₄).

Acceptance criteria: 98.0%–102.0% on the dried basis

■ **Diluent:** Methanol and water (80:20)

Solution A: 0.1% Formic acid in water

Solution B: 0.1% Formic acid in acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	75.0	25.0
2.0	55.0	45.0
14.0	42.0	58.0
24.0	0.0	100.0
35.0	0.0	100.0

Standard solution: 0.01 mg/mL of USP Deoxycholic Acid RS in *Diluent*

Sample solution: 0.01 mg/mL of Desoxycholic Acid in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Charged aerosol

Column: 4.6-mm × 15-cm; 3-μm packing L1

Flow rate: 1.0 mL/min

Autosampler temperature: 5°

Injection volume: 25 μL

Run time: 35 min

System suitability

Sample: *Standard solution*

[Note—The retention time of desoxycholic acid is about 13.0 min.]

Suitability requirements

Relative standard deviation: NMT 3.0% for six injections

Analysis**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of desoxycholic acid ($C_{24}H_{40}O_4$) in the portion of Desoxycholic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak area of desoxycholic acid from the *Sample solution*

r_S = peak area of desoxycholic acid (desoxycholic acid) from the *Standard solution*

C_S = concentration of the USP Desoxycholic Acid RS in the *Standard solution*

C_U = concentration of Desoxycholic Acid in the *Sample solution*

P = labeled purity of USP Desoxycholic Acid RS expressed as a percentage

Acceptance criteria: 97.0%–103.0% on the anhydrous basis ■_{2S} (NF34)

IMPURITIES

- **Residue on Ignition** (281)

Sample: 1.0 g

Acceptance criteria: NMT 0.2%

Change to read:

- **Limit of Lead**

[Note—

■ This test and *Acceptance criteria* are only applicable to Desoxycholic Acid of animal origin. ■_{2S} (NF34)

Select reagents having as low a lead content as practicable, and store all solutions in high-density polyethylene containers. Rinse all plastic and glassware thoroughly with warm, 50% nitric acid followed by water.]

▲▲_{NF33}

Standard solutions: [Note—Prepare these solutions on the day of use.] Transfer 10.0 and 50.0 mL of ▲ *standard lead solution TS* ▲_{NF33} into two separate 100-mL volumetric flasks, add 10 mL of 3 N *hydrochloric acid* to each, and dilute with water to volume. The third standard, 10.0 µg/mL, is taken directly from ▲ *standard lead solution TS*. ▲_{NF33}

Sample solution: Transfer 10.0 g of Desoxycholic Acid, weighed to the nearest 0.1 mg, into an evaporating dish. Add 5 mL of 25% sulfuric acid (made by adding 25 mL of *sulfuric acid* to 75 mL of water), and distribute the 25% sulfuric acid solution uniformly. Within a hood, place the dish on a steam bath to evaporate most of the water. Place the dish on a burner, and slowly pre-ash the remaining *Sample solution* by expelling most of the sulfuric acid. Place the dish in a muffle furnace that has been set at 525°, and ash the contents of the dish until the residue appears free from carbon. Cool, and cautiously wash down the inside of the evaporation dish with water. Add 5 mL of 1 N *hydrochloric acid*. Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 N *hydrochloric acid* and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer to a 10-mL volumetric flask, dilute with water to volume, and mix.

Sample blank: Prepare by ashing 5 mL of 25% sulfuric acid solution. Cool, and cautiously

wash down the inside of the evaporation dish with water. Add 5 mL of 1 N *hydrochloric acid*. Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 N *hydrochloric acid* and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer to a 10-mL volumetric flask, dilute with water to volume, and mix.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption

Analytical wavelength: 283.3 nm

Lamp: Lead electrodeless discharge

Flame: Air-acetylene

Slit width: 0.7 nm

Instrument blank: Water

Standard curve

Samples: *Standard solutions* and *Sample blank*

Plot: Corrected absorbance values versus their corresponding concentration ($\mu\text{g/mL}$).
[Note—Determine corrected absorbance values by subtracting the absorbance of the *Sample blank* from the absorbance of the *Standard solutions*.]

Analysis

Samples: *Sample solution* and *Sample blank*

[Note—Determine corrected absorbance values by subtracting the absorbance of the *Sample blank* from the absorbance of the *Sample solution*.]

From the *Standard curve*, determine the lead concentration in the *Sample solution*. Calculate the lead content in the portion of Desoxycholic Acid taken:

$$\text{Result} = (C_S \times V) / W$$

C_S = concentration of lead from the *Standard curve* ($\mu\text{g/mL}$)

V = final volume of the sample (mL)

W = weight of the sample taken (g)

Acceptance criteria: NMT 4 $\mu\text{g/g}$

Add the following:

- ● **Heavy Metals** (231), *Method II*: NMT 20 $\mu\text{g/g}$

[Note—This test and *Acceptance criteria* are only applicable to Desoxycholic Acid of synthetic origin.]

- 2S (NF34)

Add the following:

- ● **Organic Impurities**

Diluent, Mobile phase, Standard solution, and Chromatographic system: Proceed as described in the procedure for *Assay*.

Sensitivity solution: 0.5 $\mu\text{g/mL}$ of USP Deoxycholic Acid RS in *Diluent*

Sample stock solution: 1.0 mg/mL of Desoxycholic Acid in *Diluent*

Sample solution: 0.01 mg/mL Desoxycholic Acid in *Diluent* prepared from the *Sample stock solution*

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[Note—The retention time of desoxycholic acid is about 13.0 min. The relative retention times for cholic acid and desoxycholic acid are 0.56 and 1, respectively. The relative retention times for other impurities are listed in *Table 2*.]

Suitability requirements

Relative standard deviation: NMT 3.0% for six injections, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Sample stock solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Desoxycholic Acid taken:

$$\text{Result} = [r_U / (r_S \times 100 + r_T)] \times 100$$

r_U = peak area of individual impurity from the *Sample stock solution*

r_S = peak area of desoxycholic acid from the *Sample solution*

r_T = sum of peak areas of all impurities from the *Sample stock solution*

Acceptance criteria

Desoxycholic Acid of animal origin

Cholic acid: NMT 1.0%

Total impurities: NMT 2.0%

Desoxycholic Acid of synthetic origin: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria NMT (%)
3 α , 12 β -Dihydroxy-5 β -cholan-24-oic acid	0.69	0.15
3 α , 12 α -Dihydroxy-5 β -chol-9(11)-en-24-oic acid	0.87	0.15
Ethyl 3 α , 12 α -dihydroxy-5 β -cholan-24-oate	1.61	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

■ 2S (NF34)

SPECIFIC TESTS

Change to read:

- **Optical Rotation** (781S), *Specific Rotation*

Sample solution: 1% (w/v) solution in *ethanol*

Acceptance criteria ~~NLT +55°~~

▪ **Desoxycholic Acid of animal origin:** NLT +55°

Desoxycholic Acid of synthetic origin: +50° to +60° ■ 2S (NF34)

Delete the following:

▪ • ~~**Melting Range or Temperature** (741), *Class I:* 172°–175° ■ 2S (NF34)~~

Delete the following:

▪ • ~~**Loss on Drying** (731)~~

~~**Analysis:** Dry at 140° under vacuum of NMT 5 mm of mercury for 4 h.~~

~~**Acceptance criteria:** NMT 1% ■ 2S (NF34)~~

Add the following:

▪ • **Water Determination** (921), *Method Ic:* NMT 1.0% ■ 2S (NF34)

Add the following:

▪ • **Clarity of Solution**

[Note—If intended for use in preparing parenteral dosage forms, it meets the requirements of the *Clarity of Solution* test.]

Hydrazine solution: 10 mg/mL of *hydrazine sulfate*. Allow to stand 4–6 h before use.

Methenamine solution: Transfer 2.5 g of *methenamine* to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

Primary opalescent suspension: To the flask containing *Methenamine solution* add 25.0 mL of the *Hydrazine solution*, mix, and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Opalescence standard: Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, and dilute with water to volume. Use this suspension within 24 h after preparation.

Reference suspension: Transfer 5.0 mL of the *Opalescence standard* to a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: 5 mg/mL of Desoxycholic Acid in 0.1 N *sodium hydroxide*

Analysis: Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer a portion of the *Reference suspension* to a separate matching test tube. Compare the *Sample solution* and the *Reference suspension* in diffused daylight, viewing vertically against a black background (see *Nephelometry, Turbidimetry, and Visual Comparison* (855)) for 5 min after preparation of the *Reference suspension*.

Acceptance criteria: The *Sample solution* is not more opalescent than the *Reference suspension*. ■ 2S (NF34)

Add the following:

▪ • **Color of Solution**

[Note—If intended for use in preparing parenteral dosage forms, it meets the requirements of the *Color of Solution* test.]

Diluent: 27.5 mL of *hydrochloric acid* in 1000 mL of water

Standard stock solutions: Prepare two solutions, A and B, containing, respectively, the following parts of *ferric chloride CS*, *cobaltous chloride CS*, *cupric sulfate CS*, and *Diluent*.

Standard stock solution A: 2.4: 0.6: 0: 7.0

Standard stock solution B: 2.4: 1.0: 0.4: 6.2

Standard solutions

[Note—Prepare and use these solutions immediately.]

Standard solution A: Transfer 2.5 mL of *Standard stock solution A* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Standard solution B: Transfer 2.5 mL of *Standard stock solution B* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Sample solution: 5 mg/mL of Desoxycholic Acid in 0.1 N *sodium hydroxide*

Analysis: Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm, to obtain a depth of 40 mm. Similarly transfer portions of *Standard solution A* and *Standard solution B* to separate matching test tubes. Compare the color of the *Sample solution* with that of the *Standard solution A* and *Standard solution B* in diffused daylight, viewing vertically against a white background (see *Nephelometry, Turbidimetry, and Visual Comparison* (855)).

Acceptance criteria: The *Sample solution* is not more intensely colored than *Standard solution A* and *Standard solution B*. ■2S (NF34)

Add the following:

- ● **Bacterial Endotoxins Test** (85): If labeled for use in preparing parenteral dosage forms, it meets the requirements of NMT 240 Endotoxin Units/g. ■2S (NF34)

Add the following:

- ● **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): If labeled for use in preparing parenteral dosage forms, the total aerobic microbial count does not exceed 100 cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g. ■2S (NF34)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. No storage requirements specified.

Add the following:

- ● **Labeling:** Label it to indicate whether Desoxycholic Acid is derived from an animal or synthetic source. Desoxycholic Acid intended for use in preparing parenteral dosage forms is so labeled. Desoxycholic Acid of animal origin is labeled for excipient use only. ■2S (NF34)

Add the following:

- ● **USP Reference Standards** (11)
 - USP Deoxycholic Acid RS
 - USP Endotoxin RS

■2S (NF34)

Hydrogenated Castor Oil, *NF 33* page 6594. As part of the USP monograph modernization effort, it is proposed to make the following revisions:

1. Add a CAS number and chemical structure.
2. Update the monograph *Definition*.
3. Add an *Identification* test *A, Identity by Fatty Acid Composition*. The added procedure is based on a GC method of analysis performed with the Agilent DB Wax or Supelco 10 brand of G16 column. The typical retention times for methyl palmitate, methyl stearate, methyl arachidate, methyl 12-ketostearate, and methyl 12-hydroxystearate are 6.44, 8.73, 12.70, 20.01, and 23.43 min, respectively.
4. Move the test for *Melting Range or Temperature* from *Specific Tests* to the *Identification* section as test *B*.
5. In the *Assay*, add a test for *Triglyceride Composition*, which is based on analyses performed using the Phenomenex Prodigy ODS-3 or Phenomenex Ultremex C18 brand of column that contains 5- μ m packing L1. The typical retention times for glyceryl tri(12-hydroxystearate) [or tri(12-hydroxystearoyl)-glycerol], di(12-hydroxystearoyl)-(12-oxostearoyl)-glycerol, di(12-hydroxystearoyl)-palmitoyl-glycerol, di(12-hydroxystearate)-stearoyl-glycerol, and di(12-hydroxystearate)-arachidoyl-glycerol are 9.6, 10.2, 15.1, 16.9, and 18.1 min, respectively.
6. Update the *Acceptance criteria* for the *Fats and Fixed Oils* (401), *Acid Value (Free Fatty Acids)* test.
7. Add a specification for residual nickel based on the newly released Q3D Step 4 Guideline.
8. Add a specification for *Alkaline Impurities*.
9. Add a *USP Reference Standards* section, including USP Hydrogenated Castor Oil RS, USP Methyl 12-Hydroxystearate RS, USP Methyl Palmitate RS, and USP Methyl Stearate RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC1: H. Wang.)

Correspondence Number—C155322

Comment deadline: November 30, 2015

Hydrogenated Castor Oil

Add the following:



ketostearate, USP Methyl Stearate RS, and USP Methyl 12-Hydroxystearate RS, in *Diluent*

Standard solution 2: 4 mg/mL each of methyl stearate and methyl 12-hydroxystearate, from USP Methyl Stearate RS and USP Methyl 12-Hydroxystearate RS, in *Diluent*

Sample solution: Transfer 140 mg of Hydrogenated Castor Oil to a 10-mL screw-cap test tube, add 3.0 mL of *Diluent*, and mix well. Add 0.5 mL of 0.5 M sodium methoxide in methanol,¹ and mix with the sample. Allow the reaction to proceed at room temperature for 2 h. After 2 h, add 5 mL of water, and mix. Centrifuge the test tube at 1000 ×g for 5 min at 4° until a clear upper layer forms. Separate the organic layer (the upper layer), and remove the lower layer. Place an aliquot of the organic layer into an autosampler vial.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 30-m fused silica capillary bonded with a 0.25-μm layer of phase G16

Temperatures

Injection port: 240°

Detector: 250°

Column: See *Table 1* for oven program.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)	Total Time (min)
80	0	80	1	1
80	30	140	0	3
140	20	180	5	10
180	2	250	10	55

Carrier gas: Hydrogen

Flow rate: 5.0 mL/min, constant flow mode

Injection volume: 1.0 μL

Injection type: Split ratio 120:1 or 60:1

Liner: Single taper, low-pressure drop liner with deactivated wool

Run time: 55 min

System suitability

Sample: *Standard solution 1*

[Note—See *Table 2* for relative retention times.]

Table 2

Component	Relative Retention Time
Methyl palmitate (C16:0)	0.27
Methyl stearate (C18:0)	0.37
Methyl arachidate (C20:0)	0.54
Methyl 12-ketostearate	0.85
Methyl 12-hydroxystearate	1.00

Suitability requirements

Resolution: NLT 10 between any two adjacent peaks

Relative standard deviation: NMT 2.0% for the peak area ratio of methyl 12-hydroxystearate to methyl 12-ketostearate

Analysis

Samples: *Standard solution 1, Standard solution 2, and Sample solution*

Calculate the relative response factor, F , for methyl 12-hydroxystearate:

$$F = (r_S/r_R) \times (C_R/C_S)$$

r_S = peak area of methyl stearate from *Standard solution 2*

r_R = peak area of methyl 12-hydroxystearate from *Standard solution 2*

C_R = concentration of USP Methyl 12-Hydroxystearate RS in *Standard solution 2* (mg/mL)

C_S = concentration of USP Methyl Stearate RS in *Standard solution 2* (mg/mL)

Correct the peak area of methyl 12-hydroxystearate in the *Sample solution* by multiplying by F .

Calculate the percentage of each fatty acid component in the portion of sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual fatty acid methyl ester, except for the uncorrected peak area of methyl 12-hydroxystearate (or the corrected peak area of methyl 12-hydroxystearate), from the *Sample solution*

r_T = sum of all the peak areas, excluding the solvent and methyl 12-hydroxystearate peaks and including the corrected peak area of methyl hydroxystearate, from the *Sample solution*

Acceptance criteria: Hydrogenated Castor Oil exhibits the composition profile of fatty acids shown in *Table 3*.

Table 3

Component	Percentage (%)
Palmitic acid (C16:0)	≤2.0
Stearic acid (C18:0)	7.0–14.0
Arachidic acid (C20:0)	≤1.0
12-Ketostearic acid (or 12-oxostearic acid)	≤5.0
12-Hydroxystearic acid	78.0–91.0
Any other unidentified fatty acid	≤3.0

■ 2S (NF34)

Add the following:

- ● **B. Melting Range or Temperature** (741), *Class II*: 85°–88° ■ 2S (NF34)

ASSAY

Add the following:

- ● **Triglyceride Composition**

Solution A: Methanol

Solution B: 2-Propanol

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	50	50
23	0	100
25	100	0
35	100	0

Diluent: Methylene chloride

System suitability solution: 3.0 mg/mL of Hydrogenated Castor Oil in *Diluent*. [Note—Due to low solubility, sonicate the solution for about 2–3 min.]

Sample solution: 3.0 mg/mL of Hydrogenated Castor Oil in *Diluent*. [Note—Due to low solubility, sonicate the solution for about 2–3 min.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Evaporative light-scattering

Column: 4.6-mm × 25-cm; 5- μ m packing L1

Temperatures

Column: 25°

Detector: 40°

Flow rate: 1.0 mL/min

Injection volume: 5 μ L

Run time: 35 min

[Note—Depending on the different settings of the *Detector*, the *Temperature* and *Flow rate* can be adjusted as long as system suitability requirements are met.]

System suitability

Sample: *System suitability solution*

[Note—See *Table 5* for relative retention times.]

Table 5

Component	Relative Retention Time
Glyceryl tri(12-hydroxystearate) [or tri(12-hydroxystearoyl)-glycerol]	1.0
Di(12-hydroxystearoyl)-(12-oxostearoyl)-glycerol	1.1
Di(12-hydroxystearoyl)-palmitoyl-glycerol	1.7
Di(12-hydroxystearoyl)-stearoyl-glycerol	1.8
Di(12-hydroxystearoyl)-arachidoyl-glycerol	1.9

Suitability requirements

Resolution: NLT 1.5 between glyceryl tri(12-hydroxystearate) and di(12-hydroxystearoyl)-(12-oxostearoyl)-glycerol

Tailing factor: 0.8–1.8 for the glyceryl tri(12-hydroxystearate) peak

Relative standard deviation: NMT 2% for the peak area of glyceryl tri(12-hydroxystearate)

Analysis

Samples: *System suitability solution* and *Sample solution*

Calculate the percentage of each of the triglycerides in the portion of sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual triglyceride from the *Sample solution*

r_T = sum of all the peak areas, excluding the solvent peak, from the *Sample solution*

Acceptance criteria: Hydrogenated Castor Oil exhibits the composition profile shown in *Table 6*.

Table 6

Component	Percentage (%)
Glyceryl tri(12-hydroxystearate) [or tri(12-hydroxystearoyl)-glycerol]	≥70.0
Di(12-hydroxystearoyl)-(12-oxostearoyl)-glycerol	≤14.0
Di(12-hydroxystearoyl)-palmitoyl-glycerol	≤2.0
Di(12-hydroxystearoyl)-stearoyl-glycerol	10.0–23.0
Di(12-hydroxystearoyl)-arachidoyl-glycerol	≤2.0

■ 2S (NF34)

IMPURITIES

Delete the following:

● ● **Heavy Metals** (231), *Method II*: NMT 10 µg/g ● (Official 1-Dec-2015)

Add the following:

■ ● **Limit of Nickel**

[**Caution**—When using closed high-pressure digestion vessels and laboratory microwave

equipment, the safety precautions and operating instructions given by the manufacturer must be followed.]

[Note—If an alternative apparatus is used, adjustment of the instrument parameters may be necessary.]

Nickel standard stock solution: Dilute *nickel standard solution TS* two-fold with water. This solution contains the equivalent of 5 µg/mL of nickel.

Standard solutions: Transfer 25, 50, 75, and 100 µL of *Nickel standard stock solution* to four identical 25-mL volumetric flasks. To each flask add 0.5 mL of a 10-mg/mL solution of magnesium nitrate, 0.5 mL of a 100-mg/mL solution of monobasic ammonium phosphate, and 6.0 mL of nickel-free nitric acid. Dilute with water to volume, and mix well.

[Note—Content of nickel in the nickel-free nitric acid is NMT 0.005 ppm.] The *Standard solutions* contain 0.005, 0.01, 0.015, and 0.02 µg/mL of nickel, respectively.

Sample solution: Transfer about 250 mg of Hydrogenated Castor Oil to a suitable high-pressure-resistant digestion vessel (fluoropolymer or quartz glass), and add 6.0 mL of nickel-free nitric acid and 2.0 mL of 30% hydrogen peroxide. Place the closed vessel in a laboratory microwave oven, and digest using an appropriate program, e.g., 1000 W for 40 min. Allow the digestion vessel to cool before opening. Add 2.0 mL of 30% hydrogen peroxide, and repeat the digestion step. Allow the digestion vessel to cool down before opening. Quantitatively transfer to a 25-mL volumetric flask, add 0.5 mL of a 10-mg/mL solution of magnesium nitrate and 0.5 mL of a 100-mg/mL solution of monobasic ammonium phosphate. Dilute with water to volume, and mix well.

Blank solution: Place 6.0 mL of nickel-free nitric acid and 2.0 mL of 30% hydrogen peroxide in a suitable high-pressure-resistant digestion vessel. Proceed as directed under *Sample solution*, beginning with "Place the closed vessel in a laboratory microwave oven, and digest using an appropriate program, e.g., 1000 W for 40 min."

Zero solution: In a 50-mL volumetric flask, introduce 1.0 mL of a 10-mg/mL solution of magnesium nitrate, 1.0 mL of a 100-mg/mL solution of monobasic ammonium phosphate, and 12.0 mL of nickel-free nitric acid. Dilute with water to volume, and mix well.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852))

Mode: Atomic absorption, equipped with a graphite furnace, a background compensation system, and a coated tube resistant to pyrolysis

Analytical wavelength: 232.0 nm

Lamp: Nickel hollow-cathode

Temperature: Maintain the drying temperature of the furnace at 120° for 35 s after a 5-s ramp; maintain the ashing temperature at 1100° for 10 s after a 30-s ramp; maintain the cooling temperature at 800° for 5 s after a 5-s decrease; and maintain the atomization temperature at 2600° for 7 s. [Note—The temperature program may be modified to obtain optimum furnace temperatures.]

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank solution*

Concomitantly determine the absorbances of the *Samples* using the *Instrumental conditions* described above. Use the *Zero solution* to set the instrument to zero. Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of nickel, and

draw the straight line best fitting the plotted points. From this graph, determine the concentration, C_T , in $\mu\text{g/mL}$, of nickel in the *Sample solution*, and determine the concentration, C_B , in $\mu\text{g/mL}$, of nickel in the *Blank solution*. If necessary, dilute with the *Zero solution* to obtain a reading within the calibrated absorbance range.

Calculate the quantity, in μg , of nickel in each g of Hydrogenated Castor Oil taken:

$$\text{Result} = V \times (C_T - C_B) / W$$

V = volume of the *Sample solution* and the *Blank solution*, 25 mL

C_T = concentration of nickel in the *Sample solution* ($\mu\text{g/mL}$)

C_B = concentration of nickel in the *Blank solution* ($\mu\text{g/mL}$)

W = weight of Hydrogenated Castor Oil taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 20 $\mu\text{g/g}$ ■ 2S (NF34)

Add the following:

■ ● **Alkaline Impurity**

Sample: 1.0 g

Analysis: Dissolve the *Sample* by gently heating in a mixture of 1.5 mL of alcohol and 3.0 mL of toluene. Add 0.05 mL of bromophenol blue TS, and titrate with 0.01 N hydrochloric acid VS to a yellow endpoint.

Acceptance criteria: NMT 0.2 mL of 0.01 N hydrochloric acid. ■ 2S (NF34)

SPECIFIC TESTS

Delete the following:

■ ● ~~**Melting Range or Temperature, Class II (741):** 85°–88° ■ 2S (NF34)~~

- **Fats and Fixed Oils** (401), *Iodine Value:* NMT 5

- **Fats and Fixed Oils**, (401), *Saponification Value:* 176–182

Change to read:

- **Fats and Fixed Oils**, (401), *Acid Value (Free Fatty Acids)*

Sample solution: Melt 20 g in a conical flask on a steam bath, add 75 mL of hot alcohol that has previously been neutralized with 0.1 N sodium hydroxide to phenolphthalein TS, swirl, and add 1 mL of phenolphthalein TS.

Analysis: Titrate with 0.1 N sodium hydroxide VS, swirling vigorously, until the solution remains faintly pink after being shaken for 60 s.

Acceptance criteria: NMT 11.0 mL of 0.1 N sodium hydroxide VS,

■ corresponding to NMT 3.1 for acid value ■ 2S (NF34)

- **Fats and Fixed Oils** (401), *Hydroxyl Value*

Sample solution: 2 g in a glass-stoppered, 250-mL conical flask. Add 5.0 mL of a freshly prepared mixture of acetic anhydride and pyridine (1:3), and swirl to mix. Connect the flask to a reflux condenser, and heat on a steam bath for 2 h. Add 10 mL of water through the condenser, swirl to mix, heat on a steam bath for an additional 10 min, and allow to cool to room temperature. Add through the condenser 15 mL of normal butyl alcohol that has previously been neutralized to phenolphthalein, remove the condenser, wash the tip of

the condenser and the sides of the flask with an additional 10 mL of neutralized normal butyl alcohol, and add 1 mL of phenolphthalein TS.

Analysis: Titrate with 0.5 N alcoholic potassium hydroxide VS to a faint pink endpoint.

Perform a blank determination on a 5.0-mL portion of the acetic anhydride–pyridine mixture. To determine the amount of free acid in the Oil, weigh 10 g into a 250-mL conical flask, add 10 mL of pyridine that has previously been neutralized to phenolphthalein, swirl to mix, add 1 mL of phenolphthalein TS, and titrate with 0.5 N alcoholic potassium hydroxide VS to a faint pink endpoint.

Calculate the hydroxyl value in the portion of Oil taken:

$$\text{Result} = M_r \times N \times [A + (B \times W/D) - C] / W$$

M_r = molecular weight of potassium hydroxide, 56.1

N = normality of the alcoholic potassium hydroxide solution

A = volume of 0.5 N alcoholic potassium hydroxide consumed by the *Blank* (mL)

B = volume consumed in the free-acid titration (mL)

W = weight of Oil taken (g)

D = weight of Oil used in the free-acid titration (g)

C = volume consumed in the sample titration (mL)

Acceptance criteria: 154–162

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and avoid exposure to excessive heat.

Add the following:

- ● **USP Reference Standards** (11)

USP Hydrogenated Castor Oil RS

USP Methyl 12-Hydroxystearate RS

USP Methyl Palmitate RS

USP Methyl Stearate RS

■ 2S (NF34)

■ 1 0.5 M sodium methoxide in methanol is available from Sigma-Aldrich (www.sigmaaldrich.com), product #403067. Any other equivalent reagent can be used as well. ■ 2S (NF34)

BRIEFING

Magnesium Aluminum Silicate, NF 33 page 6733. The Magnesium Aluminum Silicate monograph was identified as a monograph that is in need of strengthening the *Identification* section. Although the X-ray diffraction method employed in the current *Identification* test A is definitive for the clays comprising magnesium aluminum silicate, it does not address differentiation among the four magnesium aluminum silicate *Types*. As part of the USP monograph modernization effort and on the basis of the comments received, it is proposed to make the following changes:

1. Add the *Viscosity* test to the *Identification* section of the monograph as *Identification* test B.

2. Add the requirement for *Ratio of aluminum content to magnesium content* to the *Identification* section of the monograph as *Identification test C*.
3. Add *Appearance* to the *Identification* section of the monograph as *Identification test D*.
4. Remove the reference to the adjustment of the temperature at which viscosity is measured in the *Viscosity* test. Because of poor heat transfer of the magnesium aluminum silicate dispersion, adjusting the temperature to $33 \pm 3^\circ$ will take longer than 5 min resulting in invalid viscosity data. Instead add a requirement that the sample temperature should be $33 \pm 3^\circ$.
5. Add clarification to the preparation of the *Standard solution* and the *Sample solution* in the *Aluminum Content and Magnesium Content* test.
6. Add references to a suitable blender and viscometer used in the *Viscosity* test.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC1: G. Holloway.)

Correspondence Number— C151979

Comment deadline: November 30, 2015

Magnesium Aluminum Silicate

DEFINITION

Change to read:

Magnesium Aluminum Silicate is a blend of colloidal montmorillonite and saponite that has been processed to remove grit and nonswellable ore components.

The requirements for viscosity and ratio of aluminum content to magnesium content differ for the several types of Magnesium Aluminum Silicate, as set forth in the table below.

-	Viscosity (cps)		Al Content/ Mg Content	
	Min.	Max.	Min.	Max.
IA	225	600	0.5	1.2
IB	150	450	0.5	1.2
IC	800	2200	0.5	1.2
IIA	100	300	1.4	2.8



Type	Viscosity (mPa·s)		Al Content/ Mg Content		Appearance
	Min.	Max.	Min.	Max.	
IA	225	600	0.5	1.2	Fine granules or flakes
IB	150	450	0.5	1.2	Microfine powder
IC	800	2200	0.5	1.2	Fine granules or flakes
IIA	100	300	1.4	2.8	Fine granules or flakes

■ 2S (NF34)

IDENTIFICATION

• A.

Sample: 2 g

Analysis 1: Add the *Sample* in small portions to 100 mL of water with intense agitation.

Allow to stand for 12 h to ensure complete hydration. Place 2 mL of the resulting mixture on a suitable glass slide, and allow to air-dry at room temperature to produce an oriented film. Place the slide in a vacuum desiccator over a free surface of ethylene glycol.

Evacuate the desiccator, and close the stopcock so that the ethylene glycol saturates the desiccator chamber. Allow to stand for 12 h. Record the X-ray diffraction pattern (see *X-Ray Diffraction* (941)), and calculate the *d* values.

Acceptance criteria 1: The largest peak corresponds to a *d* value between 15.0 and 17.2 Å.

Analysis 2: Prepare a random powder specimen of Magnesium Aluminum Silicate, record the X-ray diffraction pattern, and determine the *d* values in the region between 1.48 and 1.54 Å.

Acceptance criteria 2: Peaks are found at 1.492–1.504 Å and at 1.510–1.540 Å.

Add the following:

- • **B.** It meets the requirements of the test for *Viscosity* in *Specific Tests*. ■ 2S (NF34)

Add the following:

- • **C.** It meets the requirements for *Ratio of aluminum content to magnesium content* in the test for *Aluminum Content and Magnesium Content*. ■ 2S (NF34)

Add the following:

- • **D.** Its appearance corresponds to the description in the *table* in the *Definition*. ■ 2S (NF34)

ASSAY

Change to read:

• Aluminum Content and Magnesium Content

Aluminum content

Aluminum standard stock solution: Dissolve 1.000 g of *aluminum* in a mixture of 10 mL of *hydrochloric acid* and 10 mL of water by gentle heating. Transfer the solution to a 1000-mL volumetric flask, and dilute with water to volume

- dilute with water to volume, and mix. ■ 2S (NF34)

This solution contains the equivalent of 1 mg/mL of aluminum.

Aluminum standard solutions: Transfer 2-, 5-, and 10-mL aliquots of the *Aluminum standard stock solution* to separate 100-mL volumetric flasks containing 200 mg of *sodium chloride*, and dilute each with water to volume

- dilute each with water to volume, and mix. ■ 2S (NF34)

Sample stock solution: Transfer 0.200 g of Magnesium Aluminum Silicate to a 25-mL platinum crucible containing 1.0 g of *lithium metaborate*, and mix. Using a muffle furnace or a suitable burner, heat slowly at first, and ignite at 1000°–1200° for 15 min. Cool,

place the crucible in a 100-mL beaker containing 25 mL of dilute *nitric acid* (50 mg/mL), and add an additional 50 mL of the dilute acid, filling and submerging the upright crucible. Place a polyfluorocarbon-coated magnetic stirring bar into the crucible, and stir gently with a magnetic stirrer to dissolve. Pour the contents into a 250-mL beaker, and remove the crucible. Warm the solution, transfer through a rapid-flow filter paper with the aid of water into a 200-mL volumetric flask, ~~and dilute with water to volume~~

■ dilute with water to volume, and mix. ■ 2S (NF34)

Sample solution: Pipet 20 mL of the *Sample stock solution* into a 100-mL volumetric flask. Add 20 mL of a solution of *sodium chloride* (10 mg/mL), ~~and dilute with water to volume~~

■ dilute with water to volume, and mix. ■ 2S (NF34)

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometer equipped with a single-slot burner

Analytical wavelength: 309 nm

Lamp: Aluminum hollow-cathode

Flame: Oxidizing acetylene-air-nitrous oxide

Analysis

Samples: *Aluminum standard solutions* and *Sample solution*

Determine the absorbances of the *Aluminum standard solutions* and the *Sample solution*. From a linear regression equation calculated from the absorbances and concentrations of the *Aluminum standard solutions*, determine the aluminum content in the magnesium aluminum silicate.

Magnesium content

Lanthanum solution: Stir 88.30 g of *lanthanum chloride* (LaCl_3) with 500 mL of 6 N *hydrochloric acid* to dissolve, transfer with the aid of water to a 1000-mL volumetric flask, ~~and dilute with water to volume~~

■ dilute with water to volume, and mix. ■ 2S (NF34)

Magnesium standard stock solution: Place 1.000 g of *magnesium* in a 250-mL beaker containing 20 mL of water, and carefully add 20 mL of *hydrochloric acid*, warming, if necessary, to complete the reaction. Transfer the solution to a 1000-mL volumetric flask, ~~and dilute with water to volume~~

■ dilute with water to volume, and mix. ■ 2S (NF34)

This solution contains the equivalent of 1 mg/mL of magnesium. Transfer 10.0 mL of this solution to a 1000-mL volumetric flask, ~~and dilute with water to volume~~

■ dilute with water to volume, and mix. ■ 2S (NF34)

Magnesium standard solutions: Transfer 5-, 10-, 15-, and 20-mL aliquots of the *Magnesium standard stock solution* to separate 100-mL volumetric flasks. To each flask add 20.0 mL of *Lanthanum solution*, ~~and dilute with water to volume~~

■ dilute with water to volume, and mix. ■ 2S (NF34)

Sample stock solution: Use the *Sample stock solution* prepared as directed for *Aluminum content*.

Sample solution: Transfer 25 mL of the *Sample stock solution* to a 50-mL volumetric

flask, and dilute with water to volume

■ dilute with water to volume, and mix. ■ 2S (NF34)

Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 20.0 mL of *Lanthanum solution*, and dilute with water to volume

■ dilute with water to volume, and mix. ■ 2S (NF34)

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption

Analytical wavelength: 285 nm

Lamp: Magnesium hollow-cathode

Flame: Reducing flame of acetylene-air

Analysis

Samples: *Magnesium standard solutions* and *Sample solution*

Determine the absorbances of the *Sample solution* and the *Magnesium standard solutions*. From a linear regression equation calculated from the absorbances and concentrations of the *Magnesium standard solutions*, determine the magnesium content in the magnesium aluminum silicate.

Ratio of aluminum content to magnesium content

Analysis: Using the results from the *Aluminum content* and the *Magnesium content*, determine the ratio of aluminum content to magnesium content.

Acceptance criteria

Type IA: 0.5–1.2

Type IB: 0.5–1.2

Type IC: 0.5–1.2

Type IIA: 1.4–2.8

IMPURITIES

Change to read:

- **Arsenic** (211), *Method I*

Standard preparation: • Transfer 5.0 mL (5 µg of arsenic) of the *Standard Arsenic Solution* to a 25-mL volumetric flask, and add dilute hydrochloric acid (1:25) to volume. • (ERR 1-Apr-2014)

Test preparation: Transfer 13.3 g of Magnesium Aluminum Silicate to a 250-mL beaker containing 100 mL of dilute *hydrochloric acid* (1:25), mix, cover with a watch glass, and boil gently with occasional stirring for 15 min without allowing excessive foaming. Allow the insoluble material to settle, and decant the hot supernatant through a rapid-flow filter paper into a 200-mL volumetric flask, retaining as much sediment as possible in the beaker. Add 25 mL of hot dilute *hydrochloric acid* (1:25) to the residue in the beaker, stir, and heat to boiling. Allow the insoluble material to settle, and decant the supernatant through the filter into the 200-mL volumetric flask. Repeat the extraction with four additional 25-mL portions of hot dilute *hydrochloric acid* (1:25), decanting each hot supernatant through the filter into the volumetric flask. At the last extraction, transfer as

much of the insoluble material as possible onto the filter. Cool the combined filtrates to room temperature, add dilute *hydrochloric acid* (1:25) to volume, and mix. Use 25 mL for the test.

Acceptance criteria: NMT 3 µg/g; •the absorbance due to any red color from the *Test preparation* does not exceed that produced by the *Standard preparation*. •(ERR 1-Apr-2014)

Change to read:

• **Lead**

Standard preparation: On the day of use, dilute 3.0 mL of ▲*lead nitrate stock solution* TS▲NF33 with water to 100 mL. Each mL contains the equivalent of 3 µg of lead.

Sample: 10.0 g

Test preparation: Transfer the *Sample* to a 250-mL beaker containing 100 mL of dilute *hydrochloric acid* (1:25), stir, cover with a watch glass, and boil for 15 min. Cool to room temperature, and allow the insoluble matter to settle. Decant the supernatant through a rapid-flow filter paper into a 400-mL beaker. Add 25 mL of hot water to the insoluble matter in the 250-mL beaker, and stir. Allow the insoluble matter to settle, and decant the supernatant through the filter into the 400-mL beaker. Repeat the extraction with two additional 25-mL portions of water, decanting each supernatant portion through the filter into the 400-mL beaker. Wash the filter with 25 mL of hot water, collecting this filtrate in the 400-mL beaker. Concentrate the combined extracts by gentle boiling to approximately 20 mL. If a precipitate appears, add 2–3 drops of *nitric acid*, heat to boiling, and cool to room temperature. Filter the concentrated extracts through a rapid-flow filter paper into a 50-mL volumetric flask. Transfer the remaining contents of the 400-mL beaker through the filter paper and into the flask with water. Dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometer equipped with a deuterium arc background correction and a single-slot burner

Analytical wavelength: 284 nm

Lamp: Lead hollow-cathode

Flame: Oxidizing flame of air and acetylene

Acceptance criteria: The absorbance of the *Test preparation* is NMT that of the *Standard preparation* (15 µg/g).

SPECIFIC TESTS

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): Its total aerobic microbial count does not exceed 10^3 cfu/g, and it meets the requirements of the test for absence of *Escherichia coli*.
- **pH** (791)
 - Sample suspension:** 50 mg/mL
 - Acceptance criteria:** 9.0–10.0
- **Loss on Drying** (731)
 - Analysis:** Dry at 110° to constant weight.

Acceptance criteria: NMT 8.0%

Change to read:

● **Viscosity**

Sample: solution:

■ **2S (NF34)**

After determining the *Loss on Drying*, weigh a quantity of Magnesium Aluminum Silicate, equivalent to 25.0 g on the dried basis. Over a period of a few seconds, transfer the undried test specimen to a suitable 1-L blender jar containing an amount of water, maintained at a temperature of $25 \pm 2^\circ$, that is sufficient to produce a mixture weighing 500 g. Blend for 3 min, accurately timed, at 14,000–15,000 rpm (high speed).

■ **1** ■ **2S (NF34)**

■ **2S (NF34)**

[Note—Heat generated during blending causes a temperature rise to above 30° .]

Analysis: Transfer the contents of the blender to a 600-mL beaker, and allow to stand for 5 min. ~~and adjust, if necessary, to a temperature of $33 \pm 3^\circ$~~

■ The sample temperature should be $33 \pm 3^\circ$. ■ **2S (NF34)**

Using a suitable rotational viscometer

■ **2** ■ **2S (NF34)**

■ **2S (NF34)**

equipped with a spindle as specified below, operate the viscometer at 60 rpm for 6 min, accurately timed, and record the scale reading.

For Type IA, use a spindle with a cylinder 1.87 cm in diameter and 0.69 cm high attached to a shaft 0.32 cm in diameter, the distance from the top of the cylinder to the lower tip of the shaft being 2.54 cm, and the immersion depth being 5.00 cm (No. 2 spindle). If the scale reading is greater than 90% of full scale, repeat the measurement, using a spindle similar to the No. 2 spindle but with the cylinder 1.27 cm in diameter and 0.16 cm high instead (No. 3 spindle).

For Type IC, use a No. 3 spindle. If the scale reading is greater than 90% of full scale, repeat the measurement using a spindle consisting of a cylindrical shaft 0.32 cm in diameter and with an immersion depth of 4.05 cm (No. 4 spindle).

For Types IB and IIA, use a No. 2 spindle.

Acceptance criteria

Type IA: 225–600

Type IB: 150–450

Type IC: 800–2200

Type IIA: 100–300

● **Acid Demand**

Sample: After determining the *Loss on Drying*, weigh a quantity of Magnesium Aluminum Silicate equivalent to 5.00 g.

Analysis: Disperse the *Sample* in 500 mL of water with the aid of a suitable blender fitted with a 1-L jar. Using a stopwatch, designate zero time. With constant mixing, add 3.0-mL portions of 0.100 N hydrochloric acid at 5, 65, 125, 185, 245, 305, 365, 425, 485, 545,

605, 665, and 725 s, and add a 1.0-mL portion at 785 s. Determine the pH potentiometrically at 840 s.

Acceptance criteria: NMT 4.0

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label it to indicate its type.

¹ A suitable blender is available from Waring as Waring Commercial Blender Model 7009G or equivalent with 1-L glass jar and tachometer adapter, Model CAC24 or equivalent.

² A suitable viscometer is available from Brookfield as Viscometer Model LVF or LVT, or equivalent.

BRIEFING

Polyoxyl 35 Castor Oil, *NF* 33 page 6820. As part of the USP monograph modernization effort, it is proposed to make the following revisions:

1. Add chemical names and a CAS number.
2. Replace the current *Identification* test C based on a decolorization test with the test for *Identity by Fatty Acid Composition*.
3. The procedure for *Identity by Fatty Acid Composition* is based on a GC method of analysis performed with the Agilent DB-225 or Restek Rtx-225 brand of G7 column. The typical retention times for methyl palmitate, methyl stearate, methyl oleate, methyl *cis*-11-octadecenoate, methyl linoleate, methyl *cis*-11-eicosenoate, and methyl ricinoleate are 7.91, 12.73, 13.02, 13.24, 13.84, 22.11, and 29.89 min, respectively.
4. Add a specification for residual ethylene oxide and dioxane by using the procedure in *Ethylene Oxide and Dioxane* (228).
5. Add a specification for residual ethylene glycol and diethylene glycol by using the procedure in *Ethylene Glycol, Diethylene Glycol, and Triethylene Glycol in Ethoxylated Substances* (469).
6. Update the *Packaging and Storage* section.
7. Add USP Methyl Linoleate RS, USP Methyl Ricinoleate RS, USP Methyl Palmitate RS, and USP Methyl Stearate RS to the *USP Reference Standards* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC1: H. Wang.)

Correspondence Number—C155323

Comment deadline: November 30, 2015

Polyoxyl 35 Castor Oil

Add the following:

- Polyethylene glycol 35 castor oil;
Polyoxyethylene 35 castor oil [61791-12-6]. ■ 2S (*NF34*)

DEFINITION

Polyoxyl 35 Castor Oil contains mainly the tri-ricinoleate ester of ethoxylated glycerol with smaller amounts of polyethylene glycol ricinoleate and the corresponding free glycols. It results from the reaction of glycerol ricinoleate with 35 moles of ethylene oxide.

IDENTIFICATION

- **A. Infrared Absorption** (197F)
- **B.**

Sample: 0.1 g

Analysis: Dissolve the *Sample* in 10 mL of *alcoholic potassium hydroxide TS*, boil for 3 min, and evaporate to dryness. Mix the residue with 5 mL of water.

Acceptance criteria: The residue dissolves, yielding a clear solution. Add a few drops of glacial acetic acid. A white precipitate is formed.

Delete the following:

- • ~~€.~~

~~**Sample solution:** 1 in 20~~

~~**Analysis:** Add bromine TS dropwise to the *Sample solution*.~~

~~**Acceptance criteria:** The bromine is decolorized. ■ 2S (NF34)~~

Add the following:

- • **C. Identity by Fatty Acid Composition**

Diluent: *n*-Heptane

Standard solution 1: 0.2 mg/mL each of methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, methyl *cis*-11-eicosenoate, and methyl ricinoleate, from USP Methyl Palmitate RS, USP Methyl Stearate RS, USP Methyl Oleate RS, USP Methyl Linoleate RS, methyl *cis*-11-eicosenoate, and USP Methyl Ricinoleate RS, in *Diluent*

Standard solution 2: 4 mg/mL each of methyl stearate and methyl ricinoleate from USP Methyl Stearate RS and USP Methyl Ricinoleate RS in *Diluent*

Sample solution: Transfer 140 mg of Polyoxyl 35 Castor Oil to a 10-mL screw-cap test tube, add 3.0 mL of *Diluent*, and mix well. Add 0.5 mL of 0.5 M sodium methoxide in methanol,¹ and mix with the sample. Allow the reaction to proceed at room temperature for 2 h. After 2 h, add 5 mL of water, and mix. Centrifuge the test tube at 1000 ×*g* for 10–15 min until a clear upper layer forms. Separate the organic layer (the upper layer), and remove the lower layer. Place an aliquot of the organic layer into an autosampler vial.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 15-m fused silica capillary; bonded with a 0.25-μm layer of phase G7

Temperatures

Injection port: 240°

Detector: 250°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)	Total Time (min)
80	0	80	1	1
80	30	140	0	3
140	3	150	0	6.3
150	1	155	0	11.3
155	2	165	0	16.3
165	3	220	10	45

Column mode: See *Table 2* for the pressure program.

Table 2

Pressure (psi)	Pressure Ramp (psi/min)	Hold Time (min)	Total Time (min)
10	0	16	16
4	5	9 or 0 ^a	26.2 or 17.2
3	10	19 or 28	45

^a If considerable discrimination of late eluting compounds is observed, the hold time can be adjusted from 9 to 0 min. Thus the total time should be 17.2 min. Then the next step of the hold time should be 28 min.

Carrier gas: Hydrogen

Injection volume: 0.5 µL

Injection type: Split ratio, 60:1

Liner: Single taper, low-pressure drop liner with deactivated wool

Run time: 45 min

System suitability

Sample: *Standard solution 1*

[Note—See *Table 3* for relative retention times.]

Table 3

Component	Relative Retention Time
Methyl palmitate (C16:0)	0.61
Methyl stearate (C18:0)	0.98
Methyl oleate (C18:1)	1.00
Methyl linoleate (C18:2)	1.02
Methyl <i>cis</i> -11-eicosenoate (C20:1)	1.70
Methyl ricinoleate	2.30

Suitability requirements

Resolution: NLT 1.5 between methyl stearate and methyl oleate peaks

Relative standard deviation: NMT 2.0% for the peak area ratio of methyl ricinoleate to methyl linoleate

Analysis

Samples: *Standard solution 1, Standard solution 2, and Sample solution*

The peak of methyl *cis*-11-octadecenoate, which is an isomer of methyl oleate, can be resolved from the methyl oleate peak with a resolution of about 1 and a relative retention time of 1.01 with respect to methyl oleate.

Calculate the relative response factor, F , for methyl ricinoleate:

$$F = (r_S/r_R) \times (C_R/C_S)$$

r_S = peak area of methyl stearate from *Standard solution 2*

r_R = peak area of methyl ricinoleate from *Standard solution 2*

C_R = concentration of USP Methyl Ricinoleate RS in *Standard solution 2* (mg/mL)

C_S = concentration of USP Methyl Stearate RS in *Standard solution 2* (mg/mL)

Correct the peak area of methyl ricinoleate in the *Sample solution* by multiplying by F . Calculate the percentage of each fatty acid component in the portion of Polyoxyl 35 Castor Oil taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual fatty acid methyl ester except for the uncorrected peak area of methyl ricinoleate (or the corrected peak area of methyl ricinoleate) from the *Sample solution*

r_T = sum of all the peak areas, excluding the solvent and methyl ricinoleate peaks and including the corrected peak area of methyl ricinoleate from the *Sample solution*

Acceptance criteria: Polyoxyl 35 Castor Oil exhibits the composition profile of fatty acids shown in *Table 4*.

Table 4

Component	Percentage (%)
Palmitic acid (C16:0)	≤4.0
Stearic acid (C18:0)	≤5.0
Oleic acid (C18:1)	4.0–10.0
Linoleic acid (C18:2)	1.0–5.0
<i>cis</i> -11-Eicosenoic acid (C20:1)	≤1.0
Ricinoleic acid	45.0–75.0

■ 2S (NF34)

IMPURITIES

- **Residue on Ignition** (281): NMT 0.3%

Delete the following:

- **Heavy Metals** (231), *Method II*: NMT 10 µg/g • (Official 1-Dec-2015)

Add the following:

- ● **Ethylene Oxide and Dioxane** (228), *Method I*

Acceptance criteria

Ethylene oxide: NMT 1 µg/g

Dioxane: NMT 10 µg/g

■ 2S (NF34)

Add the following:

- ● **Ethylene Glycol, Diethylene Glycol, and Triethylene Glycol in Ethoxylated Substances** (469)

Acceptance criteria

Ethylene glycol: NMT 620 µg/g

Sum of diethylene glycol and ethylene glycol: NMT 2500 µg/g

■ 2S (NF34)

SPECIFIC TESTS

- **Specific Gravity** (841): 1.05–1.06

Change to read:

- **Viscosity—Capillary Methods** (911): 600–850 centipoises
 - mPa·s ■ 2S (NF34)
 at 25°, using a capillary viscometer
- **Fats and Fixed Oils** (401), *Acid Value*: NMT 2.0
- **Fats and Fixed Oils** (401), *Hydroxyl Value*: 65–80
- **Fats and Fixed Oils** (401), *Iodine Value*: 25–35
- **Fats and Fixed Oils** (401), *Saponification Value*: 60–75
- **Water Determination** (921), *Method I*: NMT 3.0%

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers,
 - protected from light and moisture. Store at room temperature, and avoid exposure to excessive heat. ■ 2S (NF34)

Change to read:

- **USP Reference Standards** (11)
 - USP Methyl Linoleate RS
 - USP Methyl Oleate RS
 - USP Methyl Palmitate RS

USP Methyl Ricinoleate RS

USP Methyl Stearate RS ■ 2S (NF34)

USP Polyoxyl 35 Castor Oil RS

■ 1 0.5 M sodium methoxide in methanol is available from Sigma-Aldrich (www.sigmaaldrich.com), product #403067. Any other equivalent reagent can be used as well. ■ 2S (NF34)

BRIEFING

Polyoxyl 40 Hydrogenated Castor Oil, *NF 33* page 6821. As part of the USP monograph modernization effort, it is proposed to make the following revisions:

1. Add chemical names and a CAS number.
2. Replace the current *Identification* test *A* based on a wet chemical test with a test for *Identity by Fatty Acid Composition*.
3. The procedure for *Identity by Fatty Acid Composition* is based on a GC method of analysis performed with the Agilent DB Wax or Supelco 10 brand of G16 column. The typical retention times for methyl palmitate, methyl stearate, methyl arachidate, methyl 12-ketostearate, and methyl 12-hydroxystearate are 6.49, 8.78, 12.83, 20.18, and 23.62 min, respectively.
4. Add a specification for residual ethylene oxide and dioxane by using the procedure in *Ethylene Oxide and Dioxane* (228).
5. Add a specification for residual nickel based on the newly released Q3D Step 4 Guideline.
6. Change the lower limit of *Hydroxyl Value* from 60 to 57.
7. Update the *Packaging and Storage* section.
8. Add a *USP Reference Standards* section including USP Methyl 12-Hydroxystearate RS, USP Methyl Palmitate RS, and USP Methyl Stearate RS.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC1: H. Wang.)

Correspondence Number—C155324

Comment deadline: November 30, 2015

Polyoxyl 40 Hydrogenated Castor Oil

Add the following:

■ Polyethylene glycol 40 hydrogenated castor oil;
 Polyoxyethylene 40 hydrogenated castor oil [61788-85-0]. ■ 2S (NF34)

DEFINITION

Change to read:

Polyoxyl 40 Hydrogenated Castor Oil contains mainly the tri-hydroxystearate ester of ethoxylated glycerol, with smaller amounts of polyethylene glycol tri-

■ 2S (NF34)

hydroxystearate and the corresponding free glycols. It results from the reaction of glycerol trihydroxystearate with 40–45 moles of ethylene oxide.

IDENTIFICATION

Delete the following:

■ • ~~A.~~

Sample: ~~0.1 g~~

Analysis: ~~Dissolve the Sample in 1 mL of water, add 9 mL of sodium chloride solution (50 mg/mL), and heat in a water bath.~~

Acceptance criteria: ~~The solution becomes turbid at 70°–85°.~~ ■ 2S (NF34)

Add the following:

■ • **A. Identity by Fatty Acid Composition**

Diluent: Chloroform

Standard solution 1: 0.4 mg/mL each of methyl palmitate, methyl arachidate, methyl 12-ketostearate, methyl stearate, and methyl 12-hydroxystearate, from USP Methyl Palmitate RS, *methyl arachidate*, methyl 12-ketostearate, USP Methyl Stearate RS, and USP Methyl 12-Hydroxystearate RS, in *Diluent*

Standard solution 2: 4 mg/mL each of methyl stearate and methyl 12-hydroxystearate from USP Methyl Stearate RS and USP Methyl 12-Hydroxystearate RS in *Diluent*

Sample solution: Transfer 140 mg of Polyoxyl 40 Hydrogenated Castor Oil to a 10-mL screw-cap test tube, add 3.0 mL of *Diluent*, and mix well. Add 0.5 mL of 0.5 M sodium methoxide in methanol,¹ and mix with the sample. Allow the reaction to proceed at room temperature for 2 h. After 2 h, add 5 mL of water, and mix. Centrifuge the test tube at 2000 ×g for 10 min at 4° until a clear upper layer forms. Separate the organic layer (the upper layer), and remove the lower layer. Place an aliquot of the organic layer into an autosampler vial.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 30-m fused silica capillary; bonded with a 0.25-μm layer of phase G16

Temperatures

Injection port: 240°

Detector: 250°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)	Total Time (min)
80	0	80	1	1
80	30	140	0	3
140	20	180	5	10
180	2	250	10	55

Carrier gas: Hydrogen

Flow rate: 5.0 mL/min, constant flow mode

Injection volume: 1.0 µL

Injection type: Split ratio, 120:1 or 60:1

Liner: Single gooseneck liner with deactivated wool

Run time: 55 min

System suitability

Sample: *Standard solution 1*

[Note—See *Table 2* for relative retention times.]

Table 2

Component	Relative Retention Time
Methyl palmitate (C16:0)	0.27
Methyl stearate (C18:0)	0.37
Methyl arachidate (C20:0)	0.54
Methyl 12-ketostearate	0.85
Methyl 12-hydroxystearate	1.00

Suitability requirements

Resolution: NLT 10 between any two adjacent peaks

Relative standard deviation: NMT 2.0% for the peak area ratio of methyl 12-hydroxystearate to methyl 12-ketostearate

Analysis

Samples: *Standard solution 1, Standard solution 2, and Sample solution*

Calculate the relative response factor, F , for methyl 12-hydroxystearate:

$$F = (r_S/r_R) \times (C_R/C_S)$$

r_S = peak area of methyl stearate from *Standard solution 2*

r_R = peak area of methyl 12-hydroxystearate from *Standard solution 2*

C_R = concentration of USP Methyl 12-Hydroxystearate RS in *Standard solution 2* (mg/mL)

C_S = concentration of USP Methyl Stearate RS in *Standard solution 2* (mg/mL)

Correct the peak area of methyl 12-hydroxystearate in the *Sample solution* by multiplying

by F .

Calculate the percentage of each fatty acid component in the portion of Polyoxyl 40 Hydrogenated Castor Oil taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual fatty acid methyl ester except for the uncorrected peak area of methyl 12-hydroxystearate (or the corrected peak area of methyl 12-hydroxystearate), from the *Sample solution*

r_T = sum of all the peak areas, excluding the solvent and methyl 12-hydroxystearate peaks and including the corrected peak area of methyl hydroxystearate, from the *Sample solution*

Acceptance criteria: Polyoxyl 40 Hydrogenated Castor Oil exhibits the composition profile of fatty acids shown in *Table 3*.

Table 3

Component	Percentage (%)
Palmitic acid (C16:0)	≤4.0
Stearic acid (C18:0)	15.0–25.0
Arachidic acid(C20:0)	≤2.0
12-Ketostearic acid (or 12-oxostearic acid)	≤5.0
12-Hydroxystearic acid	50.0–70.0

■ 2S (NF34)

● **B.**

Sample: 0.1 g

Analysis: Dissolve the *Sample* in 10 mL of *alcoholic potassium hydroxide TS*, boil for 3 min, and evaporate to dryness. Mix the residue with 5 mL of water.

Acceptance criteria: The residue dissolves, yielding a clear solution. Add a few drops of glacial acetic acid. A white precipitate is formed.

IMPURITIES

- **Residue on Ignition** (281): NMT 0.3%

Add the following:

- ● **Ethylene Oxide and Dioxane** (228), *Method I*

Acceptance criteria

Ethylene oxide: NMT 1 µg/g

Dioxane: NMT 10 µg/g

■ 2S (NF34)

Delete the following:

- ● **Heavy Metals** (231), *Method II*: NMT 10 µg/g • (Official 1-Dec-2015)

Add the following:

- ● **Limit of Nickel**

[**Caution**—When using closed high-pressure digestion vessels and laboratory microwave

equipment, the safety precautions and operating instructions given by the manufacturer must be followed.]

[Note—If an alternative apparatus is used, adjustment of the instrument parameters may be necessary.]

Nickel standard stock solution: Dilute *nickel standard solution TS* two-fold with water. This solution contains the equivalent of 5 µg/mL of nickel.

Standard solutions: Transfer 25, 50, 75, and 100 µL of *Nickel standard stock solution* to four identical 25-mL volumetric flasks. To each flask add 0.5 mL of a 10-mg/mL solution of magnesium nitrate, 0.5 mL of a 100-mg/mL solution of monobasic ammonium phosphate, and 6.0 mL of nickel-free nitric acid. Dilute with water to volume, and mix well.

[Note—Content of nickel in the nickel-free nitric acid is NMT 0.005 ppm.] The *Standard solutions* contain 0.005, 0.01, 0.015, and 0.02 µg/mL of nickel, respectively.]

Sample solution: Transfer about 250 mg of Polyoxyl 40 Hydrogenated Castor Oil to a suitable high-pressure-resistant digestion vessel (fluoropolymer or quartz glass), and add 6.0 mL of nickel-free nitric acid and 2.0 mL of 30% hydrogen peroxide. Place the closed vessel in a laboratory microwave oven, and digest using an appropriate program, e.g., 1000 W for 40 min. Allow the digestion vessel to cool before opening. Add 2.0 mL of 30% hydrogen peroxide, and repeat the digestion step. Allow the digestion vessel to cool before opening. Quantitatively transfer to a 25-mL volumetric flask, add 0.5 mL of a 10-mg/mL solution of magnesium nitrate and 0.5 mL of a 100-mg/mL solution of monobasic ammonium phosphate. Dilute with water to volume, and mix well.

Blank solution: Place 6.0 mL of nickel-free nitric acid and 2.0 mL of 30% hydrogen peroxide in a suitable high-pressure-resistant digestion vessel. Prepare as directed in the *Sample solution*, beginning with "Place the closed vessel in a laboratory microwave oven..."

Zero solution: In a 50-mL volumetric flask, introduce 1.0 mL of a 10-mg/mL solution of magnesium nitrate, 1.0 mL of a 100-mg/mL solution of monobasic ammonium phosphate, and 12.0 mL of nickel-free nitric acid. Dilute with water to volume, and mix well.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852))

Mode: Atomic absorption, equipped with a graphite furnace, a background compensation system, and a coated tube resistant to pyrolysis

Analytical wavelength: 232.0 nm

Lamp: Nickel hollow-cathode

Temperature: Maintain the drying temperature of the furnace at 120° for 35 s after a 5-s ramp; maintain the ashing temperature at 1100° for 10 s after a 30-s ramp; maintain the cooling temperature at 800° for 5 s after a 5-s decrease; and maintain the atomization temperature at 2600° for 7 s. [Note—The temperature program may be modified to obtain optimum furnace temperatures.]

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank solution*

Concomitantly determine the absorbances of the *Samples* using the *Instrumental conditions* described above. Use the *Zero solution* to set the instrument to zero. Plot the

absorbances of the *Standard solutions* versus the concentration, in $\mu\text{g/mL}$, of nickel, and draw the straight line best fitting the plotted points. From the graph so obtained, determine the concentration, C_T , in $\mu\text{g/mL}$, of nickel in the *Sample solution*, and determine the concentration, C_B , in $\mu\text{g/mL}$, of nickel in the *Blank solution*. If necessary, dilute with the *Zero solution* to obtain a reading within the calibrated absorbance range. Calculate the quantity, in μg , of nickel in each g of Polyoxyl 40 Hydrogenated Castor Oil taken:

$$\text{Result} = V \times [(C_T - C_B) / W]$$

V = volume of the *Sample solution* and the *Blank solution*, 25 mL

C_T = concentration of nickel in the *Sample solution* ($\mu\text{g/mL}$)

C_B = concentration of nickel in the *Blank solution* ($\mu\text{g/mL}$)

W = weight of Polyoxyl 40 Hydrogenated Castor Oil taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 20 $\mu\text{g/g}$ ■ 2S (NF34)

SPECIFIC TESTS

- **Congeeing Temperature** (651): 16°–26°
- **Fats and Fixed Oils** (401), *Acid Value*: NMT 2.0

Change to read:

- **Fats and Fixed Oils** (401), *Hydroxyl Value*: ~~60~~
 - 57 ■ 2S (NF34)
 - 80
- **Fats and Fixed Oils** (401), *Iodine Value*: NMT 2.0
- **Fats and Fixed Oils** (401), *Saponification Value*: 45–69
- **Water Determination** (921), *Method I*: NMT 3.0%

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers,
 - protected from light and moisture. Store at room temperature, and avoid exposure to excessive heat. ■ 2S (NF34)

Add the following:

- ● **USP Reference Standards** (11)
 - USP Methyl 12-Hydroxystearate RS
 - USP Methyl Palmitate RS
 - USP Methyl Stearate RS

■ 2S (NF34)

■ 1 0.5 M sodium methoxide in methanol is available from Sigma-Aldrich (www.sigmaaldrich.com), product #403067. Any other equivalent reagent can be used as well. ■ 2S (NF34)

BRIEFING

Polyvinyl Acetate Dispersion, *NF 33* page 6831. As part of the *USP* monograph modernization effort, and on the basis of comments and data received, it is proposed to make the following changes:

1. Add the test title for *Identification* test A.
2. For *Identification* test B, add a reference to *Infrared Absorption* (197).
3. Change the *Assay* procedure which uses a calculation for the percentage content of polyvinyl acetate based on saponification value to a calculation for the percentage content of polyvinyl acetate based on ester value.
4. In the test for *Limit of Acetic Acid/Acetate*, the improved HPLC procedure is based on analyses performed using the Aquasil C18 brand of column that contains 5- μ m packing L1. The typical retention times for malonic acid and acetic acid are 5.3 and 6.1 min, respectively.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC1: H. Wang.)

Correspondence Number—C155201

Comment deadline: November 30, 2015

Polyvinyl Acetate Dispersion

DEFINITION

Dispersion of polyvinyl acetate in water. It contains 25.0% to 30.0% of polyvinyl acetate. It may contain suitable surface active agents and stabilizers.

IDENTIFICATION

Change to read:

- A.

- **Film Formation:** ■ 2S (NF34)

Place one drop of Dispersion on a glass plate and allow to dry. A clear and homogeneous film is formed.

Change to read:

- B. Infrared Absorption

- (See *Spectrophotometric Identification Tests* (197), *Infrared Absorption*.)

- 2S (NF34)

Analysis: Place one drop of the Dispersion on a glass plate, and cover the test substance with a water-resistant crystal disk (silver chloride or KRS-5).¹ Gently press on, and then remove the crystal disk. Dry the crystal disk in a drying chamber until a homogeneous film is formed.

Acceptance criteria: The IR absorption spectrum of the film so formed exhibits maxima corresponding to the same wavelengths as those of a similar preparation of USP Polyvinyl

Acetate Dispersion RS treated in the same manner.

ASSAY

Change to read:

• Procedure

■ **Sample 1:** 10 g of Dispersion

Solvent: 50 mL of a mixture of equal volumes of *alcohol* and *petroleum ether* with a 100°–120° boiling range, which is previously neutralized with 0.1 N potassium hydroxide or 0.1 N sodium hydroxide

Analysis 1: Dissolve *Sample 1* in the *Solvent*. Add 0.5 mL of *phenolphthalein TS*, and titrate with 0.1 N potassium hydroxide or 0.1 N sodium hydroxide until the pink color persists for at least 15 s.

Calculate the acid value, I_A :

$$\text{Result} = (M_{r1} \times V \times N) / W$$

M_{r1} = molecular weight of potassium hydroxide, 56.11

V = volume of 0.1 N potassium hydroxide or 0.1 N sodium hydroxide consumed in the actual test (mL)

N = exact normality of the potassium hydroxide solution or sodium hydroxide solution

W = weight of Dispersion taken for the test (g)

■ 2S (NF34)

Sample

■ **2:** ■ 2S (NF34)

1.5 g of Dispersion

Analysis

■ **2:** ■ 2S (NF34)

Transfer *Sample*

■ **2:** ■ 2S (NF34)

to a 250-mL borosilicate glass flask fitted with a reflux condenser. Add 25.0 mL of 0.5 M *alcoholic potassium hydroxide* and a few glass beads. Attach the condenser and heat under reflux for 30 min. Add 1 mL of *phenolphthalein TS*, and titrate immediately (while still hot) with 0.5 N hydrochloric acid VS. Perform a blank determination under the same conditions (see *Titrimetry* (541), *Residual Titrations*.)

Calculate the saponification value, I_S :

$$\text{Result} = [M_{r1} \times (V_B - V_T) \times N] / W$$

M_{r1} = molecular weight of potassium hydroxide, 56.11

V_B = volume of 0.5 N hydrochloric acid consumed in the blank test (mL)

V_T = volume of 0.5 N hydrochloric acid consumed in the actual test (mL)

N = exact normality of the hydrochloric acid

W = weight of Dispersion taken for the test (g)

Calculate the percentage content of polyvinyl acetate in the portion of Dispersion taken:

$$\text{Result} = F \times (M_{r2} \times I_S / M_{r1}) \times 100$$

$$\text{Result} = F \times \{M_{r2} \times [(I_S - I_A) / M_{r1}]\} \times 100 \quad \text{2S (NF34)}$$

F = factor converting mg to g, 10^{-3} g/mg

M_{r2} = molecular weight of vinyl acetate, 86.09

I_S = saponification value

I_A = acid value 2S (NF34)

M_{r1} = molecular weight of potassium hydroxide, 56.11

Acceptance criteria: The content of polyvinyl acetate is 25.0%–30.0%.

OTHER COMPONENTS

Stabilizers or Surface Active Agents

- **Povidone**

[Note—Perform this test only if the Dispersion contains povidone.]

Sample: 0.25 g

Analysis: Perform nitrogen determination by sulfuric acid digestion on the *Sample* as directed in *Nitrogen Determination* (461), *Method II*.

Calculate the percentage content of povidone in the portion of Dispersion taken:

$$\text{Result} = N/N_V$$

N = percentage content of nitrogen

N_V = percentage content, expressed as a decimal number, of nitrogen in vinylpyrrolidone, 0.126

Acceptance criteria: The content of povidone is NMT 4.0%.

IMPURITIES

- **Residue on Ignition** (281)

Sample: 1.0 g of Dispersion

Analysis: Heat a silica crucible to redness for 30 min, allow to cool in a desiccator, and weigh. Evenly distribute the *Sample* in the crucible and weigh. Dry the crucible at 100° – 105° for 1 h and ignite in a muffle furnace at $600 \pm 25^{\circ}$, until the test substance is thoroughly charred. Continue the experiment as directed in *Residue on Ignition* (281) on the residue obtained, beginning with "Moisten the sample with a small amount (usually 1 mL) of sulfuric acid..."

Acceptance criteria: NMT 0.5%

- **Limit of Vinyl Acetate**

Solution A: *Acetonitrile*, *methanol*, and water (5:5:90)

Solution B: *Acetonitrile*, *methanol*, and water (45:5:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
40	85	15
42	0	100
48	0	100
51	100	0

Standard solution: Transfer 50 mg of *vinyl acetate* to a 100-mL volumetric flask, dissolve in and dilute with *methanol* to volume, and mix well. Dilute 5.0 mL of the solution with *Solution A* to 100 mL. Dilute 10.0 mL of this solution with *Solution A* to 100 mL. The *Standard solution* contains about 2.5 µg/mL of vinyl acetate. [Note—This solution should be analyzed within 1 h when stored at room temperature.]

System suitability solution: Transfer 50 mg of *vinyl acetate* and 50 mg of *1-vinylpyrrolidin-2-one* to a 50-mL volumetric flask, add 10 mL of *methanol*, sonicate or gently shake the flask to dissolve the materials. Dilute with *Solution A* to volume. Dilute 10 mL of this solution with *Solution A* to 100 mL. Dilute 5 mL of this solution with *Solution A* to 100 mL. The *System suitability solution* contains about 5 µg/mL each of vinyl acetate and 1-vinylpyrrolidin-2-one.

Sample solution: Transfer 250 mg of Dispersion to a 10-mL volumetric flask, add about 4 mL of *methanol*, and sonicate. After cooling to ambient temperature, dilute with water to volume, and mix. Centrifuge at 4000 ×g for 10 min, and pass through a 0.2-µm membrane filter. [Note—This solution should be analyzed within 1 h when stored at room temperature.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Columns

Precolumn: 4.0-mm × 3-cm; 5-µm packing *L1* may be used if a matrix effect is observed. [Note—The matrix effect may result in poor reproducibility of the retention times and of the peak shapes.]

Analytical: 4.0-mm × 25-cm; 5-µm packing *L1*

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for vinyl acetate and 1-vinylpyrrolidin-2-one are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 5.0 between vinyl acetate and 1-vinylpyrrolidin-2-one

Relative standard deviation: NMT 5.0% determined from the 1-vinylpyrrolidin-2-one peak

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The response of the vinyl acetate peak from the *Sample solution* is NMT that of the vinyl acetate peak from the *Standard solution*, corresponding to NMT 100 ppm of vinyl acetate.

Change to read:

• **Limit of Acetic Acid/Acetate**

Mobile phase: ~~5 mM sulfuric acid~~

Standard solution: ~~0.3 mg/mL for each of acetic acid and citric acid in *Mobile phase*~~

Sample solution: ~~Transfer 200 mg of the Dispersion to a 10-mL volumetric flask, add about 8 mL of water, and sonicate for about 10 min. Cool to ambient temperature, and dilute with water to volume.~~

Chromatographic system

~~(See *Chromatography* (621), *System Suitability*.)~~

Mode: ~~LC~~

Detector: ~~UV 205 nm~~

Column: ~~4.6 mm × 25 cm; 5-µm packing L1~~

Column clean: ~~After each injection, rinse the column with a mixture of equal volumes of *Mobile phase* and acetonitrile.~~

Column temperature: ~~Ambient~~

Flow rate: ~~1 mL/min~~

Injection size: ~~20 µL~~

System suitability

Sample: ~~*Standard solution*~~

~~[Note—The relative retention times for acetic acid and citric acid are 1.0 and 1.2, respectively.]~~

Suitability requirements

Resolution: ~~NLT 2.0 between acetic acid and citric acid~~

Relative standard deviation: ~~NMT 5.0% determined from the acetic acid peak~~

Analysis

Samples: ~~*Standard solution* and *Sample solution*~~

Acceptance criteria: ~~The response of the acetic acid peak from the *Sample solution* is NMT that of the acetic acid peak from the *Standard solution*, corresponding to NMT 1.5% of acetic acid.~~

▪ **Solution A:** 5 mM sulfuric acid

Solution B: Acetonitrile and 5 mM sulfuric acid (1:1)

Mobile phase: See *Table 2*.

TABLE 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
10.5	0	100
20	0	100
20.5	100	0
30	100	0

System suitability solution: Transfer 30 mg of *glacial acetic acid* and 30 mg of malonic acid to a 25-mL volumetric flask, dilute with *methanol* to volume, and mix well. Transfer 1 mL of the solution to a 25-mL flask, dilute with water to volume, and mix well. The solution contains 0.048 mg/mL each of acetic acid and malonic acid.

Standard solution: 0.1 mg/mL of acetic acid in water

Sample solution: Transfer 330 mg of Dispersion to a 50-mL volumetric flask, add about 5 mL of *methanol*, and dilute with water to volume which leads to a precipitation of sample. Pass the dispersion through a 0.2- μ m regenerated cellulose membrane filter.²

■ ■2S (NF34)

Use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 25°

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: 30 min

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for malonic acid and acetic acid are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between malonic acid and acetic acid, *System suitability solution*

Relative standard deviation: NMT 5.0% determined from the acetic acid peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acetic acid in the portion of Dispersion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetic acid from the *Sample solution*

r_S = peak response of acetic acid from the *Standard solution*

C_S = concentration of acetic acid in the *Standard solution* (mg/mL)

C_U = concentration of Polyvinyl Acetate Dispersion in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.5% of acetic acid ■_{2S} (NF34)

SPECIFIC TESTS

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): The total aerobic microbial count is NMT 1000 cfu/g, and the total combined molds and yeasts count is NMT 100 cfu/g.
- **pH** (791): 3.0–5.5
- **Loss on Drying** (731)
 - Sample:** 1.0 g of Dispersion
 - Analysis:** Dry the *Sample* at 110° for 5 h.
 - Acceptance criteria:** 68.5%–71.5%
- **Coagulum Content**
 - Sample:** 100 g of Dispersion
 - Analysis:** Accurately weigh a stainless steel sieve with 45- μ m openings or a suitable single-woven wire cloth with a mesh width of 45 μ m, and filter the *Sample* through it. [Note—Suitable single-woven wire cloth mesh meets the requirements set in ISO 9044.] Wash the sieve or the cloth with distilled water until a clear filtrate is obtained, and dry the sieve or the cloth to constant weight at 100°–105°.
 - Acceptance criteria:** The weight of the residue is NMT 500 mg (0.5%).

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers at a temperature below 25°. Protect from freezing.
- **Labeling:** Label it to indicate the names and quantities of any added surface active agents and stabilizers.
- **USP Reference Standards** (11)
USP Polyvinyl Acetate Dispersion RS

¹ KRS-5 consists of 42% thallium(I) bromide and 58% thallium(I) iodine by molecular weight. Suitable disks of silver chloride and of KRS-5 are available from www.photonic.saint-gobain.com, www.almazoptics.com, and www.internationalcrystal.net.

² Whatman Spartan HPLC certified syringe filter, Whatman Cat # 10463060 or equivalent filter.

BRIEFING

Potassium Benzoate, NF 33 page 6835. See the *Briefing* under *Calcium Propionate*, appearing elsewhere in this issue of *PF*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

acid to a pH of 2.5.

Mobile phase: *Solution A* and *acetonitrile* (70:30)

Diluent: Water and *acetonitrile* (50:50)

System suitability solution: 0.1 mg/mL of USP Salicylic Acid RS and 0.1 mg/mL of USP Benzoic Acid RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Benzoic Acid RS in *Diluent*

Sample solution: 0.1 mg/mL of Potassium Benzoate in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 5-μm packing *L1*

Column temperature: 25°

Flow rate: 1.0 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for benzoic acid and salicylic acid are approximately 1 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 3.0 between benzoic acid and salicylic acid, *System suitability solution*

Tailing factor: NMT 2.0 for benzoic acid, *Standard solution*

Relative standard deviation: NMT 0.5% for benzoic acid, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of potassium benzoate (C₇H₅KO₂) in the portion of Potassium Benzoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of benzoic acid from the *Sample solution*

r_S = peak area of benzoic acid from the *Standard solution*

C_S = concentration of USP Benzoic Acid RS in the *Standard solution*, corrected for purity (mg/mL)

C_U = concentration of Potassium Benzoate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of potassium benzoate, 160.21

M_{r2} = molecular weight of benzoic acid, 122.12

Acceptance criteria: 99.0%–101.0% on the anhydrous basis

IMPURITIES

Delete the following:

- **Heavy Metals** (231)

Test preparation: Dilute 4.0 g in 40 mL of water. Add, dropwise with vigorous stirring, 10 mL of 3 N hydrochloric acid, and filter. Use 25 mL of the filtrate.

Acceptance criteria: NMT 10 µg/g • (Official 1-Dec-2015)

SPECIFIC TESTS

- **Alkalinity**

Sample: 2 g

Analysis: Dissolve the *Sample* in 20 mL of hot water, and add 2 drops of *phenolphthalein TS*.

Acceptance criteria: If a pink color is produced, it is discharged by the addition of 0.20 mL of 0.10 N *sulfuric acid*.

- **Water Determination** (921), *Method I*: NMT 1.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

- **USP Reference Standards** (11)

USP Benzoic Acid RS

USP Potassium Benzoate RS

USP Salicylic Acid RS

BRIEFING

Sodium Cetostearyl Sulfate, *NF 33* page 6868. See the *Briefing* under *Calcium Propionate* appearing elsewhere in this issue of *PF*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC1: G. Holloway.)

Correspondence Number—C162766

Comment deadline: November 30, 2015

Sodium Cetostearyl Sulfate**DEFINITION**

Sodium Cetostearyl Sulfate is a mixture of sodium cetyl sulfate and sodium stearyl sulfate. It contains NLT 40.0% of sodium cetyl sulfate ($C_{16}H_{33}NaSO_4$), and the sum of the sodium cetyl sulfate content and sodium stearyl sulfate ($C_{18}H_{37}NaSO_4$) content is NLT 90.0% (both contents calculated on the anhydrous basis). It may contain a suitable buffer.

IDENTIFICATION

- **A.** The retention times of the two major peaks of *Sample solution C* correspond to those of

Chromatographic system*(See Chromatography (621), System Suitability.)***Mode:** GC**Detector:** Flame ionization**Column:** 0.25-mm × 25-m fused silica capillary; phase G2**Temperatures****Injection port:** 250°**Detector:** 250°**Column:** See Table 1.**Table 1**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
150	5	250	Duration of analysis

Carrier gas: Nitrogen**Flow rate:** 1 mL/min**Injection volume:** 1 µL**Injection type:** Split ratio 100:1**System suitability****Sample:** *System suitability solution***Suitability requirements****Resolution:** NLT 4.0 between cetyl alcohol and stearyl alcohol**Relative standard deviation:** NMT 1.5%**Analysis**

Correction for interference: Inject *Sample solution A* and *Sample solution B* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. If *Sample solution B* shows a peak at the same retention time as the internal standard peak of *Sample solution A*, calculate the ratio, *R*:

$$R = S_{CB}/S_I$$

S_{CB} = peak response of cetyl alcohol from *Sample solution B*

S_I = peak response with the same retention time as the internal standard of *Sample solution B*

If *R* is less than 300, calculate the corrected area, $S_{A(corr)}$, of the peak corresponding to the internal standard of *Sample solution A*:

$$S_{A(corr)} = S_{HA} - (S_I \times S_{CA}/S_{CB})$$

S_{HA} = peak response of the internal standard from *Sample solution A*

S_I = peak response with the same retention time as the internal standard of *Sample solution B*

S_{CA} = peak response of cetyl alcohol from *Sample solution A*

S_{CB} = peak response of cetyl alcohol from *Sample solution B*

Inject *Sample solution C* and *Sample solution D* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Carry out the *Correction for interference* in the same manner as for *Sample solution A*, and calculate the corrected area of the peak corresponding to the internal standard of *Sample solution C*, $S_{C(corr)}$.

Samples: *System suitability solution*, *Sample solution C*, and *Sample solution D*

[Note—The substances are eluted in the following order: cetyl alcohol, 1-heptadecanol (internal standard), and stearyl alcohol. Identify the cetyl alcohol and stearyl alcohol peaks in the chromatograms of the *Sample solutions* by comparison with the *System suitability solution*.]

Calculate the percentage of sodium cetyl sulfate ($C_{16}H_{33}NaSO_4$) in the portion of Sodium Cetostearyl Sulfate taken:

$$\text{Result} = (r_C \times W_{CH}) / (S_{C(corr)} \times W_C) \times F \times 100$$

r_C = peak response of cetyl alcohol from *Sample solution C*

W_{CH} = weight of the internal standard added in the preparation of *Sample solution C* (mg)

$S_{C(corr)}$ = corrected area of the peak corresponding to the internal standard of *Sample solution C*

W_C = weight of Sodium Cetostearyl Sulfate taken to prepare *Sample solution C*, calculated on the anhydrous basis (mg)

F = correction factor, 1.421

Calculate the percentage of sodium stearyl sulfate ($C_{18}H_{37}NaSO_4$) in the portion of Sodium Cetostearyl Sulfate taken:

$$\text{Result} = (B_C \times W_{CH}) / (S_{C(corr)} \times W_C) \times F \times 100$$

B_C = peak response of stearyl alcohol from *Sample solution C*

W_{CH} = weight of the internal standard added in the preparation of *Sample solution C* (mg)

$S_{C(corr)}$ = corrected area of the peak corresponding to the internal standard of *Sample solution C*

W_C = weight of Sodium Cetostearyl Sulfate taken to prepare *Sample solution C*, calculated on the anhydrous basis (mg)

F = correction factor, 1.377

Acceptance criteria

Sodium cetyl sulfate: NLT 40.0% on the anhydrous basis

Sum of sodium cetyl sulfate and sodium stearyl sulfate: NLT 90.0% on the anhydrous basis

IMPURITIES

- **Limit of Sodium Chloride and Sodium Sulfate**

Sodium chloride**Sample:** 5 g**Titrimetric system****Mode:** Direct titration**Titrant:** 0.1 N silver nitrate VS**Endpoint detection:** Potentiometric

Analysis: Dissolve the *Sample* in 50 mL of water, and add *diluted nitric acid* dropwise until the solution is neutral to blue litmus paper. To the resulting solution add 1 mL of *potassium chromate TS* and titrate with *Titrant*.

Calculate the percentage of sodium chloride (NaCl) in the portion of Sodium Cetostearyl Sulfate taken:

$$\text{Result} = (V \times N) / W \times F$$

V = volume of the *Titrant* (mL)

N = actual normality of the *Titrant*

W = weight of Sodium Cetostearyl Sulfate (g)

F = equivalence factor for sodium chloride, 5.844

Sodium sulfate

Dichloroacetic acid solution: Dilute 67 mL of *dichloroacetic acid* with water to 300 mL, and neutralize to blue litmus paper using *ammonia TS*. Cool, add 33 mL of *dichloroacetic acid*, and dilute with water to 600 mL.

Sample: 0.5 g**Titrimetric system****Mode:** Direct titration**Titrant:** 0.01 M lead nitrate VS**Endpoint detection:** Potentiometric

Analysis: Dissolve the *Sample* in 20 mL of water, warming gently if necessary, and add 1 mL of a solution containing 0.5 g/L of *dithizone* in *acetone*. If the solution is red, add 1 mL *nitric acid* dropwise until a bluish-green color is obtained. To the resulting solution add 2.0 mL of *Dichloroacetic acid solution* and 80 mL of *acetone*, and titrate with *Titrant* until a persistent orange-red color is obtained.

Calculate the percentage of sodium sulfate (Na₂SO₄) in the portion of Sodium Cetostearyl Sulfate taken:

$$\text{Result} = (V \times M) / W \times F$$

V = volume of *Titrant* (mL)

M = actual molarity of *Titrant*

W = weight of Sodium Cetostearyl Sulfate (g)

F = equivalence factor for sodium sulfate, 14.20

Acceptance criteria: The sum of the percentages of sodium chloride and sodium sulfate is NMT 8.0%.

Change to read:

- **Limit of Free Cetostearyl Alcohol**

Analysis: Examine the chromatogram of *Sample solution A*, obtained as directed in the *Assay*.

Calculate the percentage of free cetostearyl alcohol in the portion of Sodium Cetostearyl Sulfate taken:

$$\bullet \text{Result} = 100(r_A + r_B) \times W_{IS} / (S_{A(\text{corr})} \times W) \bullet (\text{ERR 1-Jun-2015})$$

- r_A = peak response of the cetyl alcohol peak from *Sample solution A*
 r_B = peak response of stearyl alcohol from *Sample solution A*
 W_{IS} = weight of the internal standard added in the preparation of *Sample solution A* (mg)
 $\bullet S_{A(\text{corr})} \bullet (\text{ERR 1-Jun-2015})$ = corrected peak area corresponding to the internal standard of *Sample solution A* (see *Assay*)
 W = weight of Sodium Cetostearyl Sulfate taken to prepare *Sample solution A* (mg)

Acceptance criteria: NMT 4.0%

SPECIFIC TESTS

- **Acidity or Alkalinity**

Sample: 500 mg

Analysis: Dissolve the *Sample* by heating in a mixture of 10 mL of water and 15 mL of 90% alcohol. Add 0.1 mL of phenolphthalein TS.

Acceptance criteria: The resulting solution is colorless. Add 0.1 mL of 0.1 N sodium hydroxide, and the resulting solution becomes red.

- **Water Determination** (921), *Method I*: NMT 1.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. No storage requirements specified.
- **Labeling:** Label it to indicate the name and concentration of any added buffer.
- **USP Reference Standards** (11)
 - USP Cetyl Alcohol RS
 - USP Stearyl Alcohol RS

BRIEFING

Sodium Succinate. Because there is currently no *NF* monograph for this excipient, a new monograph is proposed based on the data and comments received.

1. The HPLC procedure in the *Assay* is based on analyses performed with the Grace Alltima C18 or Waters Atlantis T3 C18 brand of L1 column. The typical retention times for succinic acid and fumaric acid are 4.3 and 5.0 min, respectively.
2. The HPLC procedure in the test for the *Limit of Sodium Acetate, Sodium Maleate, and*

Sodium Fumarate is based on analyses performed with the Grace Alltima C18 or Waters Atlantis T3 C18 brand of L1 column. The typical retention times for acetic acid, maleic acid, succinic acid, and fumaric acid are 3.0, 3.2, 4.2, and 5.0 min, respectively.

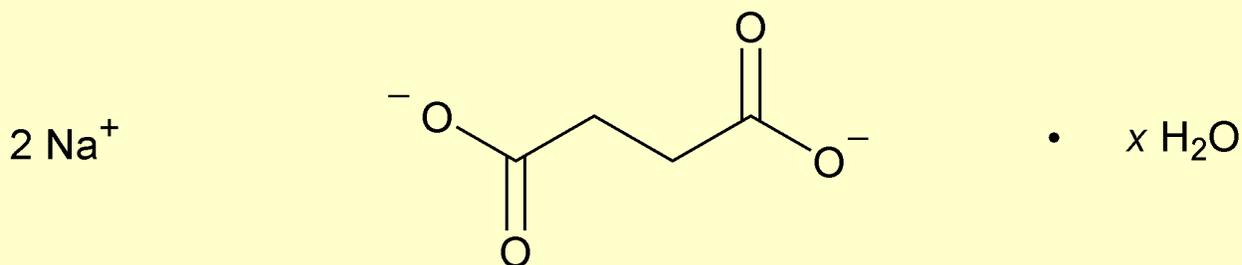
(EXC1: H. Wang.)

Correspondence Number—C151320

Comment deadline: November 30, 2015

Add the following:

▪ **Sodium Succinate**



x = 0 or 6NaOOC-CH₂CH₂-COONa (C₄H₄Na₂O₄) 162.05

Anhydrous disodium 1,4-butanedioate;

Anhydrous butanedioic acid disodium salt [150-90-3].NaOOC-CH₂CH₂-COONa·6H₂O
(C₄H₄Na₂O₄·6H₂O) 270.14

Disodium 1,4-butanedioate hexahydrate;

Butanedioic acid disodium salt hexahydrate [6106-21-4].

DEFINITION

Sodium Succinate, when dried at 120° for 2 h, contains NLT 98.0% and NMT 102.0% of disodium succinate (C₄H₄Na₂O₄).

IDENTIFICATION

- **A. Infrared Absorption** (197K) or (197A): Dry the Anhydrous Sodium Succinate or Sodium Succinate Hexahydrate sample and USP Anhydrous Sodium Succinate RS at 120° for 2 h before use.
- **B. Chromatographic Identity**

Analysis: Proceed as directed in the *Assay*.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*.
- **C. Sodium**

Analysis: Proceed as directed in *Identification Tests—General* (191), *Sodium*.

Acceptance criteria: Meets the requirements

ASSAY**• Procedure**

Solution A: Dissolve 6.8 g of monobasic potassium phosphate in 2 L of water. Adjust with phosphoric acid to a pH of 2.3. Pass under vacuum through an HNWP (nylon hydrophilic) membrane filter of 0.45- μ m pore size. This is a 25 mM potassium phosphate buffer with a pH of 2.3.

Mobile phase: Add 100 mL of methanol to 1900 mL of *Solution A* and mix well. Sonicate for 30 min and cool to room temperature.

Diluent: Add 10 mL of phosphoric acid to 1 L of water and mix well. This is a 1% phosphoric acid solution.

System suitability solution: 3.0 mg/mL of USP Anhydrous Sodium Succinate RS and 2.2 μ g/mL of USP Fumaric Acid RS in *Diluent*. Dry USP Anhydrous Sodium Succinate RS at 120° for 2 h before use.

Standard solution: 3.0 mg/mL of USP Anhydrous Sodium Succinate RS in *Diluent*. Dry USP Anhydrous Sodium Succinate RS at 120° for 2 h before use.

Sample solution: 3.0 mg/mL of Anhydrous Sodium Succinate or Sodium Succinate Hexahydrate in *Diluent*. Dry Anhydrous Sodium Succinate or Sodium Succinate Hexahydrate at 120° for 2 h before use.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 204 nm

Column: 4.6-mm \times 15-cm; 3- μ m packing L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 10 μ L

Run time: 10 min

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for succinic acid and fumaric acid are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 2.0 between succinic acid and fumaric acid, *System suitability solution*

Tailing factor: 0.8–2.0, *Standard solution*

Relative standard deviation: NMT 0.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sodium succinate ($C_4H_4Na_2O_4$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Anhydrous Sodium Succinate RS in the *Standard solution* (mg/mL)

C_U = concentration of Anhydrous Sodium Succinate or Sodium Succinate Hexahydrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• Limit of Sodium Acetate, Sodium Maleate, and Sodium Fumarate

Solution A, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Acetic acid stock solution: Transfer 37.5 mg of USP Glacial Acetic Acid RS to a 25-mL volumetric flask that contains 10 mL of *Diluent*. Dissolve and dilute with *Diluent* to volume. Transfer 1.0 mL of this solution to a 10-mL volumetric flask and dilute with *Diluent* to volume. This solution is equivalent to 200 µg/mL of sodium acetate in *Diluent*.

Maleic acid stock solution: Transfer 36.5 mg of USP Maleic Acid RS to a 50-mL volumetric flask. Dissolve and dilute with *Diluent* to volume. Transfer 1.0 mL of this solution to a 10-mL volumetric flask and dilute with *Diluent* to volume. This solution is equivalent to 100 µg/mL of sodium maleate in *Diluent*.

Fumaric acid stock solution: Transfer 36.5 mg of USP Fumaric Acid RS to a 50-mL volumetric flask. Dissolve and dilute with *Diluent* to volume. Transfer 1.0 mL of this solution to a 10-mL volumetric flask and dilute with *Diluent* to volume. This solution is equivalent to 100 µg/mL of sodium fumarate in *Diluent*.

System suitability solution: 10 mg/mL of USP Anhydrous Sodium Succinate RS, 15 µg/mL of USP Glacial Acetic Acid RS, and 7.3 µg/mL each of USP Maleic Acid RS and USP Fumaric Acid RS in *Diluent*. Dry USP Anhydrous Sodium Succinate RS at 120° for 2 h before use.

Standard solution: Transfer 1 mL each of *Acetic acid stock solution*, *Maleic acid stock solution*, and *Fumaric acid stock solution* to a 10-mL volumetric flask and dilute with *Diluent* to volume.

Sample solution: 10 mg/mL of Anhydrous Sodium Succinate or Sodium Succinate Hexahydrate in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for acetic acid, maleic acid, succinic acid, and fumaric acid are 0.7, 0.8, 1.0, and 1.2, respectively]

Suitability requirements

Resolution: NLT 1.5 between acetic acid and maleic acid; NLT 2.0 between succinic acid and fumaric acid, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Based on the *Standard solution*, identify the peaks of acetic acid, maleic acid, and fumaric acid. Compare peak areas of acetic acid, maleic acid, and fumaric acid in the *Standard solution* and the *Sample solution*.

Acceptance criteria

Sodium acetate: The peak area of acetic acid in the *Sample solution* is NMT the peak area of acetic acid in the *Standard solution*, corresponding to NMT 0.2% of sodium acetate in Sodium Succinate.

Sodium maleate: The peak area of maleic acid in the *Sample solution* is NMT the peak area of maleic acid in the *Standard solution*, corresponding to NMT 0.1% of sodium maleate in Sodium Succinate.

Sodium fumarate: The peak area of fumaric acid in the *Sample solution* is NMT the peak area of fumaric acid in the *Standard solution*, corresponding to NMT 0.1% of sodium fumarate in Sodium Succinate.

• Limit of Sulfate

Standard solution: 0.4 mL of 0.005 mol/L sulfuric acid

Sample solution: Dissolve 1.0 g of Anhydrous Sodium Succinate or Sodium Succinate Hexahydrate in 30 mL of water and neutralize with a diluted hydrochloric acid (1 in 40).

Analysis: Proceed as directed in *Chloride and Sulfate* (221), *Sulfate*.

Acceptance criteria: NMT 0.019% as SO_4

SPECIFIC TESTS

• Acidity and Alkalinity

Sample solution: Dissolve 1.0 g of Anhydrous Sodium Succinate or Sodium Succinate Hexahydrate in carbon dioxide-free water and dilute with water to 20 mL.

Analysis: Proceed as directed in *pH* (791).

Acceptance criteria: 7.0–9.0

• Loss on Drying (731)

Analysis: Proceed as directed in *Loss on Drying* (731). Dry at 120° for 2 h.

Acceptance criteria

Anhydrous Sodium Succinate: NMT 2.0%

Sodium Succinate Hexahydrate: 37.0%–41.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Store at room temperature.

• **Labeling:** Label it to state, as part of the official title, anhydrous or hexahydrate for sodium succinate.

• **USP Reference Standards** (11)

USP Anhydrous Sodium Succinate RS

USP Fumaric Acid RS

USP Glacial Acetic Acid RS

USP Maleic Acid RS

■ 2S (NF34)

BRIEFING

Carnauba Wax, *NF 33* page 6949. As part of the USP monograph modernization effort and on the basis of comments and data received, it is proposed to add an infrared *Identification* test because the current *NF* monograph lacks an *Identification* test.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC2: J. Liu.)

Correspondence Number—C125488

Comment deadline: November 30, 2015

Carnauba Wax**DEFINITION**

Carnauba Wax is obtained from the leaves of *Copernicia cerifera* Mart. (Fam. Palmae).

IDENTIFICATION**Add the following:**

- **A. Infrared Absorption** (197F) or (197A) ■ 2S (NF34)

IMPURITIES

- **Residue on Ignition** (281)

Sample: 2 g

Analysis: Heat the *Sample* in an open porcelain or platinum dish over a flame; it volatilizes without emitting an acrid odor. Ignite.

Acceptance criteria: The weight of the residue is NMT 5 mg, corresponding to NMT 0.25%.

Delete the following:

- ~~**Heavy Metals**, Method II (231): 20 ppm~~ (Official 1-Dec-2015)

SPECIFIC TESTS

- **Melting Range or Temperature** (741), *Procedure for Class II:* 80°–86°

- **Acid Value**

Sample: 3 g

Analysis: Weigh the *Sample* into a 250-mL flask attached to a reflux condenser. Add 50 mL of a mixture of isopropyl alcohol and toluene (5:4), and boil gently until the wax is completely dissolved. Remove the flask from the condenser, add 1 mL of *phenolphthalein TS*, and immediately titrate with 0.5 N alcoholic potassium hydroxide VS to a faint, reddish-yellow color. [Note—Do not allow the solution to cool. Titrate at warm temperature after refluxing.]

Calculate the acid value as the number of mg of potassium hydroxide required to neutralize the free acids in 1 g of Carnauba Wax.

Acceptance criteria: 2–7

- **Fats and Fixed Oils** (401), *Saponification Value*

Sample: Use the solution from the test for *Acid Value*.

Analysis: To the *Sample* add 15.0 mL of 0.5 N alcoholic potassium hydroxide VS, reflux for 3 h, and titrate the excess alkali with 0.5 N hydrochloric acid VS to a yellow-amber color. Perform a blank determination (see *Titrimetry* (541), *Residual Titrations*).

Calculate the ester value. The saponification value is the sum of the ester value and the acid value.

Acceptance criteria: 78–95

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Stage 4 Harmonization

This section contains monographs or chapters undergoing harmonization by the Pharmacopeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (**Stages 1** through **7**, as defined below). Stage 4 drafts are available for comments. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF*, this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

BRIEFING

(644) **Conductivity of Solutions.** The United States Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for *Conductivity of Solutions* (644), as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following document, which represents the revised OFFICIAL INQUIRY STAGE 4 document, is based in part on comments from the *Japanese Pharmacopoeia* and the *European Pharmacopoeia*. There is a growing use of electrical conductivity measurements of fluids in pharmaceutical processes, and there is a void in the *USP* general chapters on information regarding the use of this analytical tool. This is a harmonized chapter within the Pharmacopeial Discussion Group and it is ready for public comments in the three pharmacopeias.

(GCCA: A. Hernandez-Cardoso.)

Correspondence Number—C46346

Comment deadline: November 30, 2015

Add the following:

(644) CONDUCTIVITY OF SOLUTIONS

INTRODUCTION

This chapter provides information on how to apply electrical conductivity measurements (hereafter referred to as “conductivity”) of fluid solutions, including pure fluids. ♦This chapter does not replace the official *Water Conductivity* (645) procedure, which is used to ensure the ionic purity of compendial waters such as *Water for Injection*, *Purified Water*, *Pure Steam condensate*, and *Sterile Water for Injection*, among others. ♦This chapter is intended for other fluid applications when conductivity is used to measure, monitor, or control chemical dispensing, chemical purity, ionic concentration, and other applications where the ionic character of the fluid needs to be known or controlled.

Applications include, but are not limited to, solutions that may be used in clean-in-place, chromatography detection, ionic solution preparations, end point detection, dosing, fermentation, and buffer production. In some cases, conductivity measurements can be extended to pure organic fluids such as alcohols and glycols where a weak conductivity signal exists, and the signal can be significantly increased if the organics become contaminated with water or salts. ♦[Note—For additional background information, see *Theory and Practice of Electrical Conductivity Measurements of Solutions* (1644).] ♦

Conductivity is the measurement of the ability of a fluid to conduct electricity via its chemical ions. The ability of any ion to electrically conduct is directly related to its ion mobility. Conductivity is directly proportional to the concentrations of ions in the fluid, according to *Equation 1*:

$$\kappa = 1000 \sum_{\substack{\text{all} \\ \text{ions} \\ i}} C_i \lambda_i \quad [1]$$

κ = conductivity (S/cm)

C_i = concentration of chemical ion i (mol/L)

λ_i = specific molar conductance of ion i (S·cm²/mol)

Although the SI unit S/m is the appropriate SI unit for conductivity, historically the unit S/cm has been selected by industry as the accepted unit.

On the basis of *Equation 1*, conductivity is not ion selective because it responds to all ions. Furthermore, the specific molar conductance of each ion is different. As a result, unless the percentage composition of ions of the solution is limited and known, the precise concentrations of ionic species cannot be determined from conductivity measurements. However, for examples such as a solution of a single salt or acid or base, such as a caustic solution used in cleaning, the precise concentration can be directly determined. Despite the lack of ionic specificity, conductivity is a valuable laboratory and process tool for measurement and control of total ionic content because it is proportional to the sum of the concentrations of all ionic species (anions and cations) as described in *Equation 1*. Conductivity measurements cannot be applied to solids or gases, but they can be applied to the condensate of gases.

Another variable that influences conductivity measurements is the fluid temperature. As the fluid temperature increases, the ion conductance increases, making this physicochemical phenomenon the predominant reason for the temperature-compensation requirement when testing conductive fluids.

The conductivity, κ , is proportional to the conductance, K (S), of a fluid between two electrodes (*Equation 2*):

$$\kappa = K \times \left(\frac{d}{A} \right) = K \times \theta \quad [2]$$

κ = conductivity (S/cm)

K = conductance (S)

d = distance between the electrodes (cm)

A = area of the conducting electrodes (cm²)

θ = cell constant (cm⁻¹), which also equals the ratio of d/A

The resistivity ρ (Ω -cm) of the fluid is, by definition, the reciprocal of the conductivity (*Equation 3*):

$$\rho = \frac{1}{\kappa} = \frac{1}{K \times \theta} = \frac{R}{\theta} \quad [3]$$

ρ = resistivity (Ω -cm)

κ = conductivity (S/cm)

K = conductance (S)

θ = cell constant (cm^{-1})

R = resistance (Ω), which is the reciprocal of the conductance, K

APPARATUS

An electrical conductivity measurement consists of the determination of resistance of the fluid between and around the electrodes of the conductivity sensor. To achieve this measurement, the primary instrumentation is the resistance-measuring circuit and the conductivity sensor, and they are usually connected by a cable when the sensor and the user interface are separated.

The resistance measurement is made by applying an alternating current (AC, meaning the flow of electric charge periodically reverses direction) voltage (or current) to the electrodes, measuring the current (or voltage), and calculating the resistance according to Ohm's Law. The alternating source is used to prevent the polarization (collection of ions) at the electrodes. The measuring frequency of the AC signal adjusts automatically according to the measuring conditions of the instrument, and there may be multiple resistance-measuring circuits embedded in the measuring system. The resistance-measurement circuit may be embedded in the transmitter or in the sensor.

The conductivity sensor consists of at least two electrical conductors of a fixed size and geometry, separated by an electrical insulator. The electrodes, insulator, and any other wetted materials should be constructed of materials that are unreactive to fluids with which they may come into contact. Also, the sensor construction should withstand the environmental conditions (process or ambient temperature, pressure, cleaning applications) that it would be subjected to.

Most conductivity sensors have temperature devices such as a platinum resistance temperature device (RTD) or negative temperature coefficient (NTC) thermistor embedded inside the sensor, although external temperature measurement is possible. The purpose of the temperature measurement is for temperature compensation of the conductivity measurement.

CELL CONSTANT DETERMINATION

The purpose of the sensor's cell constant is to normalize the conductance (or resistance) measurement for the geometrical construction of the two electrodes.

The cell constant is determined by immersing the conductivity sensor in a solution of known conductivity. Solutions of known conductivity can be obtained by 1) preparation of specific mixtures according to national authoritative sources, 2) procurement of commercially available

certified and traceable standard solutions, or 3) comparison to other reference conductivity measuring systems. These mixtures or certified solutions can range from 5 to 200,000 $\mu\text{S}/\text{cm}$, depending on the level of accuracy desired. [Note—Conductivity measurements are not perfectly linear with concentration. Dilution of a reference solution by x-fold does not imply that the conductivity of that diluted fluid is x-fold less. Negative deviations from linearity range from 5% to 10% for commonly used reference solutions.]

The measured cell constant of the conductivity sensor must be within 5% of the value indicated by the sensor certificate.

Modern conductivity sensors normally do not change their cell constant over their lifetime. If a change of the cell constant is detected during calibration, a cleaning of the sensor is appropriate according to the manufacturer's recommendations. Following that, the calibration procedure should be repeated. Sometimes "memory effects" appear, particularly when changing from high to low concentrations if the sensor is not well flushed.

TEMPERATURE COMPENSATION

Because the conductivity of a fluid is temperature dependent, temperature compensation of the conductivity measurement is normally necessary. An appropriate temperature compensation algorithm will ensure that changes in the conductivity measurement can be ascribed to concentration changes and not temperature changes. Conductivity measurements are normally referenced to 25°. A common form of linear temperature compensation uses *Equation 4*:

$$\kappa_{25} = \frac{\kappa_T}{\left[1 + \alpha(T - 25)\right]} \quad [4]$$

κ_{25} = conductivity compensated to 25°

κ_T = conductivity at T

α = linear temperature compensation factor

T = measured temperature

A value of 0.021 for a temperature coefficient of 2.1% per 1° is commonly used for many salt solutions. Most salt-based solutions have linear compensation factors ranging from 1.9% to 2.2% per 1°. Depending on the application, other values can be used for acids, bases, and other fluids. Another known, nonlinear form of temperature compensation is described in ISO 7888:1985 *Water quality—Determination of electrical conductivity*. Depending on the fluid samples, other forms of temperature compensation may be appropriate. In cases of very low conductivity (<10 $\mu\text{S}/\text{cm}$), such as purified pharmaceutical waters, two compensations need to be made. One is for the intrinsic conductivity of water, and the other is for the other ionic species in water. These compensations are normally combined and embedded in the microprocessor-controlled conductivity measurement systems. This is not supplied in all conductivity measurement technologies.

CALIBRATION OF TEMPERATURE AND MEASUREMENT ELECTRONICS

In addition to verifying the sensor's cell constant, the embedded temperature device (or external temperature device) should be appropriately calibrated for the application to apply the temperature compensation algorithm accurately. The temperature accuracy that is required depends on the criticality of the temperature to the application. An accuracy of $\pm 1^\circ$ typically suffices.

The measurement circuit of the system is fundamentally an AC resistance measuring device. Appropriate verification and/or calibration of the measuring circuit is required. This is accomplished by disconnecting the measuring circuit from the sensor's electrodes, attaching traceable resistors of known value to the measuring circuit, and verifying that the measured resistance agrees with the resistor value to an acceptable level. A typical acceptance criterion for the resistance accuracy is $< 2\%$ of the reading at resistances $> 100 \Omega$, and increasing to 5% at lower resistances. However, the application criticality should ultimately determine the desired accuracy.

For conductivity systems that cannot have the resistance-measuring circuit disconnected from the electrodes, directly adjusting or verifying the circuit accuracy may be difficult, depending on the sensor design. An alternative method of verifying the circuit accuracy can be used by performing a sensor cell constant calibration at two (or more) measurement points for each measuring circuit that is intended to be used.

If verification/calibration of the sensor's cell constant, temperature device, and measuring circuit are done at the same service interval, the measuring circuit should be verified first, the temperature device next, and the cell constant last. Because all of these parameters are typically very stable due to modern electronics and stable sensor construction, frequent calibration (such as daily) is not usually required. Comparison to qualified reference systems is also a proper means of calibration. Calibration cycles range from 6 to 12 months.

CONDUCTIVITY MEASUREMENT OF FLUIDS

For off-line batch measurements, rinse the cleaned sensor with the fluid to be measured. Then immerse the sensor in the fluid to be measured, and record the temperature and the temperature-compensated conductivity as required. Be sure that the position of the sensor in the container does not affect the conductivity measurement, because the container walls can affect the measurement for some electrode designs.

For continuous on-line or at-line measurements, install the cleaned sensor into the pipe, tank, or other containment vessel, and flush, if necessary. Make sure proper installation procedures are applied to prevent bubbles or particles from collecting between the electrodes. Be sure that the position of the sensor in the pipe or tank does not affect the conductivity measurement, because the nearby surfaces can affect the measurement for some electrode designs.

Record the temperature and the temperature-compensated conductivity as required.

For all batch or continuous measurements, ensure that the wetted components of the sensor are compatible with the fluid and the temperature to be measured.

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An Evaluation of the Indifference Zone of the USP (905) Content Uniformity Test¹

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ABSTRACT The indifference zone, in the test for *Uniformity of Dosage Units* (905), was questioned in a recent *Stimuli* article (*PF* 37(1) [Jan.–Feb. 2011]) in which the authors found that the current test rewards batches with an off-target mean. In this *Stimuli* article, this potential issue is reviewed. This article demonstrates that the monograph potency requirement implicitly ensures that the potential issue cannot occur in practice, because an off-target batch will fail potency. On the basis of the investigations performed, and considering the difficulties involved in modifying the harmonized (905) test, we recommend retaining the current test in the chapter as-is.

INTRODUCTION

The current method for confirming content uniformity in *Uniformity of Dosage Units* (905) (1) continues to attract interest and inspires research and the possibility for improvement. The extension of (905) to applications where large sample sizes (>30) are collected is currently in debate, and several proposals on this subject have been published (2–5). Additionally, the *European Pharmacopoeia* (*Ph Eur*) has published a chapter on this subject (6). USP has established a “Content Uniformity with Large Sample Sizes Expert Panel” (CULSS EP) to develop a new chapter on statistical analysis and acceptance criteria (“Large N test”) when assessing content uniformity in sample sizes larger than those called for in the (905) test. Another task of the CULSS EP is to address a characteristic of the current (905) that was described in a *Stimuli* article in *PF* 37(1) [Jan.–Feb. 2011] (7). That article demonstrated that the indifference zone (IDZ; see below for a description of this element of the test) of (905) introduces a deviation that, for a given coverage, benefits batches with significantly off-target mean. Shen and Tsong (7) describe this deviation as a “bias”, and we will use this term throughout the remainder of this *Stimuli* article.

The purpose of this *Stimuli* article is to describe and put into perspective the possible bias identified by Shen and Tsong (7), review the history behind the introduction of the IDZ, remind the reader of the harmonization process that was a key element of the development of (905) as it stands today, and explore the consequences of removing the IDZ and/or modifying the current (905) test. Resolving this issue is critical for the CULSS EP because clarifying the *Content Uniformity* test in (905) is the starting point for developing a Large N test. Another purpose of this article is to briefly discuss the difference between batch release testing and compliance testing as described in (905). Unfortunately, there is considerable confusion among industry and regulatory agencies regarding the role of USP tests for batch release. USP has clarified that (905) is not intended for batch release and that it is the sponsors’ responsibility to arrange batch release testing to provide reasonable assurance that a released batch will comply with (905) whenever tested (8). The current plan for the CULSS EP, following the planned publication of a chapter containing the Large N test, includes development of a chapter on some approaches to perform batch release testing to ensure

compliance with (905).

THE ISSUE

The harmonized test in (905) is summarized below [assuming a manufacturing target (*T*) of 100, a limit (*L1*) for the acceptance value of 15.0, and a limit (*L2*) for individual doses of 25.0]:

- Collect a sample of 30 dosage units from the batch.
- Assay 10 units.
- Express the individual results as percentage of the label claim.
- Calculate the average (\bar{X}) and standard deviation (*s*) of the 10 results.
- Let $M = 98.5$ if $\bar{X} < 98.5$, $M = 101.5$ if $\bar{X} > 101.5$, and $M = \bar{X}$ otherwise (this is the *IDZ*).
- Calculate the acceptance value (*AV*): $AV = | M - \bar{X} | + 2.4 \cdot s$.
- The sample complies if $AV \leq 15.0$.
- If $AV > 15.0$, assay the remaining 20 units.
- Express the individual results as percentage of the label claim.
- Calculate the average (\bar{X}) and standard deviation (*s*) of the 30 results in the combined sample.
- Let $M = 98.5$ if $\bar{X} < 98.5$, $M = 101.5$ if $\bar{X} > 101.5$, and $M = \bar{X}$ otherwise.
- Calculate $AV = | M - \bar{X} | + 2.0 \cdot s$.
- The sample complies if $AV \leq 15.0$ and all results are within 75.0%–125.0% of *M*.

The operating characteristics (OC) curve for the harmonized pharmacopeial test is shown in *Figure 1*, assuming that the data follow a normal distribution. *Figure 1* shows, for a selection of batch means in the range of 86%–100% label claim (LC), the probability to pass the test in (905) vs. coverage of the interval of 85%–115% LC. This coverage is the true proportion of dosage units in the batch with a content in the range of 85%–115% LC.

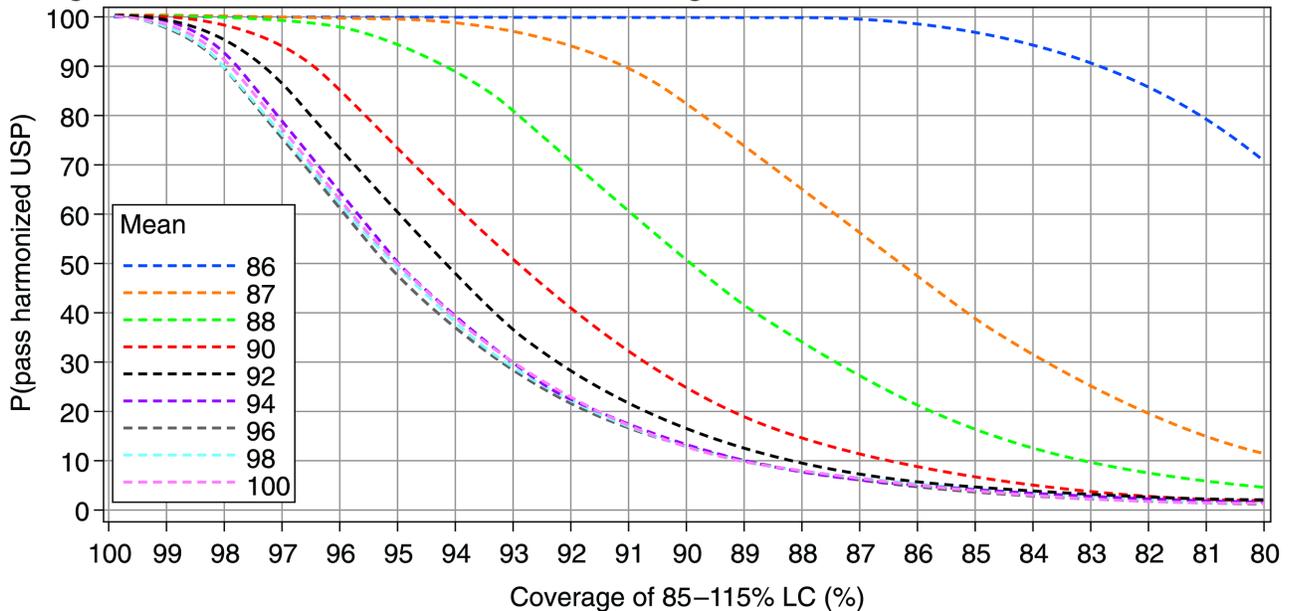


Figure 1. Harmonized *USP* test vs. coverage of 85%–115% LC.

Figure 1 illustrates the issue described by Shen and Tsong (7). As long as the batch mean is in the range of 94%–106% LC, the OC curves are similar (so the chance to pass is essentially independent of batch mean), but outside this range the probability to pass (for a given

coverage) increases with an increasing mean deviation from the target. This bias clearly appears to be an undesirable property of the test in (905).

Figure 1 shows that for a fixed coverage, the probability to pass increases with decreasing batch mean. To keep the coverage constant when the mean deviation from the target increases, the associated standard deviation must decrease. This is illustrated in Figure 2, which shows four normal distributions that all have 91% coverage of 85%–115% LC [a coverage of 91% was chosen for the illustration because this was previously studied by Shen and Tsong (7)]. The difference between the distributions is the mean; this has been set to 86%, 90%, 95%, and 100% LC in this graph. Consequently, to keep the coverage constant, the standard deviation has been modified as appropriate.

Figure 2 introduces the question of whether equal coverage directly corresponds to equal quality. If one requires that the probability to comply with the (905) test should be constant for a given coverage, regardless of the batch mean, one has implicitly decided that the four distributions in Figure 2 represent equal quality. But is this reasonable? One can argue that the batch with a mean of 100% LC is better, because patients receiving medication from this batch would more often receive the intended dose. It can also be argued that the batch with a mean of 86% LC is better, because this is much more uniform, meaning that patients will obtain a more precise dose. What is best is thus a matter of opinion on how best to balance mean and standard deviation. For this reason, it is not at all obvious that the OC curves for different batch means in the (905) test should be required to overlap; this would be a reasonable requirement only if it was agreed that the four distributions in Figure 2 are equally good.

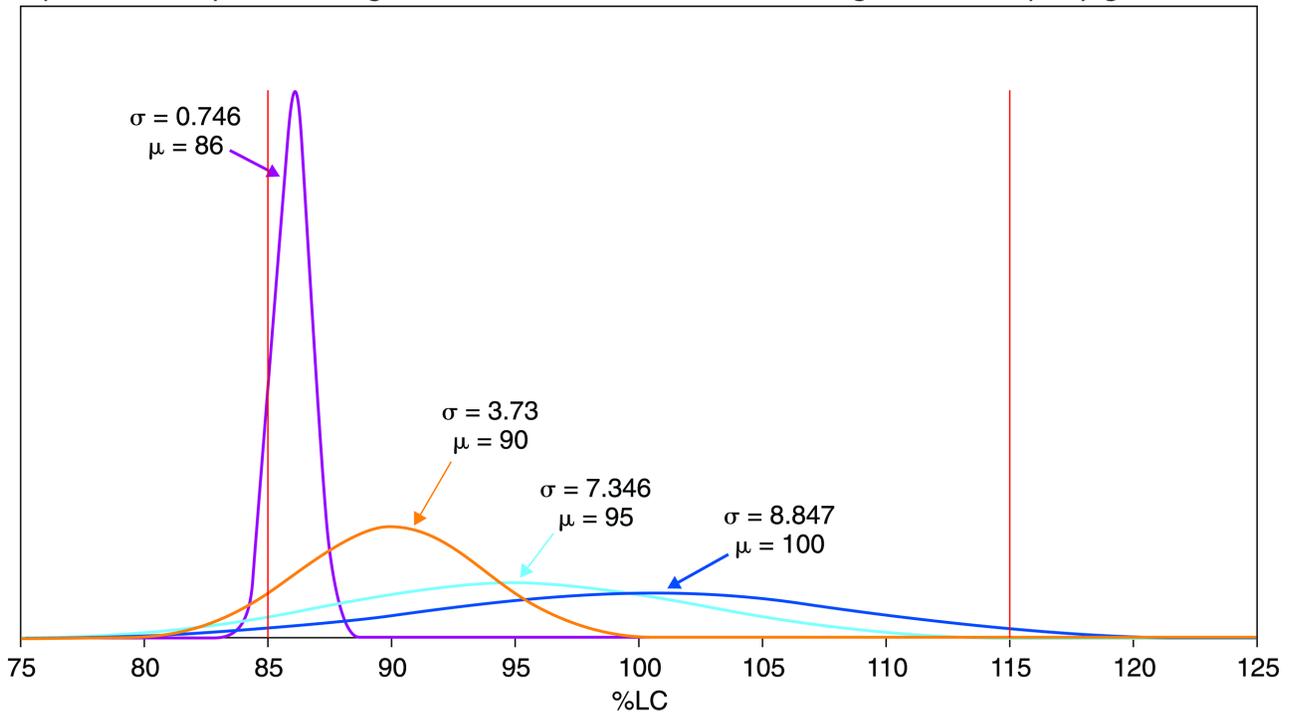


Figure 2. Normal distributions with means of 86%, 90%, 95%, and 100% LC, all with 91% coverage of 85%–115% LC.

Another approach that puts the bias into perspective is illustrated in Figure 3. This figure shows the same data as in Figure 1, but here the probability to pass the harmonized (905) is displayed against batch standard deviation.

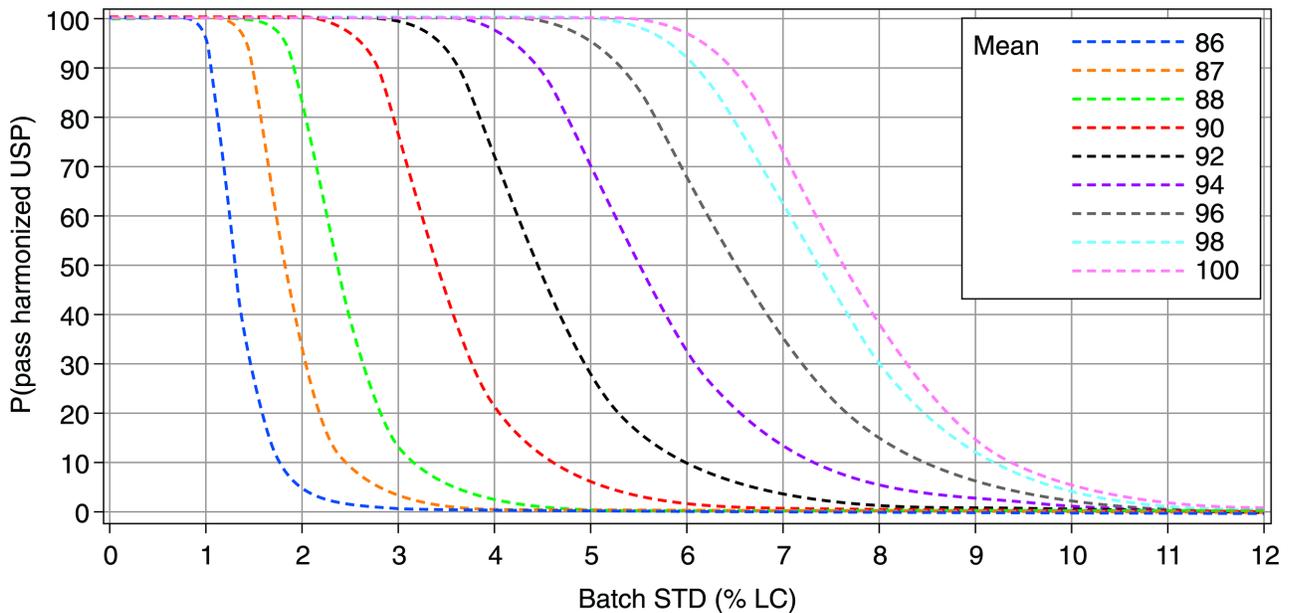


Figure 3. Harmonized USP test vs. batch standard deviation (% LC).

Figure 3 indicates that the issue with the bias observed in Figure 1 might not be a concern because the bias effect is no longer seen. Also, extremely small standard deviations are required to “allow” an off-target batch to comply with (905). Figure 3 shows, for example, that if the batch mean is 86% LC, the standard deviation must be <1% LC for the probability to pass to exceed 95%.

For completeness, Figure 4 shows the same data once more, now displaying batch mean on the x-axis and showing the probability to pass the harmonized (905) for different standard deviations (STD).

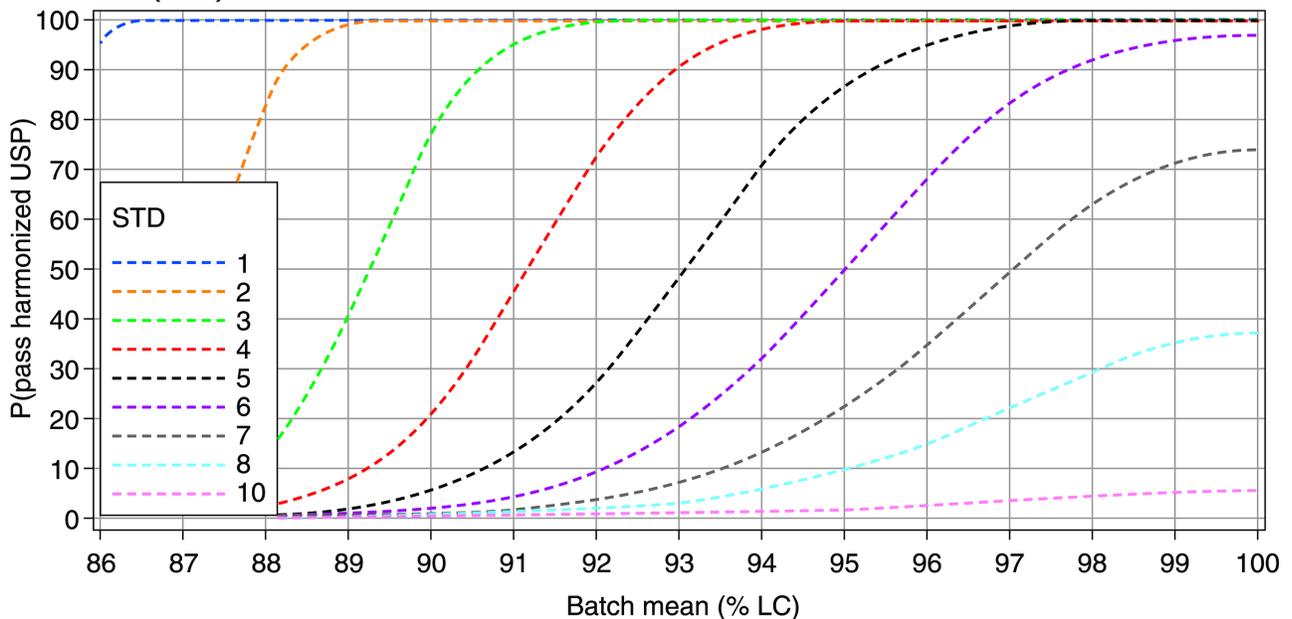


Figure 4. Harmonized USP test vs. batch mean (% LC).

A final approach to demonstrate the uniformity required is the following: for each mean in the range of 86%–100% LC, the standard deviations corresponding to 50%, 75%, 80%, 85%, 90%,

95%, 97.5%, 99%, and 99.9% probability to pass (905) were determined. The results from this exercise are shown in *Figure 5*.

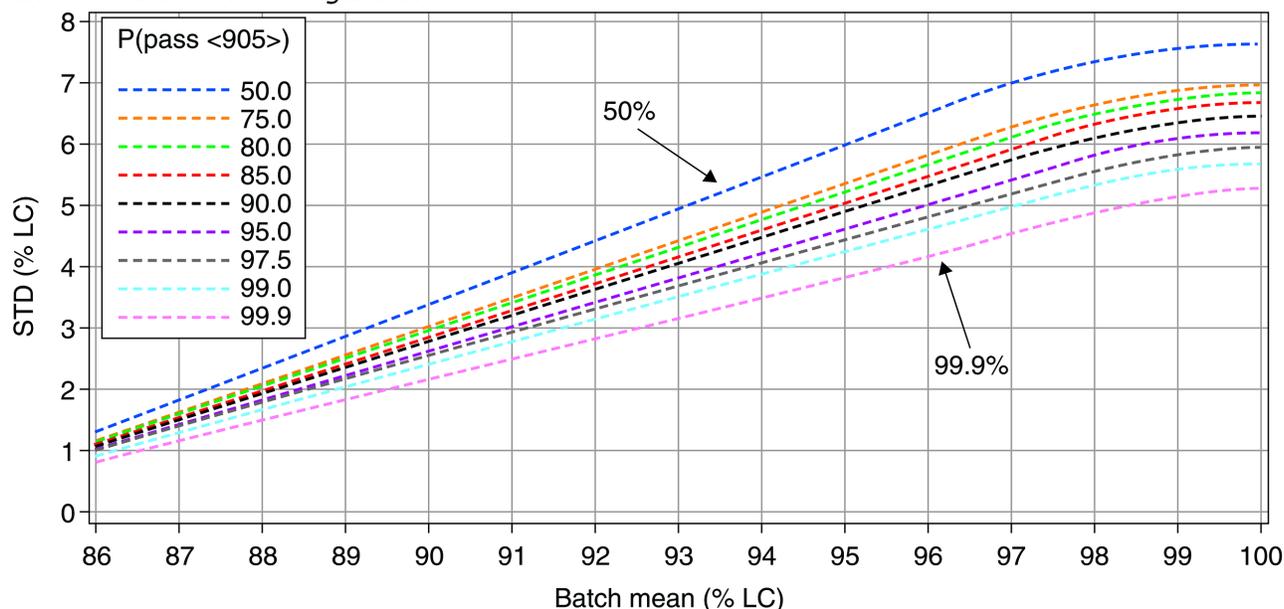


Figure 5. Standard deviation (% LC) corresponding to different probabilities to pass (905) vs. batch mean (% LC).

Figure 5 illustrates the change in standard deviation required to increase the probability to pass the uniformity test in (905) from 50% to 99.9%. Furthermore, *Figure 5* demonstrates that the change in standard deviation required varies with batch mean; when the batch mean is on target, the standard deviation range is about 5.2%–7.6% LC, whereas when off-target the range is much narrower. For an extreme batch mean of 86% of target, *Figure 5* illustrates that a marginal change in standard deviation from 0.9% to 1.2% LC would reduce the probability to pass from 99.9% to 50%.

In summary, this section has shown that although the harmonized (905) exhibits some sort of bias, it appears to have minor practical influence in that batches that would benefit from the bias require such a small standard deviation that they are unlikely to exist. Moreover, whether the bias is indeed a bias is a matter of opinion, depending on how one prefers to balance an on-target mean and low variability. This bias is reviewed below in further detail, along with possible approaches to remove the questioned characteristics of (905).

HISTORY OF (905)

This section provides a brief historical review of how the USP criterion for content uniformity has developed over the years.

In *USP 15* (from 1955, page 945) and *USP 16* (from 1960, page 941), there was a section for "Weight Variation" in *Physical Tests*, but this did not include content uniformity. The first *USP* content uniformity chapter appeared in *USP 17* (from 1965), although there were separate headings under *Physical Tests* that included "Content Uniformity" (page 905) and "Weight Variation" (page 926). All measurement of dosage units and criteria were expressed as a percentage of the "average of the tolerances" called AT (i.e., potency specifications).

All versions of the *Content Uniformity* test from *USP 17* to the most recent *USP* have consisted of two stages. Stage 1 consisted of testing 10 dosage units. If all 10 results met the

acceptance criteria, then the sample passed the test for *Content Uniformity*. If the Stage 1 criteria were not met, then an additional 20 dosage units were tested for Stage 2. If the Stage 2 criteria were met, then the sample passes the *Content Uniformity* test; otherwise, it fails. In Stage 1 of *USP 17*, if all results were between 85% and 115% of the *AT*, then the sample passed the content uniformity test. If NMT 1 result fell outside 85%–115% *AT*, then the test proceeded to Stage 2; otherwise, it failed. At Stage 2, if NMT 1 result of all 30 fell outside 85%–115% *AT*, then the sample passed the *Content Uniformity* test; otherwise, it failed. These criteria were subsequently adjusted in *USP 18* and *19*, as follows.

In *USP 18* (from 1970), a new requirement was added to the *Content Uniformity* test that no result could fall outside 75%–125% *AT* in either stage. Additionally, *USP 18* introduced new and different criteria for tablets and capsules. In Stage 1, if NMT 1 result fell outside 85%–115% *AT* for both tablets and capsules, then the sample passed the content uniformity test. For the first 10 dosage units, if NMT 2 results for tablets or 3 results for capsules fell outside 85%–115% *AT*, then the test proceeded to Stage 2; otherwise, it failed. In Stage 2, NMT 2 tablets from both Stages 1 and 2 could fall outside 85%–115% *AT* to pass the *Content Uniformity* test; whereas for capsules, NMT 3 capsules from both Stages 1 and 2 could fall outside 85%–115% *AT* to pass the content uniformity test.

In *USP 19* (from 1975), the number of decimal places in the criteria was changed to the tenths place. *USP 19* also added a correction for *Special Procedures* in the content uniformity requirement to adjust results based on the difference between the weight of the single dosage unit when using the assay vs. using the special procedure, when different analytical methods were used for assay and content uniformity.

In *USP 20* (from 1980), there were separate general chapters for weight variation and content uniformity: *Content Uniformity* (681) and *Weight Variation* (931). Chapter (905) first appeared in *USP 20–NF 15, Addendum to the Third Supplement* (official September 1, 1982). The chapter combined (681) and (931) and allowed uniformity of dosage units to be demonstrated by either weight variation or content uniformity. Weight variation could be applied if the product was a liquid-filled soft capsule or if the product contained 50 mg or more of a single active ingredient comprising 50% or more, by weight, of the dosage-form unit. The *Addendum* changed the expression of results to LC from *AT*, added a coefficient of variation ($CV = 100 \cdot s/\text{mean}$) criterion, and the number of decimal places in the allowable ranges for individual results was changed to a whole number. The test and criteria from the *Addendum* are provided in *Table 1*.

Table 1. Content Uniformity Test/Criteria before Harmonization

Stage	Number Tested	<i>USP 20</i> Content Uniformity Test/Criteria	<i>JP XIII</i> Content Uniformity Test/Criteria	<i>Ph Eur</i> Uniformity of Content of Single-Dose Preparations, Test A
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Stage	Number Tested	USP 20 Content Uniformity Test/Criteria	JP XIII Content Uniformity Test/Criteria	Ph Eur Uniformity of Content of Single-Dose Preparations, Test A
1	10	<p>Express individual results as a percentage of the label claim.</p> <p>Tablets: Pass if all results fall between 85%–115% LC and CV < 6.0%. Go to Stage 2 if NMT 1 result falls outside 85%–115% LC and no result is outside 75%–125% LC. Otherwise, the sample fails content uniformity.</p> <p>Capsules: Pass if NMT 1 result falls outside 85%–115% LC and no result falls outside 75%–125% LC and CV < 6.0%. Go to Stage 2 if 2 or 3 results are outside 85%–115% LC, no result is outside 75%–125% LC, or CV > 6.0%. Otherwise, the sample fails content uniformity.</p>	<p>Express individual results as a percentage of the label claim. Calculate the average (\bar{X}) and standard deviation (s) of the 10 results.</p> $AV = 100 \frac{-\bar{X}}{s} + 2.2 \cdot s$ <p>Pass if $AV \leq 15.0$. If $AV > 15.0$, go to Stage 2.</p>	<p>Express individual results as a percentage of the average. Calculate the average (\bar{X}) of the 10 results. Express individual results as a percentage of the average.</p> <p>Count the number of units outside 85%–115% of the average (n_{15}) and the number of units outside 75%–125% of the average (n_{25}).</p> <p>Pass if $n_{15} = 0$ and $n_{25} = 0$. If $n_{25} = 0$ and $n_{15} = 1$, then go to Stage 2.</p>

Stage	Number Tested	USP 20 Content Uniformity Test/Criteria	JP XIII Content Uniformity Test/Criteria	Ph Eur Uniformity of Content of Single-Dose Preparations, Test A
2	20	Express individual results as a percentage of the label claim. Tablets: Pass if NMT 1 result is outside 85%–115% LC, no result is outside 75%–125% LC, and CV < 7.8%. Otherwise, the sample fails content uniformity. Capsules: Pass if NMT 3 results are outside 85%–115% LC, no result is outside 75%–125% LC, and CV < 7.8%. Otherwise, the sample fails <i>Content Uniformity</i> .	Express individual results as a percentage of the label claim. Calculate the average (\bar{X}) and standard deviation (s) of the 30 results. $AV = 100 - \bar{X} + 1.9 \cdot s$ Pass if $AV \leq 15.0$ and all results are within 75%–125% LC.	Express individual results as a percentage of the average. Calculate the average (\bar{X}) of the 30 results. Express individual results as a percentage of the average. Count the number of units outside 85%–115% of the average and the number of units outside 75%–125% of the average. Pass if $n_{15} \leq 1$ and $n_{25} = 0$.

The *Addendum* also contained criteria for the situation where the average of the potency specifications was 100% LC or less. The test is the same as shown in *Table 1*.

USP 20, Addendum to the Fourth Supplement (page 915) contained criteria for the situation where the average of the potency specifications was >100% LC. In this case, if the average value of the dosage units tested is greater than or equal to the average of the potency limits, then LC in *Table 1* is replaced by LC multiplied by the average of the limits specified in the potency definition. If the average value of the dosage units tested is between 100% LC and the average of the potency limits, then LC in *Table 1* is replaced by the average of the dosage units tested.

USP 20, Fifth Supplement (page 1114) updated (905) to clarify instances of potency specifications >100.0%, by adding “expressed as a percent of label claim” to the average value of the dosage units tested. A limit was added to permit the use of the *Special Procedure* for the ratio of the calculated adjustment to the *Special Procedure* mean. If the ratio is <0.030, then no adjustment is needed.

HARMONIZATION

The current harmonized “uniformity of dosage units” (UDU) test in (905) was developed in an effort to harmonize UDU testing for content and mass/weight variation between the existing (905) UDU test (9) and the corresponding tests in the *Japanese Pharmacopeia* (JP) (10) and *Ph Eur* (11). The *USP*, *JP*, and *Ph Eur* tests that were in effect at the time are described in *Table 1*.

For the functional form of the test, the goal of the harmonization was to retain the *JP* approach to use an AV. The choice of the values of k_1 , k_2 , and the *IDZ* for the mean has been an area

of much discussion and research. The history of the establishment of these values is provided to dispel any confusion on this topic.

As mentioned above, the goal of the harmonized UDU test was to develop a test that was similar to the *USP* and *JP* tests in effect at that time, when the true mean was equal to 100%, and to also provide a test that was approximately a 50/50 compromise from an operating characteristic curve perspective for means differing from 100. The values 96% LC and 92% LC were examined because they were thought to be the maximum range of reasonable means to consider given the typical assay specifications of 90.0%–110.0% LC in the U.S. Initially, the goal was to retain separate criteria for tablets and capsules, but this would inhibit harmonization; therefore, the focus was on the previous *USP* tablet test and the *JP* test. Because the *JP* test defined its own values of k_1 and k_2 and lacked an *IDZ*, an initial examination showed that simply evaluating alternative values of k_1 and k_2 without an *IDZ* would not achieve the desired goals. Therefore, the addition of an *IDZ* was considered. An extensive matrix of possible values of k_1 , k_2 , and *IDZ* were evaluated to identify the combination that achieved the best 50/50 compromise between the previous *USP* tablet test and *JP* test. The initial proposal that best met the desired goals was $k_1 = 2.3$, $k_2 = 2.0$, and *IDZ* = 1.5. After negotiation at an International Conference on Harmonisation (ICH) meeting (Japan, 1999) where separate criteria for capsules ($k_1 = 2.4$ and $k_2 = 1.9$, and *IDZ* = 3.5) were considered, the final parameters of the test were established as they are today: $k_1 = 2.4$, $k_2 = 2.0$, and *IDZ* = 1.5.

The following two graphs (*Figures 6 and 7*) show that the goal of the harmonization process for content uniformity was achieved with the current *USP* test (shown as *Harm UDU* in graphs).

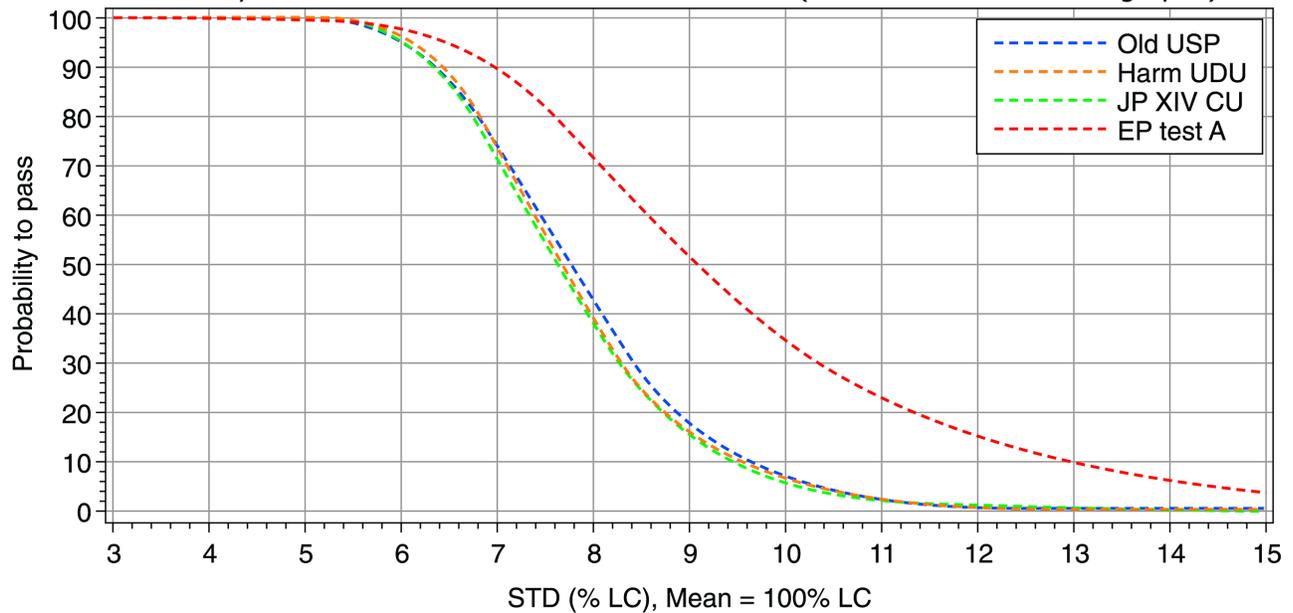


Figure 6. Comparison of harmonized UDU (*Harm UDU*) test and regional tests in effect at the time for harmonization (batch mean at 100% LC).

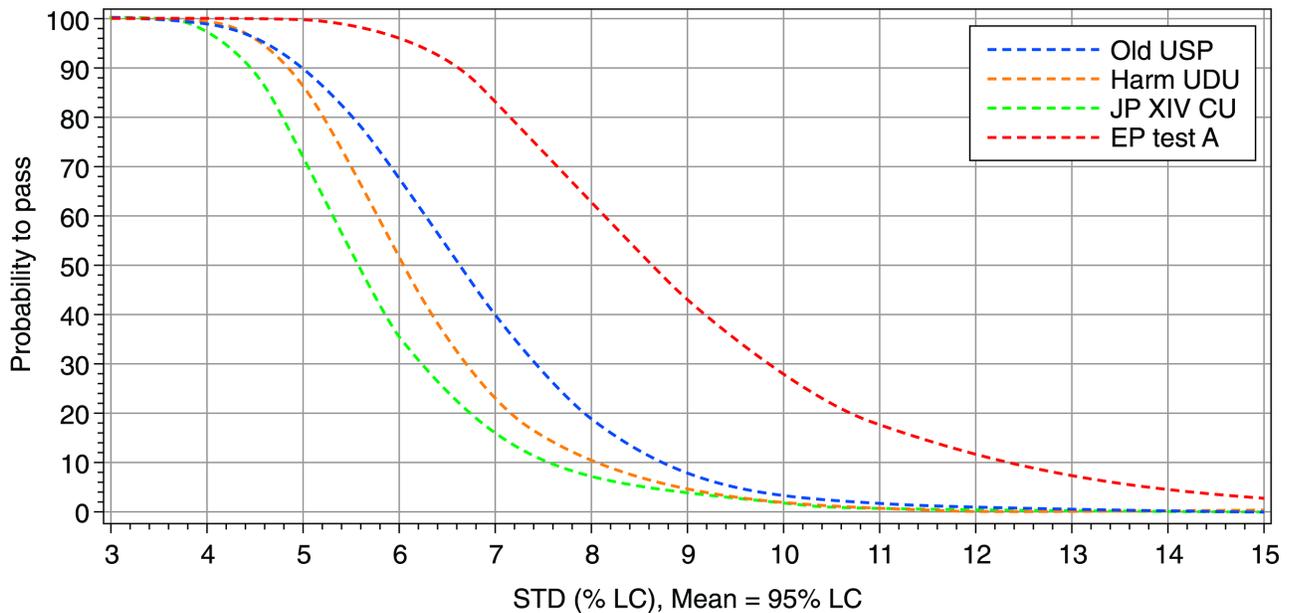


Figure 7. Comparison of harmonized UDU (*Harm UDU*) test and regional tests in effect at the time for harmonization (batch mean at 95% LC).

Figures 6 and 7 show that the harmonized UDU test is almost always tighter than the *Ph Eur* test A, is virtually identical to the old *JP* and *USP* tests when batch mean is on target, and is a reasonable compromise between old *JP* and *USP* tests for off-target batch means.

It is noted that despite the appearance of being tolerance interval factors, the selection of the values of *k1* and *k2* was not to achieve any stated confidence or coverage. As discussed, the parameters of the test were derived to develop a test criteria that was a 50/50 compromise between the old *JP* and *USP* tests. The introduction of the *IDZ* was a key element in achieving this compromise. A removal of the *IDZ* will make the resulting test tighter, therefore inhibiting the original goal of harmonization, unless some modification is introduced to counter this unwanted shift. This issue will be studied in the next section.

INVESTIGATING POTENTIAL SOLUTIONS

Shen and Tsong (7) suggest in their *Stimuli* article that the *IDZ* be removed from (905) to avoid the bias. In Figure 8, the OC curves for (905) with the *IDZ* removed are shown. As seen in the graph, this approach does resolve the bias issue; the OC curves for means $\leq 96\%$ LC overlap, and there is no tendency for a large deviation from the target to be associated with a greater chance to fulfill the testing requirements.

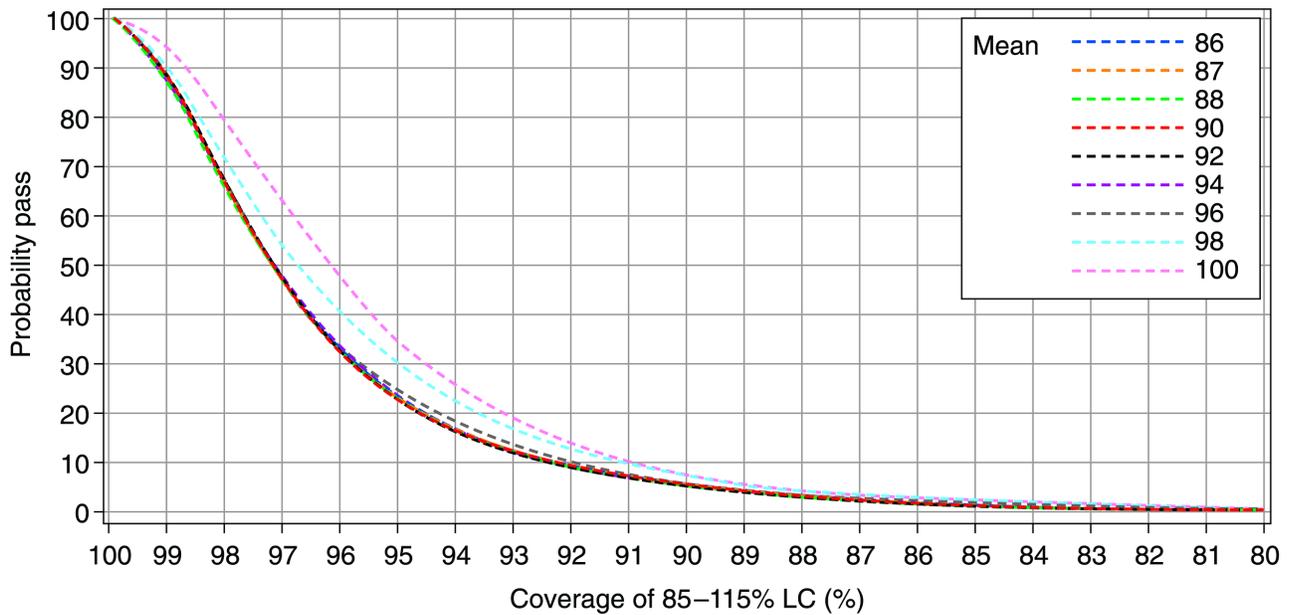


Figure 8. Operating characteristics of the harmonized *USP* test without *IDZ* vs. coverage of 85%–115% LC for different batch means.

As described above, the current (905) was constructed to be a compromise between the old (905) and the *JP* content uniformity test in effect at that time. A natural question is whether this compromise still exists after removal of the *IDZ*. To explore this, *Figure 9* compares the (905) test with the *IDZ* (current *USP*, *solid lines*) to the same test but without the *IDZ* (*dotted lines*). The two tests are compared for means of 100%, 96%, and 92% LC (the same means that were used in the development of the harmonized *USP* test).

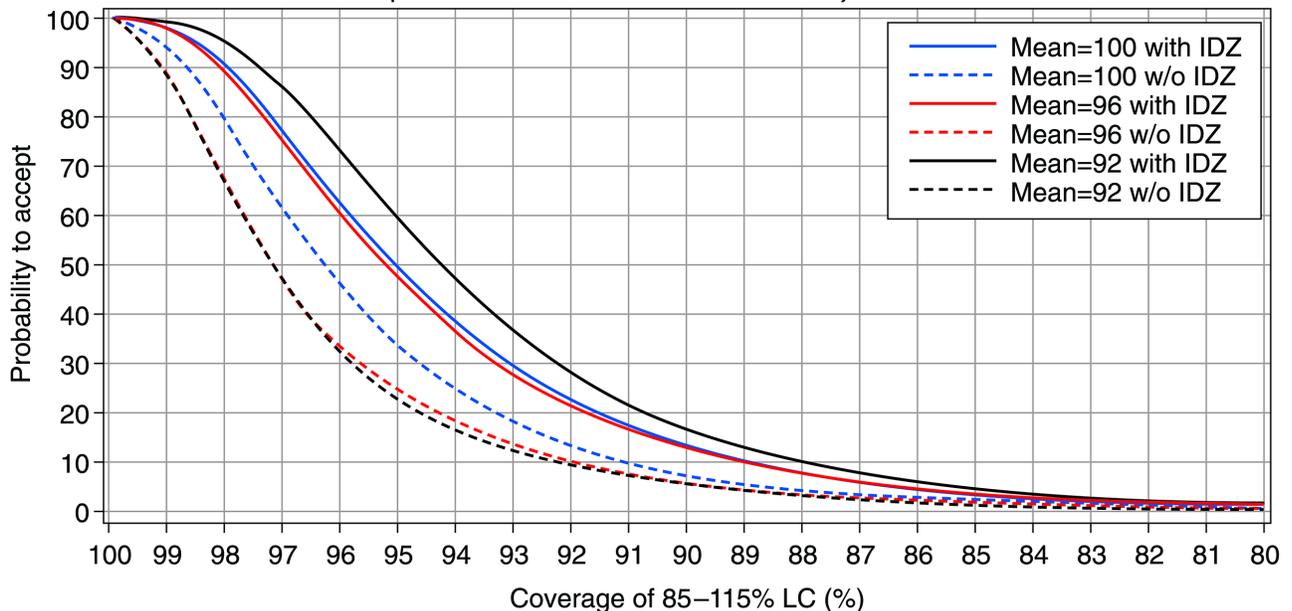


Figure 9. Operating characteristics of harmonized *USP* test with and without (*w/o*) *IDZ* vs. coverage of 85–115% LC for mean = 92%, 96%, and 100% LC.

Figure 9 clearly shows that for all batch means, removing the *IDZ* will lead to a significant tightening of the current requirements. For example, if the true mean is at target, to have 90% probability to fulfill requirements, the required coverage of 85%–115% LC increases from 98.0% to 98.6%. This illustrates that if the *IDZ* is removed, something needs to be changed to retain

the same tightness as that of the current requirement; otherwise, the compromise reached during the harmonization effort would no longer be maintained.

One alternative to avoid the claimed bias while keeping the *IDZ* would be to include some additional requirement. Because the bias appears only when the true batch mean deviates significantly from target, a natural addition would be to add a requirement on the sample average. Indeed, there is already an implicit requirement on the average because the potency is typically required to be within 90%–110% LC. This means that in practice, the test in (905) should be seen in the light of the need to also fulfill the potency requirement. To review the effect of this combined requirement ((905) and potency), *Figure 10* shows the OC curves for the current (905) when a requirement is added that the average be within 90%–110% LC.

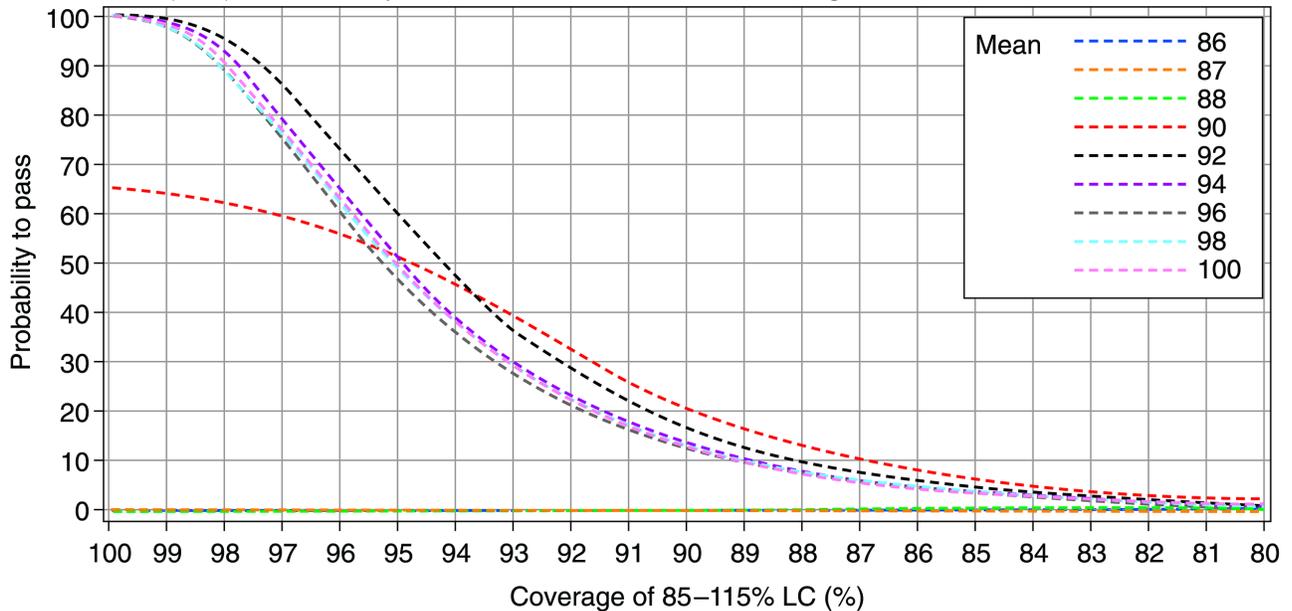


Figure 10. Operating characteristics of the harmonized *USP* test with the additional requirement that the average is within 90–110% LC vs. coverage of 85%–115% LC.

Figure 10 illustrates that that the potency requirement solves the majority of the bias issue (there is still some minor bias for batch means in the range of 90%–92% LC). This analysis explains why the perceived bias has not been an issue in practice; the potency requirement has ensured that the bias has never played a role.

To fully remove the bias, the potency requirement on the average would need to be further tightened. In *Figure 11*, the performance of the current (905) combined with a requirement that the average is within 92.0%–108.0% LC is shown. As seen in *Figure 11*, the additional requirement completely removes the bias because the operating characteristics of the modified *USP* test remain practically unchanged when the batch mean is in the range of 92%–108% LC.

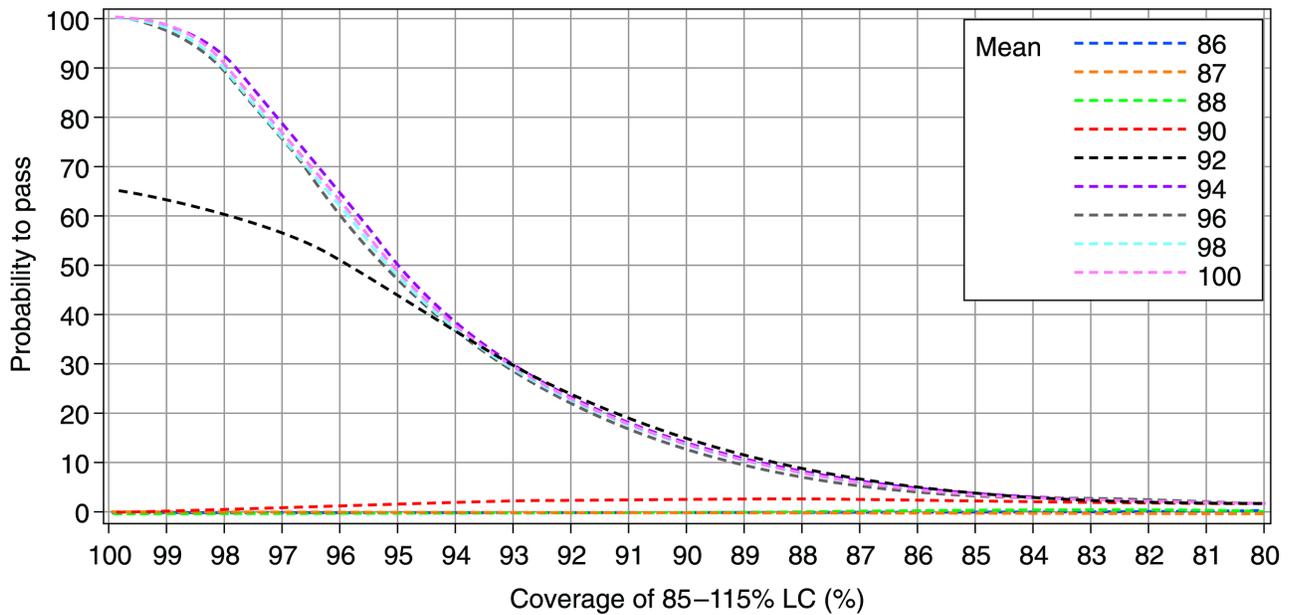


Figure 11. Operating characteristics of a harmonized *USP* test with an additional requirement that the average is within 92-108% LC vs. coverage of 85%-115% LC.

To remove the bias while keeping the *IDZ* in the test, one option is thus to add a requirement for the sample average. Another option is to remove the *IDZ*, but one must compensate for this by changing the values of k_1 and k_2 . In *Figure 12*, the effect of changing the value of k_1 while keeping $k_2 = 2.0$ is shown. In this graph, the mean is at 95% LC, because this is when the (905) test is tightest, both with and without *IDZ* (compare *Figures 1* and *7*). The figure shows the OC curve of the harmonized test in (905) (*solid line*) compared to those of the same test without *IDZ* but with different values of k_1 (*dotted lines*). This graph demonstrates that it is not possible to change only the value k_1 to achieve a match.

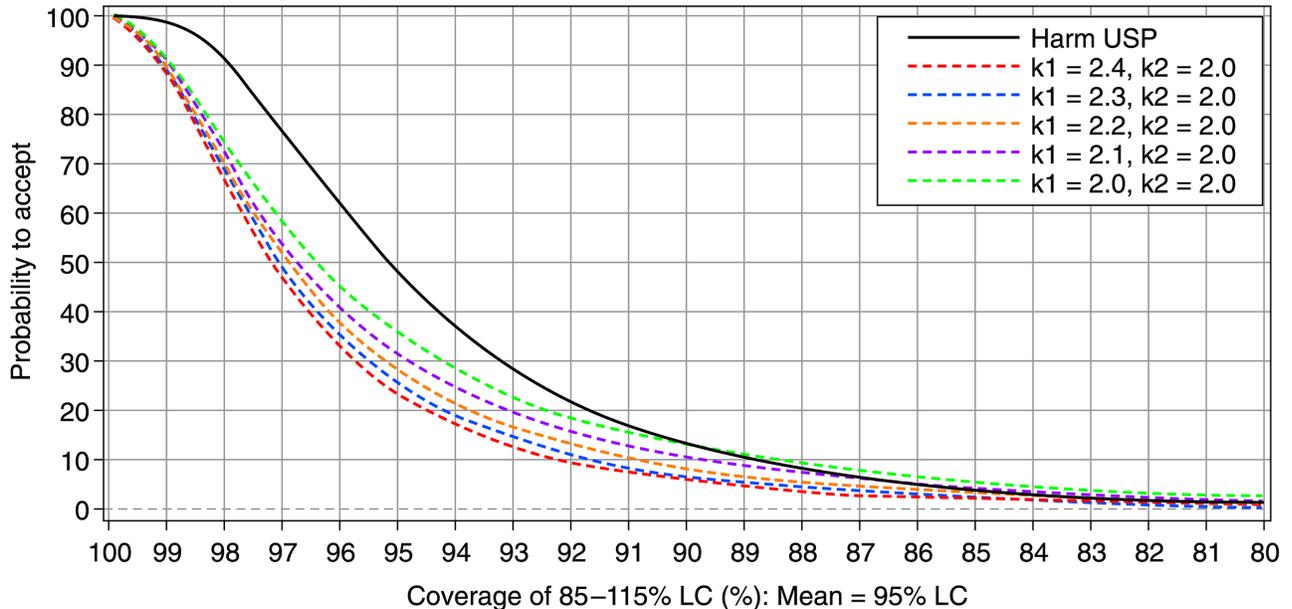


Figure 12. Operating characteristics of a harmonized *USP* (*Harm USP*) test compared to (905) without *IDZ*, $k_2 = 2.0$, and different k_1 's vs. coverage of 85%-115% LC (mean = 95% LC).

In *Figure 13*, the option of changing the value of k_2 while keeping $k_1 = 2.4$ is explored in the same manner as in *Figure 12*. This analysis shows that reducing the value of k_2 to 1.7 results in

an almost perfect match of the test without *IDZ* to the harmonized *USP* test, when the mean is at 95% LC.

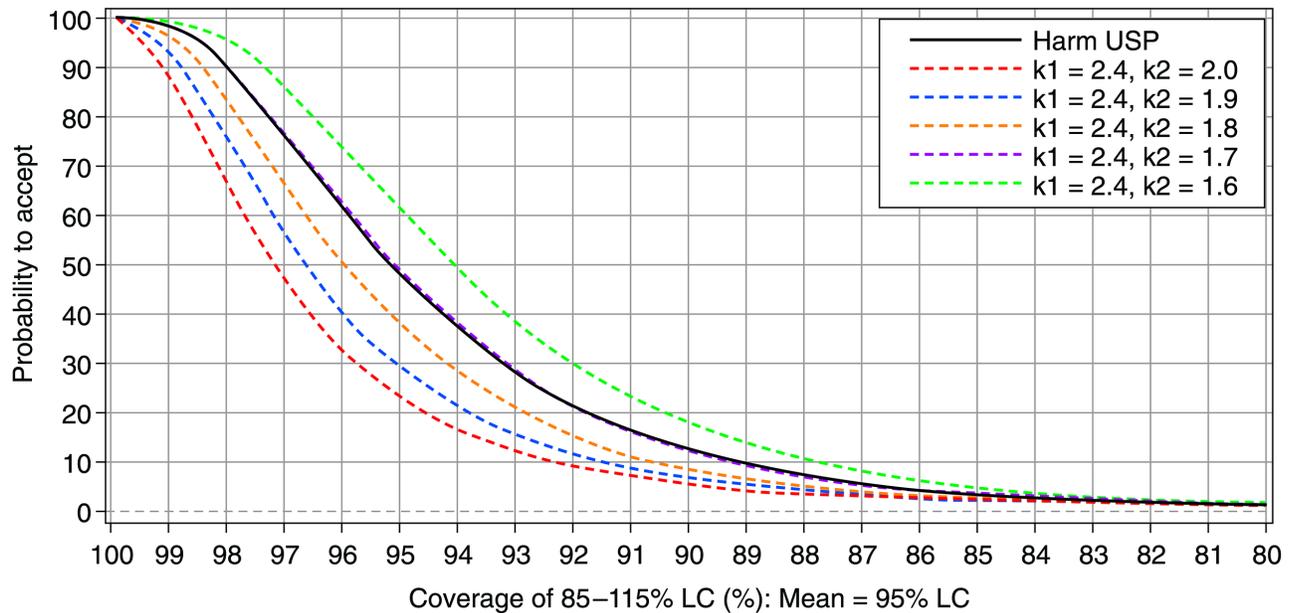


Figure 13. Operating characteristics of the harmonized *USP* test compared to (905) without *IDZ*, $k_1 = 2.4$, and different k_2 's vs. coverage of 85%–115% LC (mean = 95% LC).

Next is explored how this altered test (harmonized *USP* without *IDZ* and $k_2 = 1.7$) with different choices for the value of k_1 compares to the current (905) when the mean is at target; this is shown in *Figure 14*.

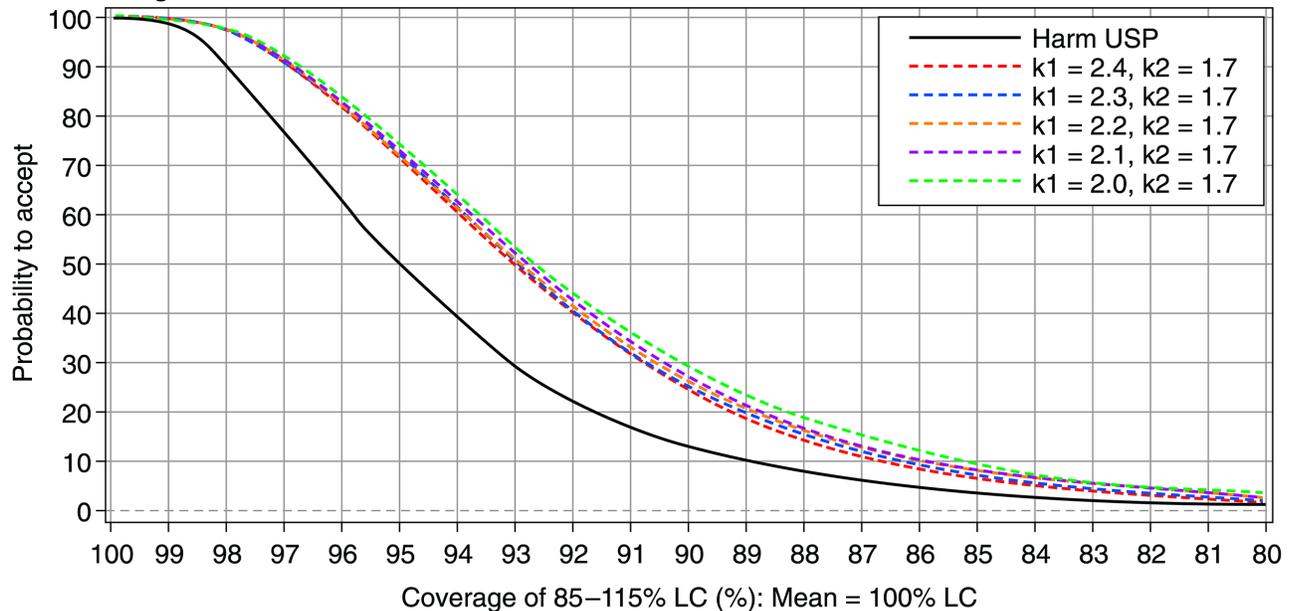


Figure 14. Operating characteristics of the harmonized *USP* (*Harm USP*) test compared to (905) without *IDZ*, $k_2 = 1.7$, and different k_1 's vs. coverage of 85%–115% LC (mean = 100% LC).

Figure 14 clearly shows that the test without *IDZ* is more relaxed than the current test and that no modification of k_1 can solve this. This is a general finding; if we determine k_1 or k_2 for an off-target mean so the test without *IDZ* matches the test with *IDZ*, then the test without *IDZ* will be more relaxed for means that are close to target. Similarly, if we determine a value of

k_1 or k_2 for a match of OC curves when the mean is at target, the test without *IDZ* will be tighter than the current test when the mean is off-target. As discussed above, this is the same challenge that the developers of the harmonized *Content Uniformity* test faced (12–14); their solution was to introduce the *IDZ* and select this, together with the k_1 and k_2 values, to achieve the compromise between the *USP* and *JP* tests.

There are, of course, other alternatives for adjusting the test without an *IDZ* so that its outcome matches the current (905). One such alternative is to let the values of k_1 and k_2 depend on the observed sample average, or to consider another balance between the first- and second-stage sample sizes. Such alternatives suggest that, apart from adding an explicit requirement on the sample average, there is no simple modification of the current (905) that can balance the potential removal of the *IDZ*.

BATCH RELEASE VS. *USP* TESTING

Understanding the purpose of the test is a necessary precondition when evaluating the current harmonized *Content Uniformity* test and any potential modifications. Unfortunately, there is considerable confusion among industry and regulatory agencies regarding the role of *USP* tests for batch release. *USP* is clear that the results of *USP* tests apply only to the sample tested; no inference is intended. Specifically, *USP General Notices Section 3.10 (8)* states, “The similarity to statistical procedures may seem to suggest an intent to make inference to some larger group of units, but in all cases, statements about whether the compendial standard is met apply only to the units tested.” *USP* tests are, therefore, not intended to be informative about the properties of the batch the sample from which it originated. This intention, however, does not conflict with the fact that a sample from the batch is often evaluated against (905) at the time of batch release, because the sample should comply with *USP*. Thus, failure of content uniformity or of any *USP* test at the time of the intended batch release is a warning that needs to be investigated. Importantly, the contrary is not the case—compliance of one sample from the batch with (905) is not typically sufficient evidence to conclude that the batch properties are sufficient to control the risk of future samples not complying with *USP*.

Another important aspect of *USP* tests is that any sample, if within its stated shelf life and if stored properly, should meet all *USP* requirements for that product when tested. Although it is not realistic to expect all possible samples of size 10–30 (typical pharmacopeial sample sizes) from a batch that may contain millions of units to meet the pharmacopeial requirements, the probability of failing is a business risk to be controlled. Failure raises the issue of adulteration or misbranding under the Federal Food, Drug, and Cosmetic Act.

The relationship of *USP* tests to batch release tests is now clear. It is the responsibility of the manufacturer to determine how to assess a batch so that, if released, it has a sufficiently high probability of passing the *USP* tests when tested, where “sufficiently high” is then a business decision that must be made. For content uniformity, the manufacturer may accomplish this with a content uniformity test that includes a sample size appropriate for this purpose (15,16), or they may use process analytical technologies or other approaches without a specific content uniformity test at release.

The use of OC curves to evaluate *USP* tests in this paper and by others is useful as demonstrated above, although no inference is intended. The OC curves are from the company perspective: for batches of given characteristics, what is the risk of samples from those batches failing the *USP* test?

One consequence of this relationship is that the pharmacopeial test will have less stringent acceptance criteria than a properly designed batch release test. Complaints that the

pharmacopeial test is not stringent enough may be due to falsely considering the *USP* test as a batch release test. One of the authors, when working for *USP*, was often asked what is learned from the batch, based on the pharmacopeial test, reflecting this confusion. It appears that Shen and Tsong (7) also have confused pharmacopeial testing and batch release testing. They note that the *USP* test is "incapable of inferring the characteristics of the population (the lot)". They then propose to replace the test in (905) with a significantly tighter test, such as a parametric two-sided tolerance interval or two parametric one-sided tolerance intervals; use of these tests as a pharmacopeial test is not reasonable for the reason described above. However, using one of these tests, with sample size and tightness determined by the manufacturer, as a batch release test to ensure that the batch complies with the test in (905), whenever tested, could be a reasonable approach.

To assist companies faced with these content uniformity issues, the CULSS EP is planning two additional general chapters. The first will present some methods and acceptance criteria for *USP* testing of content uniformity in sample sizes larger than those specified in (905). The second will address some approaches to setting batch release specifications to ensure a high probability of meeting the (905) uniformity standard when tested.

CONCLUSIONS

As detailed above, the observation from Shen and Tsong (7) that due to the *IDZ* included in *USP* (905), the operating characteristics of the test vary with sample mean and favors off-target lots have been discussed extensively. This claimed bias is only apparent when the probability to pass (905) is displayed vs. the coverage of the batch. After comparing the characteristics of batches with different means but identical coverage, we learn that it is a matter of opinion of which has the better quality. Should one favor good uniformity or being on target, and how should these be balanced? One can conclude that the observed bias can be either good or bad, depending on what is desired.

The harmonization process between the *United States*, *European*, and *Japanese Pharmacopeias* aimed to establish common acceptance criteria for content uniformity testing. This resulted in an effort to find a requirement of the *JP* type (a tolerance interval test) that could provide a 50/50 compromise (regardless of true batch mean) between the *JP* and *USP* content uniformity tests in effect at the time. This turned out to be a challenging exercise that could only be solved by introducing an *IDZ* of appropriate size. As a consequence, it is concluded that the *IDZ* cannot simply be removed without destroying the compromise between the pharmacopeias; we believe that this is not warranted.

The current (905) has been used for several years without the claimed bias being an issue in practice. As explained above, this is shown to be due to the potency requirement which specifies that the mean should be within 90%–110% LC; this implicitly ensures that significantly off-target batches will not be released. It is, however, somewhat unattractive to ensure content uniformity by two independent pharmacopeial requirements. Perhaps consideration could be given toward adding a requirement on the sample average to the current test in (905). It was shown above that 92%–108% LC could be suitable, and that this would not tighten the current requirements but remove any possibility that off-target material could be released. Finally, the option to remove the *IDZ* and instead modify the stage *k1* and *k2* values was reviewed. This showed, as was found in the development work during the harmonization process, that although it is simple to find new *k* values to match the OC curve of the current test for a particular batch mean, the resulting test will be tighter or wider than the current standard for other mean values.

In summary:

- One can question if the claimed bias is indeed a bias. The same coverage does not mean equal uniformity. Variability is directly linked to uniformity. What appears to be a bias from a coverage perspective is merely the content uniformity test being true to its character and favoring low variability/better uniformity.
- Because of the potency requirement, the claimed bias is not a problem from a practical point of view; there is no relevant risk that significantly off-target batches will be released.
- If desired, the addition of a 92.0%–108.0% LC acceptance criterion for the sample average to the test in (905) will explicitly ensure that the bias is removed.
- Removal of the *IDZ* from the (905) test will result in a tighter requirement, and some modification of the test will be required to retain its desired properties; this is not simply a task of modifying the *k* values.
- It is important to retain the harmonization of content uniformity testing between pharmacopeias.
- It does not appear that there are any significant reasons to modify the current harmonized (905) test.

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¹ This article represents only the authors' opinions and not necessarily those of their employers.

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STIMULI TO THE REVISION PROCESS

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USP Responses to Comments on *Stimuli* Article *Proposed New USP General Information Chapter: Shear Cell Methodology for Powder Flow Testing* (1063)

Bruno Hancock,^a David Goldfarb,^a Steve Hoag,^a Geoff Zhang,^a Jim Prescott,^b Andres Orlando,^b Galina Holloway^c

ABSTRACT In *Pharmaceutical Forum* 39(3) [May–Jun 2013], the General Chapters—Physical Analysis Expert Committee of the 2010–2015 United States Pharmacopeia (USP) revision cycle and USP staff published a *Stimuli* article (1) describing the use of shear cells for testing the powder flow properties of selected pharmaceutical ingredients. The article expanded on the brief description of the shear cell apparatus, which is used in one of the four methods listed in *Powder Flow* (1174), and laid the foundation for a proposed new general chapter, *Shear Cell Methodology for Powder Flow Testing* (1063). The authors focused on the three most popular shear cell types: translational (Jenike type); annular (Schulze type); and rotational (Peschl type). The ultimate objective was to identify and build up areas of consensus that could be included in the general chapter. Following publication of the original *Stimuli* article, three commenters submitted their comments. This *Stimuli* article responds to these public comments.

INTRODUCTION

In 2013, the General Chapters—Physical Analysis Expert Committee of the 2010–2015 United States Pharmacopeia (USP) revision cycle and USP staff published a *Stimuli* article titled “*Proposed New USP General Information Chapter: Shear Cell Methodology for Powder Flow Testing* (1063)” (1). Comments about the article were received from three commenters during the public comment period. The Expert Committee and USP staff have considered these comments and have responded by way of this article.

The original *Stimuli* article was seeking specific input from users in the pharmaceutical industry regarding the following questions:

1. Would identification of a pharmaceutical reference material be of benefit to shear cell users in the industry? If so, what material should that be?
2. Would a “round-robin” test of standard pharmaceutical materials using different shear cells under various specified conditions be of value to the industry? If so, specifically what value would be provided and which materials should be tested and why?

On the basis of comments received and after much discussion, the Expert Committee concluded that the certified reference material, BCR-116 limestone, should suffice, even though the use of a less cohesive and more pharmaceutically relevant material (such as microcrystalline cellulose) as a reference material was suggested. The BCR limestone is relatively cohesive and is not representative of more free-flowing powders; however, shear cell analysis is most often needed when characterizing the flow of poorly flowing (cohesive) materials. In addition, the BCR limestone is widely available at modest cost and it is a well-controlled material that has been established as a worldwide standard for since 1992. Finally, there are other examples of nonpharmaceutical materials being used as standards for physical measurements, such as

polystyrene beads for particle sizing measurements.

Representative data for several pharmaceutical powders (including microcrystalline cellulose) have now been added to the proposed General Chapter.

The overall consensus in the comments and further discussion with the Expert Committee was that several round-robin studies have already been performed (2–4), and little value would be added by doing another similar study.

COMMENTS

Comment summary #1:

The commenter noted that the shear cell method is currently mentioned in a general overview chapter in each of three pharmacopeias: *Powder Flow* (1174) (USP); 2.9.36. *Powder Flow* (European Pharmacopoeia); and *Powder Flow* (Japanese Pharmacopoeia), without any technical details or guidance for the application. The commenter recommended that a more detailed chapter about flowability testing using a ring shear tester would be highly appreciated. But even more importantly, it would be valuable to have such a chapter under the umbrella of pharmacopeial harmonization.

Response: USP plans to share the proposed general chapter with the other pharmacopeias, and will ask them to evaluate whether a harmonized chapter would be of value.

Comment summary #2:

The commenter had a concern that the *Stimuli* article abstract suggested that (1063) is to be included in *Excipient Performance* (1059), which primarily deals with excipients. Because the commenter mentioned not being familiar with how the chapters are used, is there a possibility that this test method, or even other test methods, are not applied to active pharmaceutical ingredients or formulations by the end users, due to the perception generated by where the information resides?

Response: The general chapter will not be restricted to use with excipients and is intended to be applicable to all powder materials (including formulated products, excipients, and drug substances).

Comment summary #3:

In the interest of objectivity and full disclosure, the commenter suggested disclosing that Jenike & Johanson, Inc., by whom one of the Physical Analysis Expert Committee members is employed, is the exclusive distributor in the United States for the Schulze annular ring shear cell. In addition, the commenter recommended that as many as possible of the manufacturers of shear cells are included in the review and discussion around this *Stimuli* article, to ensure that the descriptions relating to their specific equipment and preferred testing protocols are correctly represented.

Response: Comments not incorporated. Where a *Stimuli* article is authored by a USP Expert Committee, it is not USP's practice to list all affiliations of the Expert Committee members, who are required to abide by strict conflict of interest rules in order to serve on an Expert Committee. During the drafting of the general chapter, input was sought from a variety of subject matter experts including users and those associated with the manufacture and distribution of shear cells. Additionally, comments on the *Stimuli* article have been received from both users and the suppliers of the common types of shear cells described in the general chapter.

Comment summary #4:

The commenter considered the questions regarding the reference material and round-robin testing to be very pertinent. Because there is, within the ASTM, an increased interest in such validations, the commenter recommended liaising with them to discuss options.

Response: Please see the recommendation of the authors with respect to the development of a pharmaceutical reference standard and a round-robin testing program.

Comment summary #5:

In the *Background* section of the *Stimuli* article, the commenter recommended changing "significant progress in data interpretation" to "significant progress in data interpretation and engineering."

Response: Comment not incorporated. The final version of the general chapter does not contain the *Background* section.

Comment summary #6:

In the *Background* section of the *Stimuli* article, the commenter suggested enhancing "quasi-static mode" by including the phrase "transition from static to dynamic flow," or something similar.

Response: Comment not incorporated. The final version of the general chapter does not contain the *Background* section. However, the Expert Committee also concluded that the original text was sufficiently clear to the majority of readers.

Comment summary #7:

In the *Background* section of the *Stimuli* article, the commenter agreed with the statement, "A properly designed and operated shear cell has the advantage of providing device-independent results," and suggested including several qualifications, especially relating to the relative size of the particles (and the proportion of large particles) with respect to the dimensions of the shear cell.

Response: Comment not incorporated. The final version of the general chapter does not contain the *Background* section. However, the Expert Committee included the suggested qualifications in the *Sample Preparation* section of the general chapter.

Comment summary #8:

In the *Background* section of the *Stimuli* article, the commenter recommended that it should also be noted that the use of shear data for evaluating processing behavior in unit operations other than silos/hoppers is not widespread as, in general, the stress regimes employed by a shear cell do not coincide with those in the majority of powder processing environments, as duly noted in (1174).

Response: Comment not incorporated. The final version of the general chapter does not contain the *Background* section. The Expert Committee considered further discussion of the potential uses or limitations of shear cell data to be beyond the scope of the general chapter, which focuses on best practices for instrument operation.

Comment summary #9:

In the *Scope* section, the commenter suggested making it clear that the angle of internal friction, unconfined yield strength, and cohesion are also derived parameters, based on results derived from a mathematical line fitting exercise that was performed to fit the shear stress/normal stress data pairs as a function of a specific treatment of the pre-shear data.

Thus, to suggest that these are not derived parameters is incorrect.

Response: Comment incorporated. The word "derived" has been replaced with the word "related" to avoid any further misunderstandings. This text now appears in the *Introduction* section of the general chapter.

Comment summary #10:

In the *Theory and Principles* section, the commenter recommended ending the sentence, "The yield locus for a given powder..." with the caveat "...amongst others," as there are other powder properties that could affect the flow behavior, such as electrostatic charging.

Response: Comment not incorporated. The original sentence is retained as it clearly states that the factors listed are only examples, and it is not intended to be an exhaustive listing.

Comment summary #11:

In the *Theory and Principles* section, the commenter suggested modifying the sentence beginning, "Hence, determining the yield locus..." as it is only the quasi static (static to dynamic transition) that it is representative of, and other states are not represented by shear tests, such as behavior in mixers or filling of dies, for example.

Response: Comment not incorporated. The original sentence is retained as it clearly states that any measurements should be made under conditions that are representative of the manufacturing process that is of interest. Further discussion of the potential uses or limitations of shear cell data have not been included, as they are beyond the scope of <1063>, which focuses on best practices for instrument operation.

Comment summary #12:

In the *Theory and Principles* section, the commenter recommended describing the consistency of the powder bed very carefully. Consistent bulk density will only be achieved in the most precisely prepared bed by using multiple compression steps. The consistency of the shear zone is achieved by pre-shearing, and there should not be any confusion about this.

Response: This point is clearly stated in <1063> as follows, "...preparation of a uniform powder bed...is the first critical step of shear cell testing." The uniform density is achieved, not only through proper/consistent initial fill methods, but also as part of preconsolidation (if used) and pre-shear steps. Additional details on how to achieve this will vary from one material to another and between instruments, and are beyond the scope of the *Theory and Principles* section of a general chapter on the technique.

Comment summary #13:

In the *Theory and Principles* section, the commenter suggested that there needs to be a sentence to the effect that "this procedure is repeated several times over a range of stresses to allow the plotting of a yield locus." This new sentence should follow the sentence beginning, "A shear stress is applied..."

Response: Comment incorporated.

Comment summary #14:

Regarding the text in the *Description of Shear Cell Components and Designs* section, the commenter stated, "Whilst the application of a load to the powder will undoubtedly be as uniform as possible for each given design, there is no way of knowing if this is actually the case or if the powder is in a net uniform consolidated state (bulk density) as was mentioned earlier."

Response: The Expert Committee agreed with the commenter's statement and clarified that the text in this section only refers to the uniform application of a normal stress.

Comment summary #15:

The commenter expressed concern that in the *Description of Shear Cell Components and Designs* section, there is no mention of the effect of blade length or other influencing factors [see Schmitt R, Feise H. Influence of tester geometry, speed and procedure on the results from a ring shear tester. *Part Part Syst Charact.* 2004;21(5):403-410].

Response: Comment not incorporated. The general chapter describes the design of three types of shear cells (including baffles and surface features such as blades or fins) in a generic manner. As each brand of shear cell is unique in its design, it is not feasible to describe the impact of minor construction details in a general chapter, even if they may influence the results. Such features can vary from brand to brand, from model to model, and from year to year, and they are the subject of ongoing study and refinement.

Comment summary #16:

In the *Description of Shear Cell Components and Designs* section, with regard to wall friction and infinite travel testers, the commenter would like to see some comment regarding how the powder tracks against the grain of the wall coupon.

Response: Comment not incorporated. In the *Test Procedure* section it states "wall-friction... tests can be conducted with the three types of shear cells described in this chapter as long as directionality (if any) of the wall surface relative to the cell movement is taken into account."

Comment summary #17:

In the *Sample Preparation* section, the commenter suggested mentioning the proportion of larger particles present in the bulk sample, and also said that the increase in bias will have a contributory effect from the reduction in the shear surface/volume with respect to the diameter.

Response: Comment incorporated. A statement has been added noting the importance of the proportion of large particles in the sample.

Comment summary #18:

In the *Sample Preparation* section, the commenter pointed out that the sentence about shear strength of fibrous or flaky solids being due to interlocking is also equally applicable to powders with large (relative to the cell size) particles and more free-flowing powders.

Response: Comment incorporated. This sentence in the *Sample Preparation* section has been modified to include this point.

Comment summary #19:

In the *Sample Preparation* section, the commenter suggested that the sentence about controlling environmental conditions should also include the preparation, as well as the sample handling and storage.

Response: Comment not incorporated. This point is covered by the following sentence: "Powder samples must be handled and tested under conditions that are relevant from a practical standpoint."

Comment summary #20:

In the *Sample Preparation* section, the commenter proposed modifying the statement about

testing some powders immediately to include typical processing storage/wait time during manufacture to best replicate what is going to happen during manufacture.

Response: Comment not incorporated. This point is adequately covered by the existing text that states in several locations that the conditions for sample handling, storage, and testing should be both tightly controlled and relevant from a practical standpoint.

Comment summary #21:

In the *Instrument Preparation* section, the commenter suggested reflecting the following: Although in some instances the effect of strain rate is not believed to be influential (Schmitt and Feise), the instrument must still be properly calibrated to ensure that shearing is undertaken at the required strain rate and that this rate is also constant and invariant (within the applicable tolerances).

Response: Comment incorporated. The following sentence has been added to this section: "The rate of any changes in force and displacement will need to be confirmed as part of this calibration."

Comment summary #22:

In the *Instrument Preparation* section, the commenter noted that the statements about orientation of the wall coupons are extremely difficult to apply to infinite travel testers, as mentioned previously.

Response: Comment not incorporated. A discussion of the pros and cons of using each type of tester for wall friction evaluations is beyond the scope of this general chapter. In general terms, all three types of shear cells can be used for wall friction testing, provided that the coupons are oriented in a manner that is representative of real-life processing conditions.

Comment summary #23:

In the *Instrument Preparation* section, the commenter suggested emphasizing the importance of cleaning certain components of shear cells by revising the sentence to say, "particularly wall coupons."

Response: Comment not incorporated. The importance of cleaning all of the shear cell components is clearly stated in the general chapter text.

Comment summary #24:

In the *Selection of Test Conditions* section, the commenter noted that the testing procedure in the article by Ackers (5) also gives some suggested consolidating/normal stresses to use during testing.

Response: The Expert Committee agreed with the commenter and stated that additional suggestions for the selection of pre-shear stresses can be obtained from several sources in the literature, and the general chapter text allows for this.

Comment summary #25:

In the *Selection of Test Conditions* section, the commenter noted that the discussion of extended hold times is too brief and should be more detailed, as it is currently a confusing way of introducing this concept.

Response: Comment incorporated. Changes have been made to the text in an attempt to improve the clarity of this point.

Comment summary #26:

In the *Selection of Test Conditions* section, the commenter recommended adding a statement that powders with very small unconfined yield strengths may require an expanded range of normal stresses to limit the reliance on extrapolation of the yield locus and to review its linearity. This is equally important as the statement that powders with high unconfined yield strength or internal friction may require a narrower range.

Response: Comment incorporated. The following sentence has been added to address this concern: "The range of normal shear stress levels should be sufficient to allow meaningful fitting and extrapolation of the data to determine the unconfined yield stress and other related parameters."

Comment summary #27:

The commenter commented on the section *Test Procedure* as follows: "Interestingly, many of the shear testers do not record the actual applied normal stress at the point of incipient failure—it is often simply assumed to be the set-point value. In all instances, the applied normal stress should be 'controlled' and recorded. 1. The first point is, due to the nature of powders and commented on earlier, unlikely to be possible. Equally, if the even distribution of powder is 'essential,' how is this achieved? Is it tester specific? 2. This is not strictly accurate as the FT4 Powder Rheometer shear cell also performs a pre-consolidation step. 3. The whole area of pre-shearing is more complex than is mentioned here. It is recommended that this is discussed in more detail with the manufacturers. In this vein, care should also be taken to avoid under-shearing the sample—particularly pertinent for freer flowing materials."

Response: Comment incorporated. The text of the general chapter has been updated to clearly state that the applied normal stress should be both controlled and recorded during the test procedure. Over- or under-consolidation would not result in a proper preshear, although under-consolidation is easily detected because a plateau is not reached, whereas over-consolidation is not easily detectable without breaking from the procedure and intentionally creating a failed test. Because this is more difficult to detect and requires increased skill, the note emphasizing that care must be taken not to over- or under-consolidate the specimen was added to the specific step in the *Test Procedure* section. In addition, the use of a powder preconsolidation step is clarified in the section on instrument operation.

Comment summary #28:

In the *Test Procedure* section, the commenter recommended adding a description of the steps relevant to the operation of a rotational tester. The commenter noted that the four steps listed realistically apply to the translational cell only—there is no mention of re-application of the consolidating stress, intermediate pre-shear and testing at a lower stress, as would be normal practice in an infinite (rotational) tester. The commenter thought that given the fact that the vast majority of shear cells in use are of this variety, this was a serious omission.

Response: Comment incorporated. The text describing the steps has been revised to ensure that the different approaches needed for the different instrument types are clearly described.

Comment summary #29:

In the *Test Procedure* section, the commenter suggested that timed tests (mentioned in the last paragraph of this section) should be incorporated into a separate section about this part of shear testing, to simplify the description.

Response: Comment not incorporated. A detailed description of time-consolidation tests is

beyond the current scope of the general chapter. It may be considered for future inclusion.

Comment summary #30:

In the *Data Analysis and Calculations* section, the commenter requested adding the concept of 'pro-rating.'

Response: Comment incorporated. A brief description of the pro-rating approach has been added.

Comment summary #31:

In the *Data Analysis and Calculations* section, the commenter recommended adding a description of the form of the curve used in the flow function schematic, and a description of what type of curve and what sort of curve-fitting protocol is used in the wall friction schematic that shows a curved wall yield locus.

Response: Comment not incorporated. A specific form or shape (linear, curved) is not assumed for any flow function. The schematic is intended to be illustrative for understanding and not for inferring any particular shape. The experimental data points should all fall on a common curve, but because of normal experimental error, a curve-fitting procedure is usually used to create the yield locus. Common mathematical curve-fitting approaches (for example, regression analysis) are acceptable in the majority of cases. As with all data analysis, the user needs to use their judgment on what degree of fit is acceptable and should be aware of potential curve-fitting problems (such as a negative y-axis intercept in the case of the yield locus). These general principles of "good data analysis" are not specifically described in this general chapter as they may reasonably vary from one occasion to another.

Comment summary #32:

The commenter suggested that the statement about choosing the most appropriate test should appear at the start of any full document, not in the *Data Analysis and Calculations* section.

Response: Comment incorporated. The *Introduction* section has been carefully reviewed and updated to ensure that appropriate guidance on test method selection is included.

Comment summary #33:

In the *Data Analysis and Calculations* section, the commenter requested changing the phrase "...equipment that is holding the powder" to "...equipment that contains..."

Response: Comment not incorporated. However, this phrase has been updated to eliminate the word "holding."

Comment summary #34:

In the *Data Analysis and Calculations* section, the commenter recommended balancing the statement about operator performance with the potential for variability in the test powder, which can vary between tests and can be susceptible to humidity, temperature, electrostatics, and other factors.

Response: Comment incorporated. This point is now explicitly stated in several locations in the updated general chapter.

Comment summary #35:

In the *Reference Materials and Reproducibility* section, the commenter suggested including a note stating that CRM-116 can also be supplied by some of the shear cell manufacturers themselves in a more usable volume than the minimum quantity supplied by the European

Commission.

Response: Comment incorporated.

Comment summary #36:

In the *Reference Materials and Reproducibility* section, the commenter noted that the statement that suggests that pharmaceutical powders can be more cohesive than CRM-116 seems a little odd in isolation. Although this is true in some instances, there are many pharmaceutical powders—particularly some of the more common excipients—that are very much more free flowing than the limestone.

Response: Comment incorporated. This statement has been removed from the revised general chapter text.

Comment summary #37:

In the *Appendix*, the commenter recommended adding units (degrees) for Angle of internal friction.

Response: Comment incorporated.

Comment summary #38:

In the *Appendix*, the commenter suggested including the point that Bulk density may be a function of the sample preparation prior to any test.

Response: Comment incorporated. The revised text states that this property is a function of the sample history and applied normal stress.

Comment summary #39:

In the *Appendix*, the commenter recommended adding units (Pa, kPa) for Cohesion.

Response: Comment incorporated.

Comment summary #40:

In the *Appendix*, the commenter recommended adding units (degrees) for Effective angle of friction.

Response: Comment incorporated.

Comment summary #41:

In the *Appendix*, the commenter recommended adding a symbol (FF) for Flow function.

Response: Comment not incorporated. No symbol is used in the final version of the general chapter for the Flow function term.

Comment summary #42:

In the *Appendix*, the commenter recommended adding the FT4 rotational cell to the *Comments* column for Preconsolidation because it is also a step that is used for the FT4 rotational cell to limit travel of the shear head into the powder and reduce any wall effects.

Response: Comment not incorporated. However the table has been updated to reflect this point; this topic has also been expanded in the *Test Procedure* section.

Comment summary #43:

In the *Appendix*, the commenter recommended adding the FT4 powder rheometer shear cell accessory to the *Comments* column for Rotational shear cell.

Response: Comment incorporated.

Comment summary #44:

In the *Appendix*, the commenter suggested changing the current symbol for Unconfined yield strength to f_c .

Response: Comment incorporated.

Comment summary #45:

In the *Appendix*, the commenter recommended removing the references to Mass flow, Ratholing, and Funnel flow. Although absolutely relevant in the wider context, they are not described in detail in the body of the text and are a distraction and potential source of confusion.

Response: Comment not incorporated. These technical terms are used in the main body of the text, and thus they are defined in the *Appendix* for the reader's benefit.

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4. Berry RJ, Bradley MSA, McGregor RG. Brookfield powder flow tester – Results of round robin tests with CRM-116 limestone powder. *Proc Inst Mech Eng E: J Process Mech Engineering*. First published April 7, 2014, 0954408914525387.
5. Akers, RJ. The certification of a limestone powder for Jenike shear testing CRM 116. Luxembourg: Commission of the European Communities; 1992.

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The Use of Endotoxin as an Analyte in Biopharmaceutical Product Hold-Time Studies

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ABSTRACT

Low lipopolysaccharide recovery (LLR) has been reported in biopharmaceutical formulation matrices containing divalent-cation-chelating buffers and polysorbate. This poor recovery of biological activity as measured by the Bacterial Endotoxins Test (BET) led to questions regarding the utility of the *Bacterial Endotoxins Test* (85) as a robust predictor of pyrogenicity and patient safety. Our data demonstrate that the biological activity of laboratory-prepared endotoxin was fully recovered by *Limulus* amoebocyte lysate (LAL) assays and was pyrogenic in rabbits when spiked into undiluted matrices containing citrate and polysorbate. In sharp contrast, the activities of spikes of bacterial lipopolysaccharide (LPS) into the same poor-recovery conditions were rapidly lost to detection by LAL reagent and by rabbit pyrogen studies. Apparently, endotoxins, which contain LPS and other cell-wall components, have a conformation that allows recovery of activity to be resistant to LLR conditions and yet recoverable by LAL and pyrogen tests, demonstrating that natural endotoxins are a clinically relevant predictor of endotoxin activity, pyrogenicity, and thus, patient safety. These data plus the historical perspective on drugs formulated in chelating buffers and polysorbate support the contention that hold-time studies for parenteral drugs are not necessary because, in the unlikely case of endotoxin contamination, the BET will detect naturally occurring endotoxin through appropriate dilution and end-product testing as required by the compendial *USP-NF*. The data presented herein demonstrate that endotoxins, defined as cell-wall fragments of Gram-negative bacteria (GNB), are preferable to the purified LPS standards when used as analytes for endotoxin hold-time studies in undiluted biologics, should such studies be indicated.

INTRODUCTION

Because of its convenience and reliability, bacterial lipopolysaccharide (LPS) has historically been used for positive controls during the development of testing protocols for the detection of endotoxins in parenterals. The advent and ongoing availability of a global Reference Endotoxin Standard (RSE) enabled uniform sensitivity determination of *Limulus* amoebocyte lysate (LAL) reagents and calibration of secondary LPS standards, known as Control Standard Endotoxins (CSE). The use of these LPS preparations has served the parenteral industry well as primary and secondary calibration standards for suitability (inhibition/enhancements) studies and routine use of the assay. However, LPS is not endotoxin. Rather, it is the active entity in the endotoxin complex that resides within the cell walls of Gram-negative bacteria (GNB). Poor recovery of LPS activity, as measured by the Bacterial Endotoxins Test (BET) was reported in hold-time studies for a group of monoclonal antibodies (mAbs) that were formulated in the presence of divalent-cation-chelating buffers ("chelators") and polysorbate; replacement of magnesium cations did not mitigate this interference (1,2). Chen (2) further reported that a three-rabbit pyrogen test was reactive to a nominal LPS value of 7.5 Endotoxin units (EU)/kg. The term "low endotoxin recovery" (LER) was coined to describe this phenomenon. In contrast,

valid recovery of the LPS was achieved when the same product matrix was diluted in water for BET, according to suitability tests, and subjected to the *USP Bacterial Endotoxins Test* (85) BET.

Inferences were immediately drawn from the initial work of Chen and Vinther (1) that there is a potential public health concern because of the poor recovery of LPS activity, suggesting that the BET assay may not be a good predictor of pyrogenicity in parenteral products because of potential underquantification. As a result, Food and Drug Administration (FDA) reviewers have requested, in communications with parenteral firms, that firms conduct LPS hold-time challenge studies and rabbit pyrogen tests on new biological formulations that might be susceptible to LER conditions.

Notably, a number of investigators, including Chen (2), Bolden et al. (3,4), Platco (5,6), and Dubczak (7) have reported that a valid recovery of activity as measured by BET was achieved when laboratory-prepared endotoxin, rather than the purified LPS, was introduced to the undiluted mAb as the challenge material for the same hold-time studies.

Data presented below indicate that the so-called LER phenomenon is a unique form of BET interference that is caused by an alteration in LPS activity when it is introduced into an undiluted citrate/polysorbate matrix. Data will further show that endotoxin and LPS behave differently in experimental conditions representing this phenomenon. Conclusions drawn from the data suggest that LPS is unsuitable for non-BET hold-time studies where chelator/polysorbate formulae alter the activity of LPS, and that endotoxin prepared in the laboratory is the most appropriate analyte for these studies. Therefore, the purpose of this discussion is to support the proposition that LPS is an inappropriate surrogate for natural endotoxin when demonstrating the stability of assayable endotoxins.

THE BACTERIAL ENDOTOXINS TEST

The *USP* chapter (85) requires the user to demonstrate suitability of the chosen method with the material under test. Suitability is also known as the "test for interfering factors" or inhibition/enhancement testing.

Dilution in water for BET is the easiest and most common way to overcome test interferences. To conduct the compendial suitability study, a known amount of LPS is spiked into a diluted or otherwise prepared sample, not undiluted product, and activity is subsequently recovered from that preparation. As such, compendial suitability studies do not answer questions of: 1) possible inherent ability of the product formulation in its undiluted form to destroy, alter, or mask LPS activity; and 2) co-dilution of LPS with the product. See McCullough (8) for a discussion of suitability testing.

BET Test Interferences

Investigators from both FDA and industry have reported poor recovery of LPS activity in undiluted and inadequately diluted product (9–12). These reports consistently suggest that:

1. Most products, when tested undiluted, exhibit a level of interference with recovery of LPS activity using LAL reagent, and
2. The extent of the interference can be dependent on many product and assay attributes including, but not limited to, product matrix, product batch-to-batch variability, experimental design, data interpretation, vendor-specific lysate formulation, and BET method.

The following factors can significantly affect the ability of the compendial assay to recover LPS in suitability studies (13–15):

- Non-neutral pH range
- Insufficient divalent cations
- Chelating agents
- Surfactants
- Proteins
- Agents that denature proteins
- Container extractables
- Lipids and liposomes
- Metal ions

Each of these known interferences has a mitigation strategy involving dilution in water for BET or chemical intervention.

LPS Aggregation Interference

LPS aggregation is rapidly reduced when introduced into an undiluted matrix containing a chelating agent and a detergent. Data presented below indicate that the so-called LER phenomenon, redefined here as “low LPS recovery” (LLR), is a unique form of BET interference that is caused by LPS changes rather than inhibition of the LAL cascade. LLR relates to an altered state of the LPS analyte that results in diminished LAL activity and rabbit pyrogenicity. This discussion will show that endotoxin and LPS behave differently in an aqueous environment of chelator, detergent, and drug substance.

Tsuchiya (16) recently reviewed the ways that LPS is altered in the presence of dispersing agents. LPS aggregates can be dissociated by the detergent sodium deoxycholate (NaD) to monomers (17). Rabbit pyrogenicity was reduced coincidentally with reductions in LPS aggregation sizes. LPS pyrogenicity was completely lost in 0.5% NaD but was recovered after the NaD concentration was reduced by dilution or dialysis (16,17).

In summary, LPS is an amphipathic molecule that exists in an aggregated form in water (18). The LPS, in natural endotoxin, aggregates and forms micelles differently than those of purified LPS, because LPS in natural endotoxin is embedded in other GNB cell-wall components. Endotoxin micelles are larger and more spherical than LPS structures (19). Presumably, the size, shape, and the presence of other cell-wall components renders them more resistant to LLR conditions.

REDEFINING TERMS RELATED TO ENDOTOXIN AND LPS RECOVERY

Purified LPS does not exist in nature, and therefore cannot be present as a contaminant during parenteral product manufacture or in parenteral drug products. Although RSE and CSE are called “endotoxins”, RSE and CSE are preparations of purified LPS that are the biologically active portion of the natural endotoxin complex, but they are not endotoxins. The compendial RSE is an allocated number of vials of the 3rd International Standard prepared in 2012 for the World Health Organization by the National Institutes for Biological Standards and Control (NISBC) in England. This standard preparation contains highly purified LPS, lactose, and polyethylene glycol (PEG). The intended use of the International Standard is as a calibrant for

(85) (20).

The RSE is used for three purposes in the harmonized pharmacopeial chapter for BET to:

1. Assign a sensitivity to gel clot lysate or to confirm a standard curve range for quantitative BET methods
2. Standardize LPS/CSE secondary calibration standards
3. Execute suitability testing (inhibition/enhancement) as required by *USP* (85). However, LAL users almost always use CSE, a secondary calibration standard, for this purpose to conserve RSE.

The authors recognized the need for more precise definitions of endotoxin-related terms to address LLR issues and for consistency in further public discussions and publications. For clarity, we offer the following endotoxin definitions. We caution the reader on three fronts: 1) the terms "endotoxin", "LPS", and "RSE/CSE" are not interchangeable, because they have specific technical meanings; 2) the terms "activity" and "potency" are not synonymous, because they are defined by different units; and 3) the terms "standardization" and "characterization" are not synonymous.

Bacterial Endotoxins and LPS

The terms endotoxin, LPS, and RSE/CSE are not interchangeable, because they have specific technical meanings. Bacterial endotoxins, often termed "naturally occurring endotoxins" (NOE), are components of the outer cell walls of GNB. Endotoxins exist as vesicles containing the active LPS molecule, outer membrane surface proteins, lipoproteins, and phospholipids. Endotoxins found in pharmaceutical products are cell-wall fragments shed as the result of the normal GNB life cycle. Although control of endotoxins in pharmaceutical manufacturing has improved considerably since the introduction of the BET, whole GNB and/or cell-wall fragments containing endotoxins may enter the manufacturing process via raw materials, particularly those from natural sources, processing equipment that was not cleaned and dried properly, and water. Endotoxins may be prepared in the laboratory from a GNB of known origin under a set of well-documented and well-controlled conditions. For the purposes of this discussion, the terms "endotoxins", "laboratory-derived endotoxins", and "naturally occurring endotoxins" are synonymous, because these are the analytes of clinical relevance, namely pyrogenicity, and LPS refers to the primary and secondary calibration standards.

LPS is the pyrogenic component of the endotoxin molecule. LPS, however, is not endotoxin, because it is only one component of the cell-wall fragment. Given its highly purified status, it is used primarily as a calibrant to standardize LAL reagents and to perform compendial suitability studies.

For the purpose of this article the term, endotoxin refers to laboratory-derived endotoxin, and the term LPS refers to the primary and secondary calibration standards.

Activity, Potency, and Concentration

The terms activity and potency are not synonymous, because they are defined by different units and therefore define different attributes. Historically, the term, activity has been defined as the biological effect of an endotoxin or LPS preparation on the fever response in mammals as

measured by the *Pyrogen Test* (151) (21). Since the advent of the BET, the definition has been expanded to include the effect of LPS or endotoxin on the LAL cascade as measured in any of the (85) assays. The EU is the unit of measure of activity in a BET assay.

The potency, or specific activity, of an LPS preparation is the level of activity per unit weight, usually expressed as EU/ng or EU/pg. Purified LPS preparations from different GNB may have different potencies, but in each case, their presence, after addition to a liquid preparation, is measured in terms of their activity in EU/mL. Determination of the potency of a CSE is conducted by comparing the activity of the primary standard (RSE) in EU/mL with the activity of the CSE in ng/mL. The units for the resulting potency determination for the CSE are EU/ng. Potency and activity are clearly related but are not synonymous. Because laboratory-derived endotoxin preparations are liquid, they are not measured by potency but rather by concentration, which is expressed as activity per unit volume, or EU/mL. The measure of activity for BET assays is EU/mL in a sample preparation. BET assays are not sensitive to the origin of the activity (LPS or endotoxin), and they cannot distinguish between bacterial species or preparations by a level of activity. To the BET assay, 1 EU = 1 EU, regardless of the origin.

Standardization and Characterization

As measured by a BET assay, standardization is a term used to describe the process of comparing the activities of the primary standard in EU/mL, and the secondary standard, in ng/mL is used to assign a relative activity to the secondary standard in EU/mL. Characterization is the assignment of a biological activity concentration value (EU/mL) to a liquid laboratory-derived endotoxin preparation.

REPLICATING LOW-RECOVERY PHENOMENON

LER was reported after experiments that were undertaken to fulfill one firm's broad interpretation of Question 3 of the 2012 FDA BET Guidance, indicating that assayable endotoxin must remain detectable during sample storage and hold times (22). Chen and Vinther (1) reported that when CSE was added to their undiluted mAb product that was formulated in a citrate/polysorbate matrix at a nominal value of 5 EU/mL, approximately 80% of the activity of the LPS spike was not recoverable within the first 4 h of storage at 2–8°. This effect was greater when samples were kept at room temperature, but at either temperature, essentially no LPS activity was recovered at 24 h. Chen (2) further reported that in a three-rabbit test, the rabbits were reactive to a nominal value of 7.5 EU of LPS/kg of the product matrix, whereas the LAL test remained nonreactive (<0.125 EU/mL for gel clot and <0.05 EU/mL for kinetic chromogenic assay).

Because protein solutions formulated in a chelator with a surfactant are common in biotechnology products, a number of industry BET scientists including Jay Bolden (Eli Lilly and Company), Cheryl Platco (Merck Research Laboratories), and John Dubczak (Charles River Laboratories) have independently attempted to replicate the LER response and rabbit pyrogen data originally reported by Chen and Vinther (1,2).

At the 2014 Bacterial Endotoxin Summit (7), Dubczak presented unpublished data on the rapid decay of the RSE in the LER polysorbate 20/citrate matrix. The results shown in *Figure 1* indicate that 80% of the RSE activity is lost in 15 min. Ninety percent of the RSE activity is lost within 30 min. These data demonstrate that, in the absence of proteins, the polysorbate 20/citrate matrix is capable of producing a rapid and extreme loss of LPS activity. Because of

this rapid loss of activity, LER investigational studies using LPS must consider an immediate mitigation/neutralization strategy to obtain a more accurate zero time measurement.

RSE Decay in Tween/Citrate/Saline Matrix

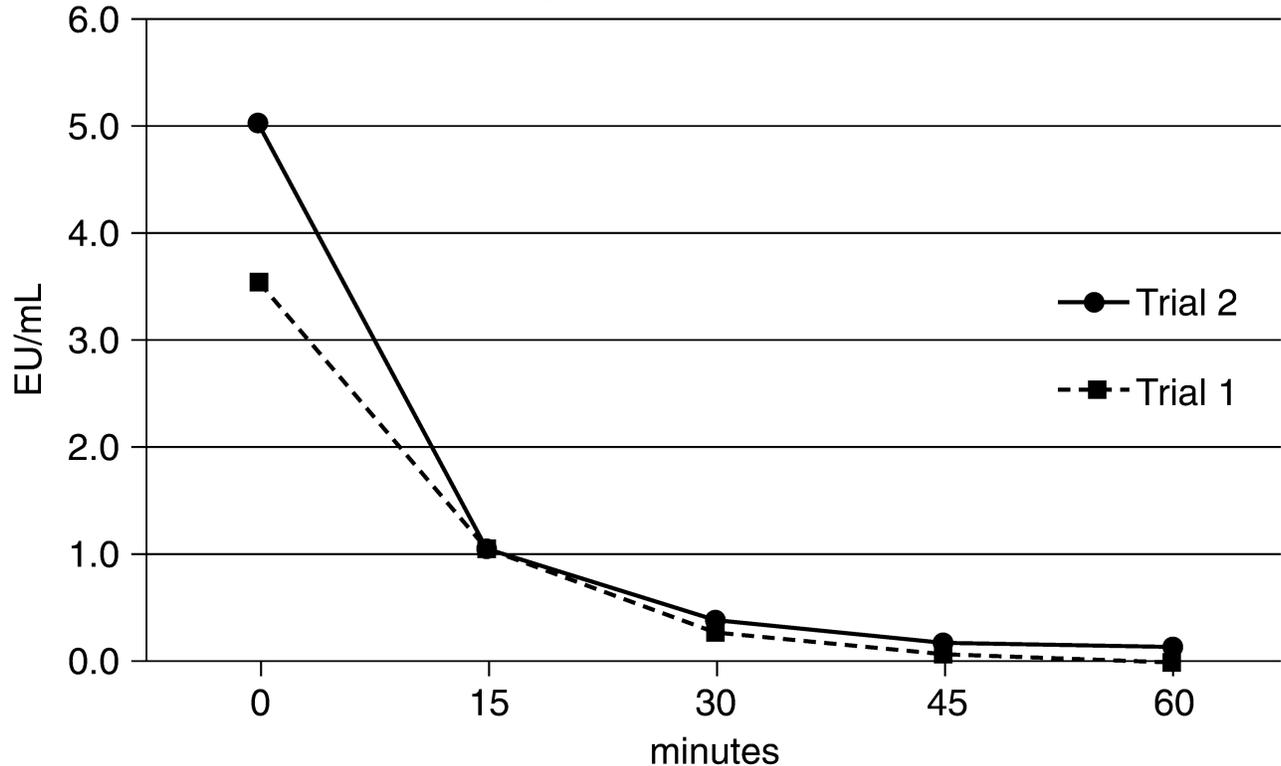


Figure 1. RSE decay in a polysorbate 20/citrate LER matrix.

Bolden et al. (3), Platco (5), and Dubczak (7) have consistently reported that the LER phenomenon is effectively mitigated when endotoxins, prepared from various GNB, were used for the analyte instead of LPS. In 2011, Bowers and Lynn (23) published a method for the laboratory preparation of endotoxin. Dubczak’s and Bolden’s preparation methods were different, but all methods yielded a characterized and stable endotoxin stock preparation for use in hold-time studies.

Figure 2 shows the results of challenge studies conducted by Bolden and colleagues (3) where both laboratory-derived endotoxin and LPS were used to spike approximately 18 EU/mL into three different batches of a low-protein concentration, mAb formulated in a citrate/polysorbate 80 buffer. Samples were stored in the final product glass container at 2–8° (3,4). The *solid lines* represent activity in EU/mL when endotoxin was used as the analyte for the study; the *broken lines* represent activity in EU/mL when LPS (CSE) was used as the analyte. A significant drop in recovery of LPS activity was seen at day 2 and beyond, whereas recoveries of endotoxin activity were approximately 100%.

Recovery of LPS and Endotoxin Activity in a Low Protein Concentration Monoclonal in a Citrate/polysorbate 80 Buffer

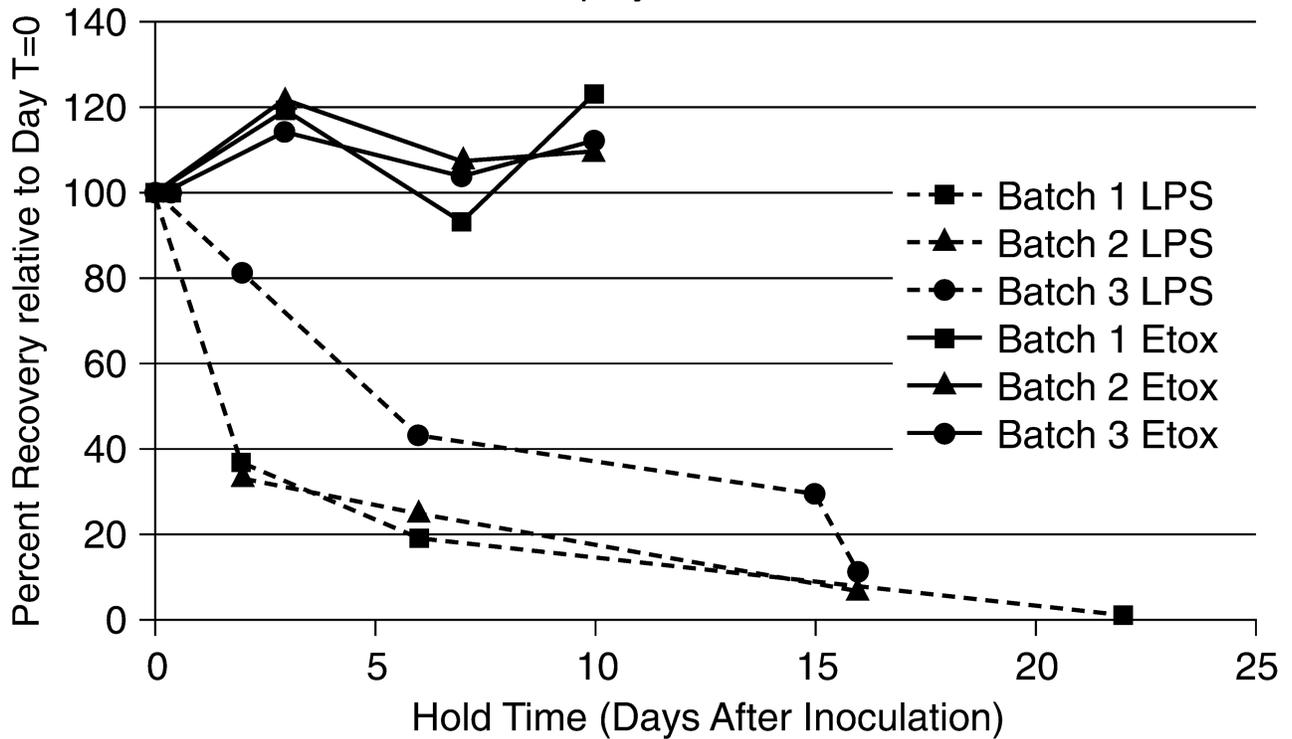


Figure 2. Recovery of LPS and endotoxin activity in challenge studies.

Platco (5,6) also studied the relative recoveries of LPS and endotoxin activity (nominal spike, 7.5 EU/mL) in two different mAb preparations formulated in a citrate/polysorbate buffer system. Spiked solutions were held at 2–8° in borosilicate glass. *Figure 3* presents Platco’s results, where the *solid lines* represent endotoxin recoveries, and the *broken lines* represent LPS recoveries.

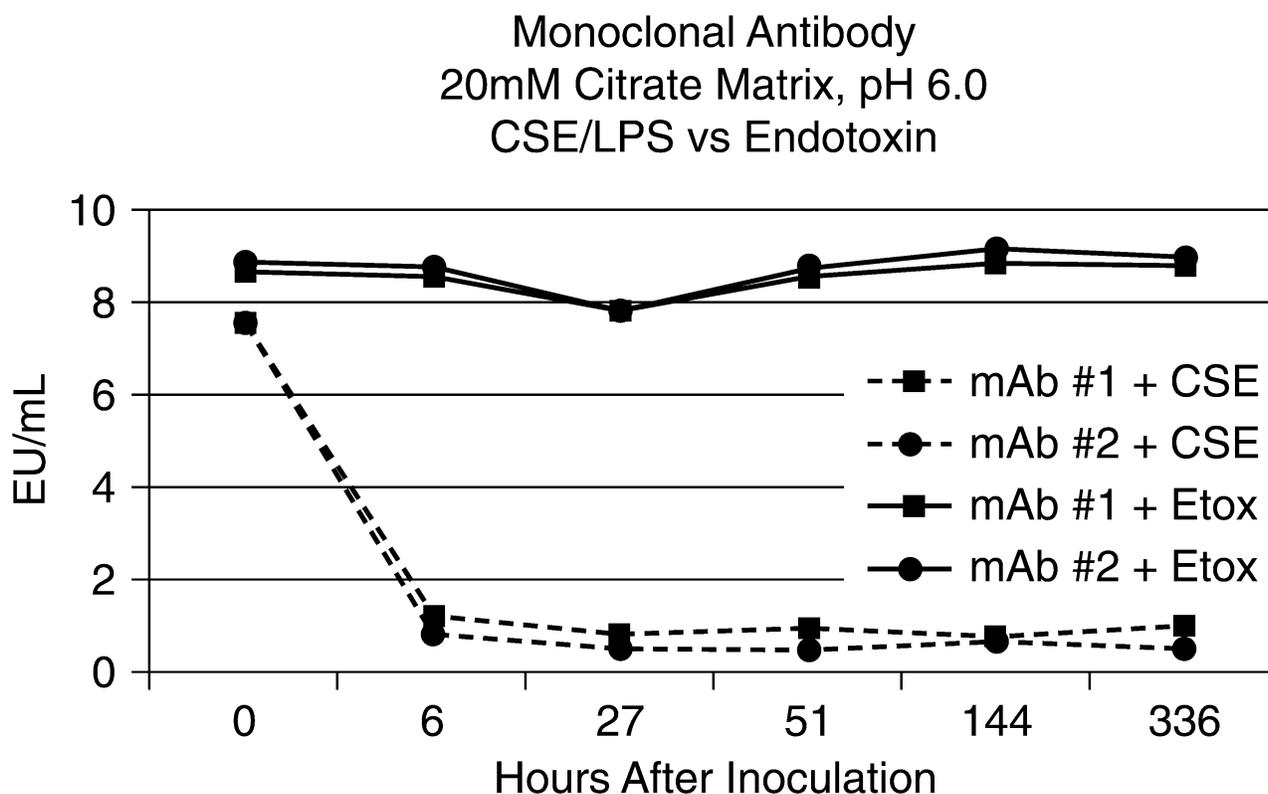


Figure 3. Recovery of LPS and endotoxin activity in two mAb preparations.

Figure 3 reveals a significant and permanent drop in activity at a point between $T = 0$ and $T = 6$ h using LPS as the spike, which is consistent with data reported by Chen and Vinther (1) and Bolden et al. (3,4). However, consistent with Bolden's observations in Figure 1 and other published studies, recoveries of endotoxin activity were repeatedly observed to be approximately 100% for the full 336-h (14-day) term of the challenge study (3-7). Platco (5) reported that a similar phenomenon was observed with other mAb formulations, and that the use of endotoxin as the analyte for these formulations mitigated recovery issues for all other matrices studied.

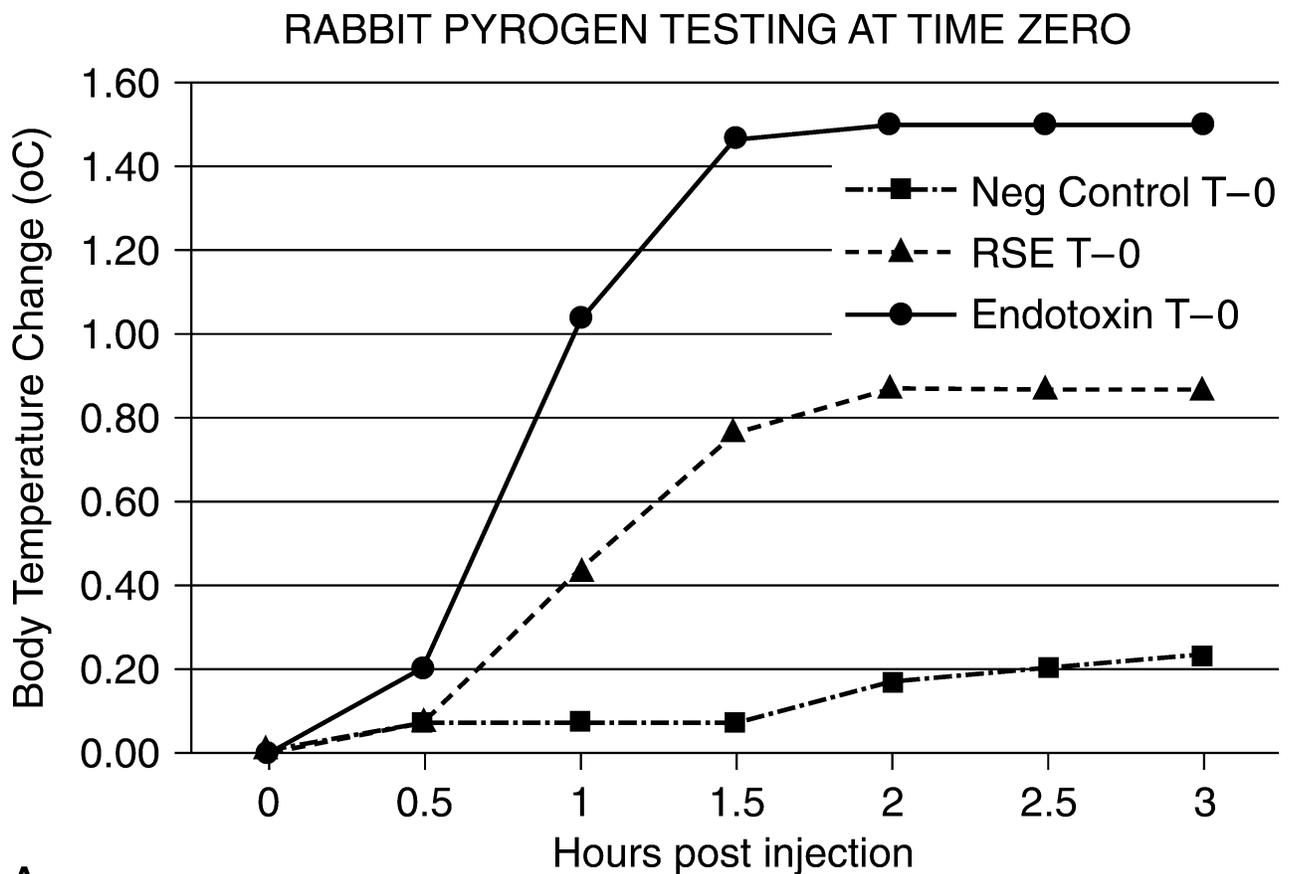
LER and the USP Rabbit Pyrogen Test

Chapter (151) is a compendial test for pyrogens that requires the measurement of rabbit temperature for 3 h after injection with the material under test. The test is initially performed on three rabbits. If no rabbit shows an individual temperature rise of 0.5° or more over baseline, the product meets the requirements for the absence of pyrogens. However, if any rabbit shows an individual temperature rise of 0.5° or more, the test is continued on five more rabbits for a total of eight. This extension is allowed because of the highly variable nature of this biological test system. If NMT three of the eight rabbits show individual rises in temperature of 0.5° or more, and if the sum of the eight-rabbit maximum temperature increases does not exceed 3.3° , the material under test meets the requirements for the absence of pyrogens.

Dubczak (7) extended his experimental work with LPS and endotoxin to compare the relative activities of these preparations to both rabbit and BET tests. Data from these experiments are seen in Figures 4a and 4b. The experimental design for the rabbit/BET comparison was as follows:

1. Polysorbate 20/citrate buffer with no active ingredient was used for all testing.

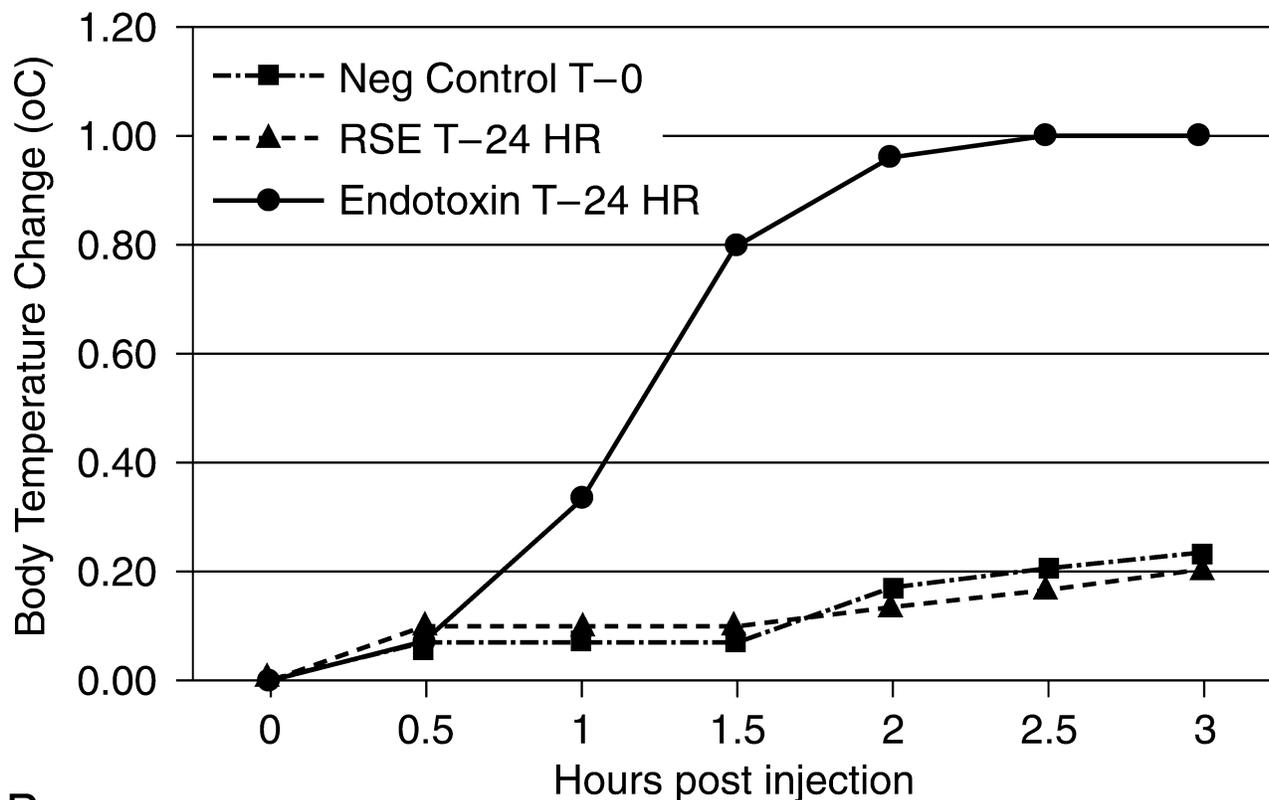
2. RSE was used to spike the matrix. It was reconstituted and vortex mixed according to the package insert.
3. Parallel sets of experiments were conducted: one with RSE and the other with bacterial endotoxin prepared from *Enterobacter cloacae*, ATCC 7256.
4. The stock concentration for both analytes (LPS and endotoxin) was nominally 40 EU/mL.
5. A negative control of normal saline was used to establish the baseline for the rabbit test.
6. The rabbit injection dose was 1.5 mL/kg, which equals about 60 EU/kg for a young adult rabbit.
7. BET and rabbit testing were coordinated to assure that rabbit injection and the LAL assay were initiated at precisely the same time, resulting in true zero-time testing.
8. The BET assay was a standard kinetic chromogenic assay.



A

Figure 4a. Rabbit pyrogen test at $T = 0$ for a citrate/polysorbate 20 buffer spiked with both endotoxin and RSE.

RABBIT PYROGEN TESTING AT 24 HOURS



B

Figure 4b. Rabbit pyrogen test at $T = 24$ h for a citrate/polysorbate 20 buffer spiked with both endotoxin and RSE.

Dubczak's (7) rabbit data at 24 h are in conflict with the three-rabbit data reported by Chen (2). At the beginning of the study ($T = 0$), rabbits responded to both endotoxin and RSE preparations stored in citrate/polysorbate buffer. Given that the rabbits received the same level of RSE and endotoxin, the temperature rise was consistently higher at 24 h with endotoxin than with RSE, which did not differ from the control (*Figure 4b*). Furthermore, at 24 h, the rabbit responded only to the preparation containing endotoxin, demonstrating that after the hold time, the RSE became not only nondetectable by the BET assay but also nonreactive in the rabbit, presumably due to buffer-induced disaggregation of RSE activity. The chromogenic BET results for these experiments are provided in *Table 1*.

Table 1. LAL Results for LAL/Rabbit Studies (*Figures 4a and 4b*)

Material	$T = 0$	$T = 24$
RSE/LPS	34.3 EU/mL	<0.5 EU/mL
Endotoxin	31.7 EU/mL	12.2 EU/mL

LAL data for the RSE and five endotoxins are described in *Figures 5a and 5b* (24). These data confirm that the RSE lost both pyrogenicity and LAL activity after the 24-h hold period. Both pyrogenicity and LAL activity were retained for all of the endotoxin preparations after 24 h. Taken together, these data confirm that: 1) endotoxin is a better analyte for any hold-time study involving biotechnology product matrices than the surrogate LPS, and 2) the BET is an excellent predictor of pyrogenicity.

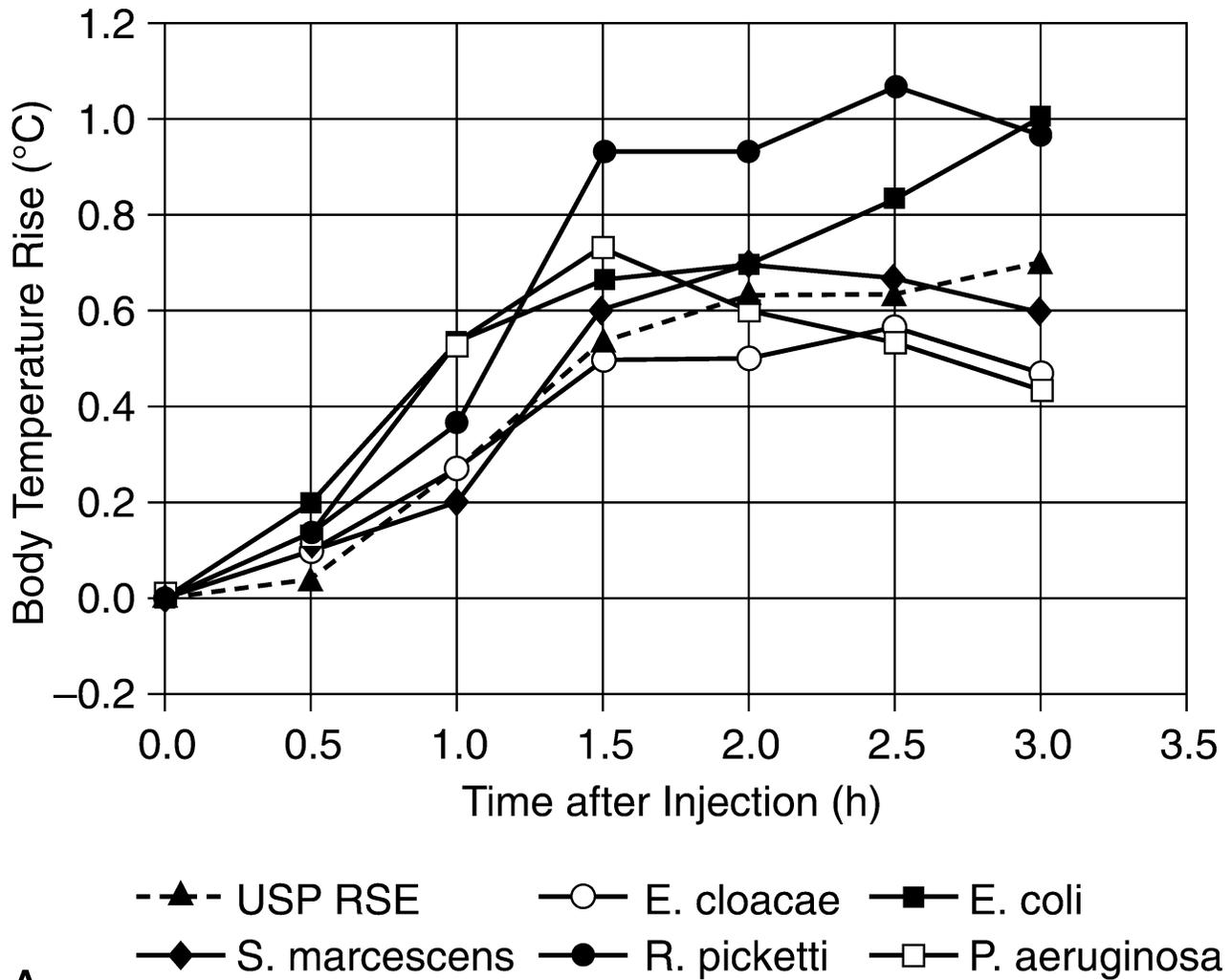


Figure 5a. Average temperature rise with a three-rabbit test in a polysorbate 20/citrate/NaCl matrix at $T = 0$. *S. marcescens*, *Serratia marcescens*; *E. cloacae*, *Enterobacter cloacae*; *R. picketti*, *Ralstonia picketti*; *E. coli*, *Escherichia coli*; *P. aeruginosa*, *Pseudomonas aeruginosa*.

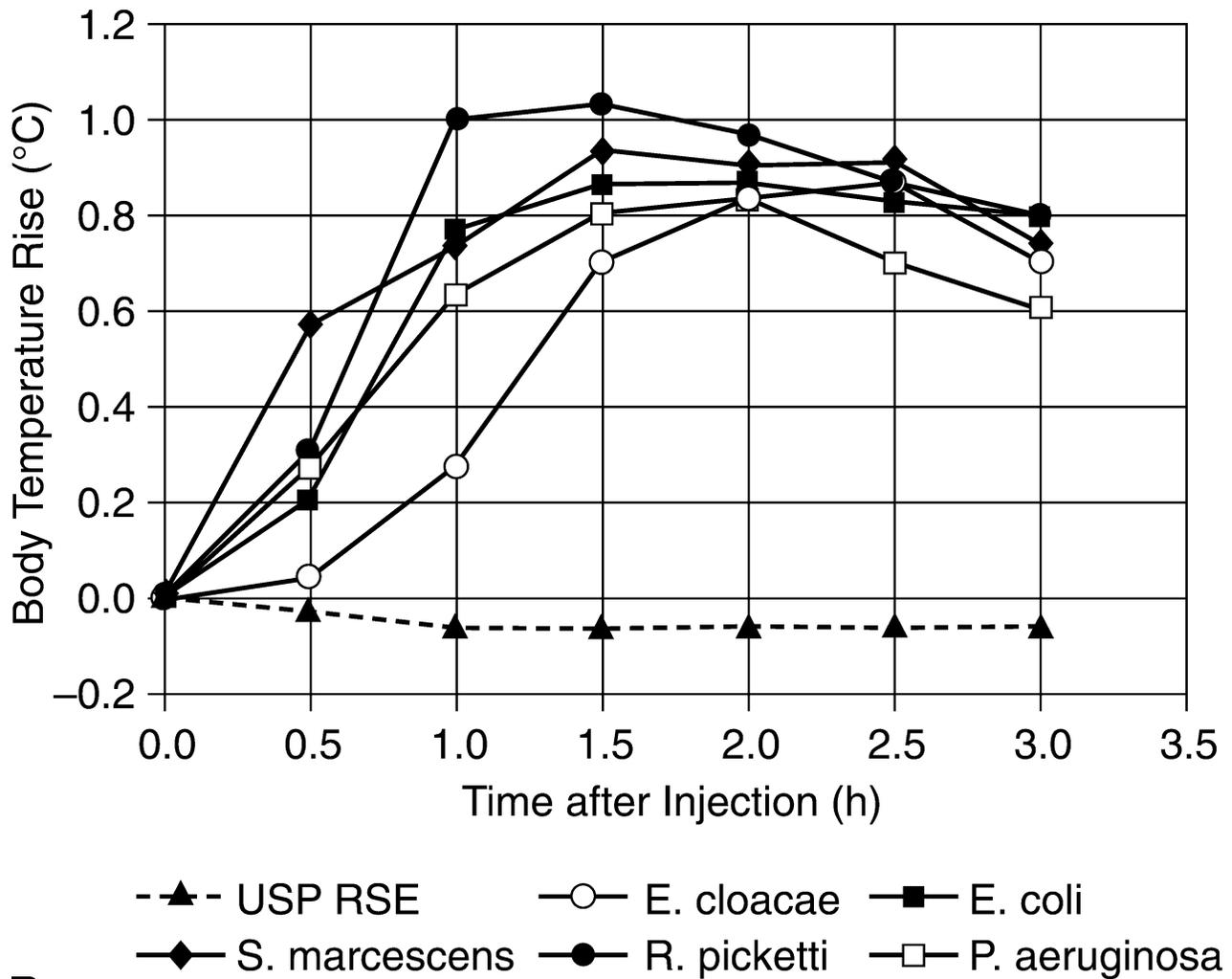
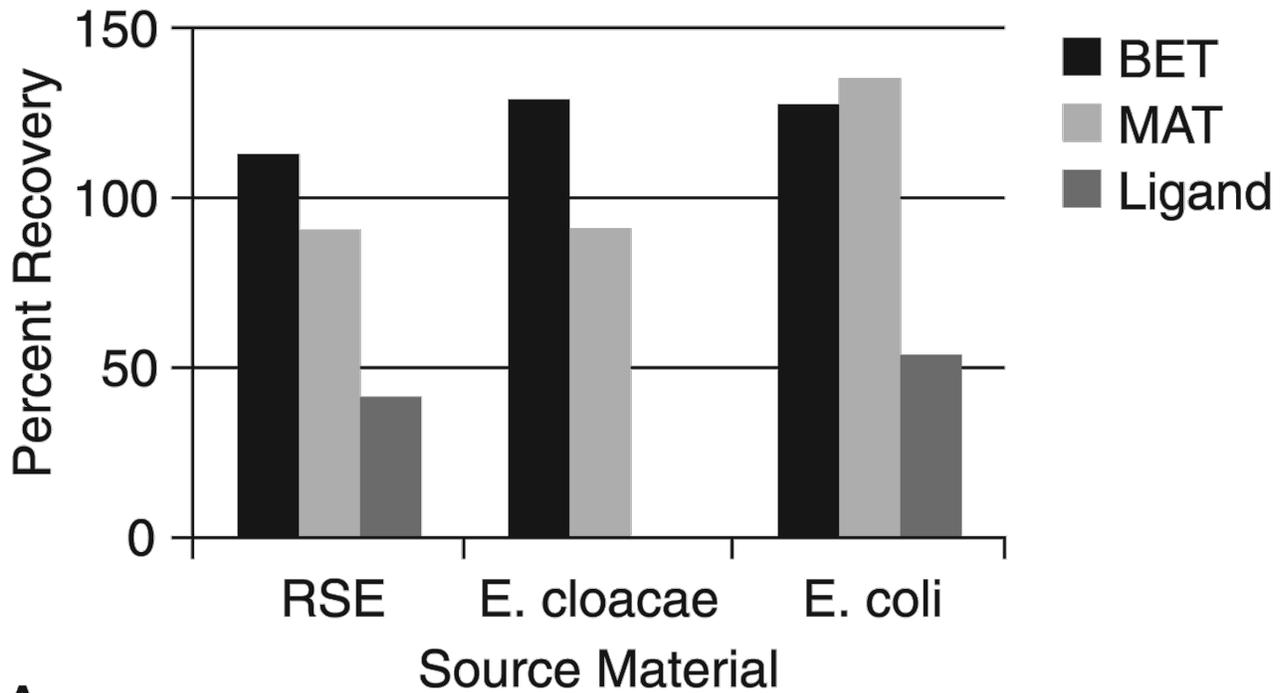


Figure 5b. Average temperature rise in a polysorbate 20/citrate/NaCl matrix at $T = 24$ h. *S. marcescens*, *Serratia marcescens*; *E. cloacae*, *Enterobacter cloacae*; *R. picketti*, *Ralstonia picketti*; *E. coli*, *Escherichia coli*; *P. aeruginosa*, *Pseudomonas aeruginosa*.

Alternative Methods for Endotoxin Detection

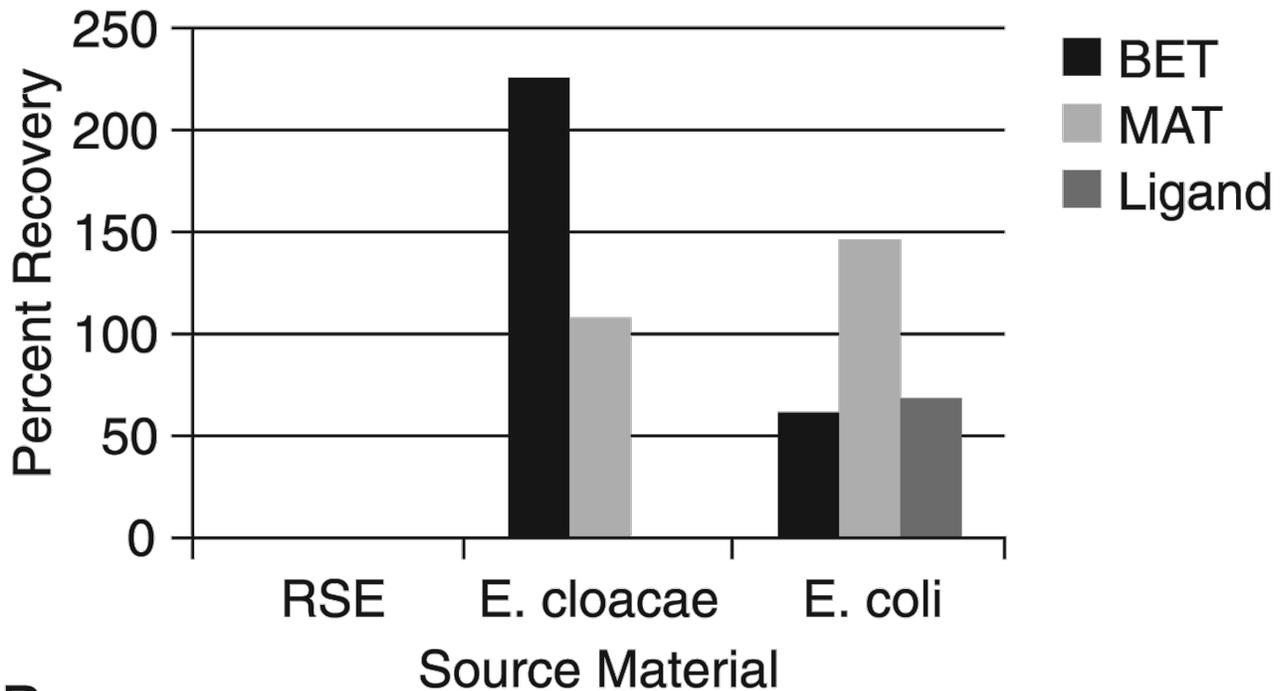
Dubczak (7) concurrently studied the LLR effect using a chromogenic LAL assay, the monocyte activation test (MAT), and a commercially available ligand assay. Comparative data are presented in Figures 6a–6c for a normal saline diluent control, a polysorbate 20/citrate buffer, and a protein formulated in polysorbate 20/citrate, respectively. These studies used laboratory-derived endotoxins from *E. cloacae* and *E. coli* and RSE. All assays were conducted according to the manufacturers’ instructions. All LAL, MAT, and ligand test samples were stored at 2–8° in borosilicate glass containers and analyzed at zero time and 24 h concurrently; i.e., all samples were executed on the same analysis plate and analyzed against the same standard curve. For Figures 6a–6c, the solid black bars represent activity measured by an LAL chromogenic assay, striped bars represent activity by the MAT assay, and dotted bars represent activity as measured by the ligand assay.



A

Figure 6a. Normal saline (NaCl) control, nominal spike value = 50 EU/mL.

Figure 6a shows the relative percent recovery after a 24-h hold in normal saline, as measured by the three test methods. Given the nominal spike value of 50 EU/mL, the data indicate that both the BET and the MAT recovered endotoxin at approximately 100%. In contrast, the ligand test recovered <50% of the RSE, approximately 50% of the *E. coli* endotoxin but none of the *E. cloacae* endotoxin.



B

Figure 6b. Citrate/polysorbate 20/NaCl, nominal spike value = 50 EU/mL.

Experiments in *Figure 6b* measure the recovery of activity of RSE, as well as *E. coli* and *E. cloacae* endotoxin in a citrate/polysorbate/NaCl buffer after a hold of 24 h at 2–8°. The BET and MAT detected both the *E. coli* and *E. cloacae* endotoxins, but the ligand test detected only the *E. coli* endotoxin. None of the tests detected RSE after the 24-h hold time.

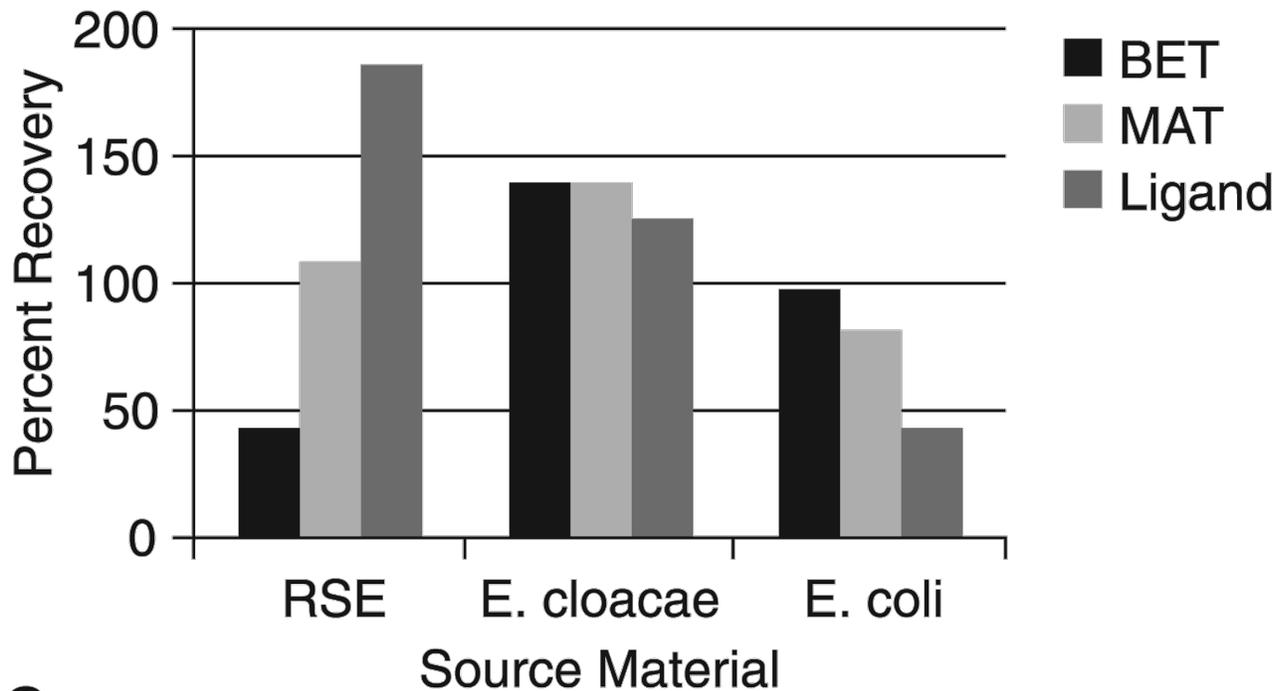


Figure 6c. Product protein in citrate/polysorbate 20/NaCl matrix, nominal spike value = 50 EU/mL.

Data in *Figure 6c* suggest that the presence of drug substance protein in the formulation matrix provides some level of protection from the LLR effect, because the presence of the drug substance improved recovery of *E. cloacae* endotoxin activity in the ligand assay.

Data from these studies suggest:

1. The LAL test is the most consistent of the three tests in the detection of endotoxin activity in each of the three matrices.
2. The endotoxin preparations are consistently detected in the saline and the product matrix.
3. The RSE standard is recovered for the 24-h hold time relative to the nominal spike in the control matrix and in the citrate/polysorbate/protein matrix but not in the matrix containing citrate/polysorbate.

Experimental Design

Because of the biological nature of LAL reagents, the compendia and regulatory authorities recognize some variability in test results. However, when undertaking any BET study, particularly hold-time studies, strict attention must be paid to experimental design and data interpretation. For example:

- When measuring $T = 0$, the laboratory must be attentive to the fact that the LLR phenomenon can happen very quickly after addition of the LPS spike; therefore,

measurement should be taken immediately after the addition of the LPS.

- Bolden et al. (3) described an association between variability in y -intercept and the extent of the LER effect, demonstrating the clear impact of standard curve control on the accuracy of any tests result. When corrected for the y -intercept variability, the LER effect was significantly reduced, demonstrating that experimental design and attention to control strategies for inherent assay variability are important factors when screening for LER as well as for routine testing. For an in-depth discussion of standard curve effects on the accuracy of test results, see McCullough (8).
- Rabbit pyrogen testing is notoriously subject to many variables. When performing pyrogen tests, be certain that the requirements and interpretation described in (151) are strictly held, and that the person performing the test is experienced to avoid false-positive results.

DISCUSSION

Biological formulations containing protein, chelating buffer, and polysorbate have been in production since the late 1980s and have been tested/released using the compendial BET assays with no reports of pyrogenic outbreaks, suggesting that assayable endotoxin in these products has been detected appropriately for about 30 years. It was only after one company's broad interpretation of Question 3 in the current FDA Guidance that the suggestion of underquantitation of endotoxin activity by the BET assay was made using LPS as a surrogate for contaminating endotoxin.

Studies conducted by Bolden et al. (3,4), Platco (5,6), and Dubczak (7) replicated earlier reports of loss of LPS activity by a product matrix consisting of polysorbate and citrate or phosphate buffers. Parallel studies using LPS and naturally derived endotoxin demonstrated that endotoxin is the appropriate analyte for conducting challenge studies in these formulations, because they are the more appropriate surrogates for real-life, cell-wall fragment contaminants. Endotoxin activity was recovered within acceptable assay variability when endotoxin was used as the analyte in LLR conditions and the experimental design and assays were tightly controlled. Although LPS preparations may be well characterized by the *USP-NF Pharmacopeia* for specific uses as a standard for biological activity (pyrogenicity) and as a calibration standard for the BET assay, LPS bears little resemblance to the endotoxin analyte that might be present during pharmaceutical manufacturing. In retrospect, to expect purified LPS activity to react similarly to endotoxin contamination in all situations is both unrealistic and unreasonable.

CONCLUSION

Chelating buffers with detergents, such as citrate and polysorbate, are excellent carriers for delivering vital therapeutic agents, particularly mAbs. Although LPS is the convenient form of endotoxin used for experimentation, it is rapidly rendered nonreactive in the BET assay and nonpyrogenic in the presence of such carriers. The proper term for this phenomenon is LLR. Fortunately, naturally occurring endotoxin, the actual pyrogenic contaminant from Gram-negative bioburden, remains LAL active and pyrogenic in rabbits under prescribed LLR storage conditions. Although the presence of endotoxin is improbable, 30 years of history plus data derived from controlled experiments indicate that endotoxin contamination in drug product would clearly be detected by verified USP methodology. We conclude that LLR poses no public health concern, that the phenomenon presents no threat to the utility, accuracy, and repeatability of the BET, and that a continued requirement to execute LLR experiments using

the surrogate LPS analyte is a resource-laden exercise with little value.

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Pharmacopeial Forum is covered in *Current Contents/Life Sciences* and in the *Science Citation Index (SCI)*, in *International Pharmaceutical Abstracts*, and in *Current Awareness in Biological Sciences*.

The United States Pharmacopeial Convention comprises representatives from academic institutions; health practitioner and scientific associations; consumer organizations; manufacturer and trade associations; government bodies and associations; and non-governmental standards-setting and conformity assessment bodies. It publishes the U.S. Pharmacopeia and National Formulary, the legally recognized compendia of standards for drugs and products of other health care technologies. The USP and NF include assays and tests for the determination of strength, quality, and purity and requirements for packaging and labeling. **Pharmacopeial Forum's Public Review and Comment Process**

This section presents an overview of the public review and comment process, conducted through *Pharmacopeial Forum (PF)*, for the development of official standards in the *United States Pharmacopeia* and the *National Formulary (USP-NF)*.

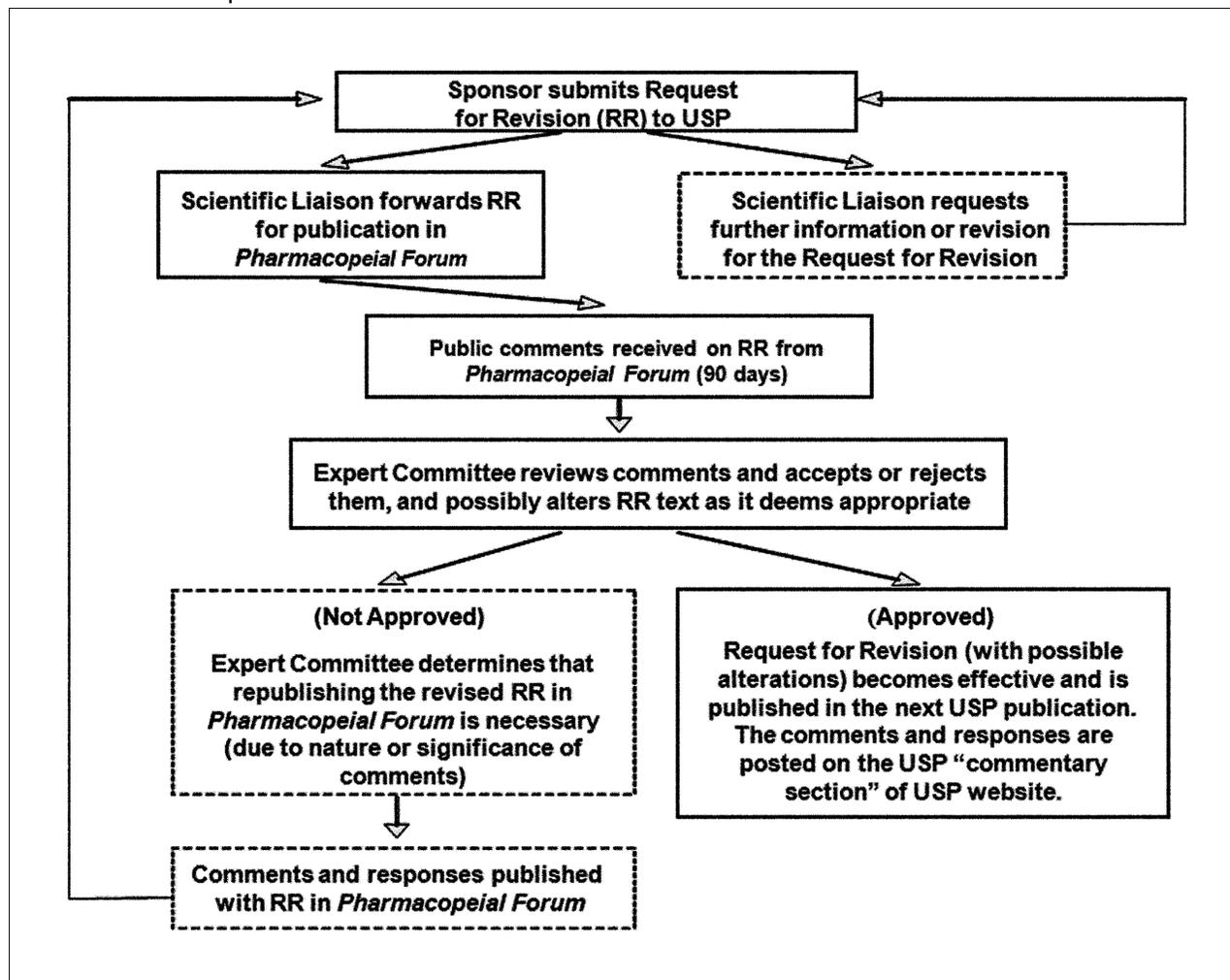
USP publishes *PF* on a bimonthly basis to provide an opportunity to review and comment on new or revised standards.

There are two types of proposed revisions in *PF*:

1. **Proposed Revisions**—New or revised standards targeted for adoption through USP's Standard Revision Process. USP's Revision Process calls for publication of a proposed revision in *PF* for a 90-day notice and comment period. After the comment period and subsequent review of comments and approval by the relevant USP Expert Committee, the official standard is published in the next available *USP-NF* or *Supplement*. If comments received are significant, or if the Expert Committee's consideration of comments results in significant additional changes, the Expert Committee may determine that republishing in *PF* is necessary prior to the revision becoming official. See the *In-Process Revision* section for current proposed revisions.
2. **Proposed Interim Revision Announcements**—New or revised standards that become official through an accelerated process in accordance with USP's Guideline on Accelerated Revisions (available on the USP Web site). *Interim Revision Announcements (IRAs)* allow for a revision to become official prior to the next *USP-NF* or *Supplement*. *IRAs* are first presented for a 90-day public comment period in the *Proposed Interim Revision Announcement* section of the *PF*. Note that final *IRAs*, as well as *Errata*, and *Revision Bulletins*, which also are defined in the Accelerated Revision Guideline, appear only on the USP Web site.

USP welcomes comments and data on proposed revisions. A summary of comments received, along with USP’s responses, will be published in the *Revisions and Commentary* section of the USP website (<http://www.usp.org/USPNF/revisions/>).

The chart below shows the public review and comment process and its relationship to standards development.



Questions on the process should be addressed to the USP Executive Secretariat, United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852 (e-mail: execsec@usp.org).

HOW TO USE PF

The various sections of *PF* are briefly described below. A more detailed description of each section is provided at the beginning of that section. A general description of the types and amount of information expected in a *Request for Revision* is available in the *Guideline for Submitting Requests for Revision to the USP–NF* on the USP Web site (www.usp.org/USPNF/submitMonograph/subGuide.html). Note that the Expert Committee listing and the Scientific Staff Directory also are located on the USP Web site (see below for links).

Proposed and Adopted Revisions to the USP–NF

Section	Content	How Readers Can Respond
<p>Proposed Interim Revision Announcements</p>	<p>Proposals for <i>Interim Revision Announcements (IRAs)</i> that will be published as official <i>USP</i> or <i>NF</i> standards BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Expert Committee and Scientific Liaisons who handled the monograph or general chapter.</p>	<p>Review material and send comments within 90 days of the <i>PF</i> publication in which the standard was proposed (or per the comment deadline listed in the Briefing section). Direct comments to the USP Scientific Liaison using the contact information provided at the end of each <i>Proposed IRA</i></p>
<p>In-Process Revision</p>	<p>Proposals for standards that will be published as official in a future <i>USP-NF</i> book or <i>Supplement</i>. BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Expert Committee and USP Scientific Liaisons who handle the monograph or general chapter.</p>	<p>Review material and send comments within 90 days of the <i>PF</i> publication in which the revision was proposed (or per the comment deadline listed in the Briefing section). Direct comments to the USP Scientific Liaison using the contact information provided at the end of each proposed revision. For general inquiries or in cases where a Scientific Liaison is not identified, use the general USP telephone number 301-881-0666 or fax number 301-998-6839 or stdsmonographs@usp.org.</p>

Section	Content	How Readers Can Respond
Stage 4 Harmonization	<p>Revision proposals from the Pharmacopoeial Discussion Group (PDG), which comprises the European Pharmacopoeia, the Japanese Pharmacopoeia, and USP. The Stage 4 draft and the briefing are published in the forum of each pharmacopoeia for public comment. The draft is published in its entirety.</p> <p>BRIEFING: Scientific rationale for the potential inclusion or change or for the proposed change.</p>	<p>Review material and provide comments to the USP Scientific Liaison using the contact information provided at the end of each Stage 4 Harmonization. Individuals who wish to correspond with the European and Japanese Pharmacopoeias concerning monographs in the Stage 4 period of international harmonization should address their comments to the coordinating pharmacopoeia, with a copy to USP.</p> <p>PhEur Secretariat Ms. Lynn Kelso-Eleuterio Central Secretariat European Pharmacopoeia Department</p> <p>European Directorate for the Health Care Council of Europe 7, Allée Kastner CS 30026 67081 Strasbourg France Tel: +33 (3) 88 41 31 48 Fax: +33 (3) 88 41 27 71 lynn.kelso@edqm.eu</p> <p>JP Secretariat Mr. Issei Takayama Technical Officer Pharmaceuticals and Medical Devices Agency (PMDA) Shin-kasumigaseki Building 3-3-2, Kasumigaseki, Chiyoda-ku Tokyo, 100-0013 Japan Phone: +81-3-3506-9431 Fax: +81-3-3506-9440 takayama-issei@pmda.go.jp</p>
Stimuli to the Revision Process	<p>Articles on standards development topics authored by the USP Council of Experts, USP staff, or other interested parties on which USP desires public input prior to further development.</p>	<p>Review material and provide comments to the recipient indicated (usually footnoted in each <i>Stimuli</i> article).</p>

Other Sections

Expert Committees

A listing of the 2015–2020 Expert Committees that work on the development of USP compendial standards (<http://www.usp.org/expert-committees>)

Staff Directory

Names and contact information of key USP scientific staff members who work on the development of USP compendial standards

(<http://www.usp.org/USPNF/devProcess/contactScientists.html>)

Proposed Interim Revision Announcements

This section includes proposals for *Interim Revision Announcements (IRAs)* that will be published as official *USP* or *NF* standards. There is a 90-day comment period for these proposals (see <http://www.usp.org/USPNF/pf/pfRedesign.html> for the *PF* comment schedule). Readers should review material in this section and provide comments to the Scientific Liaison using the contact information appearing at the end of each proposed *IRA*. Information on how to comment can be found under the "Participation" section of www.usp.org. It is important to send comments promptly so that the Expert Committee members can consider reader's input as they are deciding whether to advance standards to official status. The approved official text will be published under *IRAs* in the "New Official Text" section of USP's Web site.

Each proposal is preceded by a Briefing that indicates the proposed revisions.

Symbols—New text is enclosed in symbols and set off from the current official text as shown in the following example: *new text*. Where the symbols appear together with no enclosed text, such as ***, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the date the proposal, if approved, will become official as an *IRA*. For example, *(IRA 1-Apr-2011)

BRIEFING

Ketorolac Tromethamine, *USP 38* page 4010. On the basis of comments received, it is proposed to make the following changes:

1. Replace the *UV Identification* test *B* with an orthogonal identification test based on HPLC retention time agreement from the *Assay*.
2. Reinstate the individual unspecified impurity limit to the test for *Organic Impurities*, which was inadvertently omitted during the monograph redesign.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

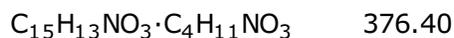
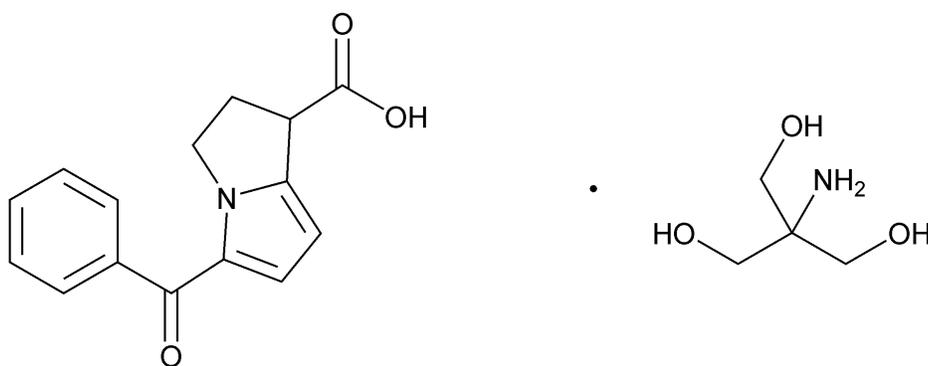
The comment period for this revision ends on Jan 31, 2016. In the absence of significant adverse comments, it is proposed to implement this revision through an *Interim Revision Announcement*, with an official date of May 1, 2016.

(CHM2: H. Cai.)

Correspondence Number—C161928

Comment deadline: January 31, 2016

Ketorolac Tromethamine



1*H*-Pyrrolizine-1-carboxylic acid, 5-benzoyl-2,3-dihydro, (±)-, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1);
 (±)-5-Benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1) [74103-07-4].

DEFINITION

Ketorolac Tromethamine contains NLT 98.5% and NMT 101.5% of ketorolac tromethamine ($\text{C}_{15}\text{H}_{13}\text{NO}_3 \cdot \text{C}_4\text{H}_{11}\text{NO}_3$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)

Delete the following:

- ~~• **B. Ultraviolet Absorption** (197U)~~

~~**Sample solution:** 10 µg/mL~~

~~**Medium:** Methanol~~

~~**Acceptance criteria:** Meets the requirements (IRA 1-May-2016)~~

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. (IRA 1-May-2016)

- **C. Thin-Layer Chromatography, Tromethamine Test**

Diluent: Dichloromethane and methanol (2:1)

Standard solution: 5 mg/mL of USP Ketorolac Tromethamine RS in *Diluent*

Sample solution: 5 mg/mL of Ketorolac Tromethamine in *Diluent*

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 40 µL

Developing solvent system: Dichloromethane, acetone, and glacial acetic acid

(95:5:2)

Spray reagent: Freshly prepared alcoholic solution containing 30 mg/mL of *ninhydrin*

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow the solvent to evaporate. Spray the plate with *Spray reagent*, and heat the plate at about 150° for 2–5 min.

Acceptance criteria: Yellow spots with pink to purple borders develop on the plate in the areas where the *Standard solution* and the *Sample solution* were applied.

ASSAY

• Procedure

Protect all the solutions from light.

Buffer: 5.75 g/L of *monobasic ammonium phosphate*. Adjust with *phosphoric acid* to a pH of 3.0.

Mobile phase: *Tetrahydrofuran* and *Buffer* (30:70)

Diluent: *Tetrahydrofuran* and water (30:70)

System suitability solution: In a 250-mL separator, mix 100 mL of water, 100 mL of *dichloromethane*, 30 mg of USP Ketorolac Tromethamine RS, and 1 mL of 1 N hydrochloric acid. Insert the stopper, shake, and allow the layers to separate. Transfer the lower *dichloromethane* layer to a stoppered borosilicate glass flask, and discard the upper layer. Expose the *dichloromethane* solution to direct sunlight for 10–15 min. Transfer 1.0 mL of the solution to a vial, evaporate in a current of air or in a stream of nitrogen to dryness, add 1.0 mL of *Diluent*, and swirl to dissolve. [Note—This solution may be stored under refrigeration and used as long as the chromatogram obtained as directed for *Analysis* is suitable for identifying the peaks due to the ketorolac 1-keto analog and ketorolac 1-hydroxy analog, and for the measurement of the resolution between the ketorolac 1-keto analog and ketorolac.]

Standard solution: 0.4 mg/mL of USP Ketorolac Tromethamine RS in *Diluent*

Sample solution: 0.4 mg/mL of Ketorolac Tromethamine in *Diluent*

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 313 nm

Column: 4.6-mm × 25-cm; 5- μ m packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for the ketorolac 1-hydroxy analog, the ketorolac 1-keto analog, and ketorolac are about 0.63, 0.89, and 1.0, respectively. Make adjustments, if necessary, to achieve a retention time for ketorolac of about 8–12 min.]

Suitability requirements

Resolution: NLT 1.5 between ketorolac 1-keto analog and ketorolac, *System suitability solution*

Column efficiency: NLT 5500 theoretical plates, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$) in the portion of Ketorolac Tromethamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Ketorolac Tromethamine RS in the *Standard solution* (mg/mL)

C_U = concentration of Ketorolac Tromethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

Delete the following:

- ~~• **Heavy Metals, Method II** (231): NMT 20 ppm (Official 1-Jan-2018)~~

Change to read:

- **Organic Impurities**

Mobile phase, Diluent, System suitability solution, Standard solution, and Sample solution: Prepare as directed in the *Assay*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 313 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 μL

Run time: 3 times the retention time of ketorolac

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Ketorolac Tromethamine taken:

$$\text{Result} = (r_U/r_T) \times F \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity having a 0.54 relative retention time	0.54	2.2	0.5
Ketorolac 1-hydroxy analog	0.63	0.67	0.1
Impurity having a 0.66 relative retention time	0.66	0.91	0.5
Ketorolac 1-keto analog	0.89	0.52	0.1
* Individual unspecified impurity	—	1.0	0.5*(IRA 1-May-2016)
Ketorolac tromethamine	1.0	1.0	—
Total impurities	—	—	1.0

SPECIFIC TESTS

- **pH** (791)

Sample solution: 10 mg/mL

Acceptance criteria: 5.7–6.7

- **Loss on Drying** (731)

Analysis: Dry under vacuum at 60° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

- **USP Reference Standards** (11)

USP Ketorolac Tromethamine RS

In-Process Revision

This section contains proposals for adoption as official *USP* or *NF* standards (either proposed *new* standards or proposed *revisions* of current *USP* or *NF* standards). These may be any of the following: (1) proposed revisions placed directly under *In-Process Revision*, (2) modifications of revisions previously proposed under *In-Process Revision*, or (3) proposed revisions for articles

awaiting approval by FDA. Readers should review material in this section and provide comments to the Scientific Liaison using the contact information appearing at the end of each proposal. Information on how to comment can be found under the "Participation" section of www.usp.org. It is important to send comments promptly, before the comment deadline listed after each title.

Briefings Each Proposal is preceded by a Briefing in the following format:

Briefing
Name of Item , citations of the most recent USP publications in which this item appeared. Rationale for the revision. Other relevant information. (For example, if a chromatographic method is being proposed, column specifications and retention times for compounds of interest.) Finally, the Expert Committee acronym (the name of the Scientific Liaison who handled the particular monograph or general chapter, and the USP tracking correspondence number, as shown in the example below:
(Expert Committee Acronym: Liaison Name.)
Correspondence Number—CXXXXX

Symbols Proposed revisions are shown with language proposed for deletion or replacement crossed out. All *USP-NF* revisions use the following symbols that indicate the final destination of the official text: * new text * if slated for an *IRA*; ▲ new text ▲ if slated for *USP-NF*; ■ new text ■ if slated for a *Supplement* to *USP-NF*; ▶ new text ◀ for articles awaiting approval by FDA. The same symbols indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as * * , or ■ ■ , or ▲ ▲ , or ▶ ▶ , it means that text has been deleted and no new text was proposed to replace it.

In revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA*, *Supplement*, or the *USP* or *NF* as the publication where the revision will appear if approved. For example, ■_{2S} (*USP 34*) indicates that the proposed revision is slated for the *Second Supplement to USP 34*, and ▲_{USP35} and ▲_{NF30} indicates that the revisions are proposed for *USP 35* and *NF 30*, respectively. In the case of revisions for articles awaiting approval by FDA, the symbols are followed by TBD indicating that the official publication is To Be Determined. For example, ▶ (TBD) does not yet have a target official publication.

Official Title Changes Where the specification "**Monograph title change**" is found, it indicates that the official title stated after that specification will be substituted for the former title in the appropriate places throughout that monograph once this revision becomes official.

BRIEFING

⟨151⟩ **Pyrogen Test**, *USP 38* page 211. To encourage the use of in vitro alternatives to in vivo animal tests, a revision is proposed to introduce text into the *Introduction* of the chapter that allows the use of a validated, equivalent in vitro pyrogen or bacterial endotoxin test in place of the in vivo rabbit pyrogen test, where appropriate. There is no impact on any of the monographs that reference this chapter.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

(GCM: R.Tirumalai.)

Correspondence Number—C164217

Comment deadline: January 31, 2016

(151) PYROGEN TEST

Change to read:



INTRODUCTION ▲*USP40*

The pyrogen test is designed to limit to an acceptable level the risks of febrile reaction in the patient to the administration, by injection, of the product concerned. The test involves measuring the rise in temperature of rabbits following the intravenous injection of a test solution and is designed for products that can be tolerated by the test rabbit in a dose not to exceed 10 mL/kg injected intravenously within a period of not more than 10 min. For products that require preliminary preparation or are subject to special conditions of administration, follow the additional directions given in the individual monograph or, in the case of antibiotics or biologics, the additional directions given in the federal regulations (see *Biologics* (1041)).

▲ A validated, equivalent in vitro pyrogen or bacterial endotoxin test may be used in place of the in vivo rabbit pyrogen test,¹ where appropriate. ▲*USP40*

▲*USP40*

APPARATUS AND DILUENTS

Render the syringes, needles, and glassware free from pyrogens by heating at 250° for NLT 30 min or by any other suitable method. Treat all diluents and solutions for washing and rinsing of devices or parenteral injection assemblies in a manner that will assure that they are sterile and pyrogen-free. Periodically perform control pyrogen tests on representative portions of the diluents and solutions for washing or rinsing of the apparatus. Where *Sodium Chloride Injection* is specified as a diluent, use Injection containing 0.9% of sodium chloride (NaCl).

TEMPERATURE RECORDING

Use an accurate temperature-sensing device such as a clinical thermometer, or thermistor probes or similar probes that have been calibrated to assure an accuracy of $\pm 0.1^\circ$ and have been tested to determine that a maximum reading is reached in less than 5 min. Insert the temperature-sensing probe into the rectum of the test rabbit to a depth of NLT 7.5 cm, and, after a period of time NLT that previously determined as sufficient, record the rabbit's body temperature.

TEST ANIMALS

Use healthy, mature rabbits. House the rabbits individually in an area of uniform temperature between 20° and 23° and free from disturbances likely to excite them. The temperature varies

NMT $\pm 3^\circ$ from the selected temperature. Before using a rabbit for the first time in a pyrogen test, condition it NMT 7 days before use by a sham test that includes all of the steps as directed in *Procedure* except injection. Do not use a rabbit for pyrogen testing more frequently than once every 48 h, nor prior to 2 weeks following a maximum rise of its temperature of 0.6° or more while being subjected to the pyrogen test, or following its having been given a test specimen that was adjudged pyrogenic.

PROCEDURE

Perform the test in a separate area designated solely for pyrogen testing and under environmental conditions similar to those under which the animals are housed and free from disturbances likely to excite them. Withhold all food from the rabbits used during the period of the test. Access to water is allowed at all times, but may be restricted during the test. If rectal temperature-measuring probes remain inserted throughout the testing period, restrain the rabbits with light-fitting neck stocks that allow the rabbits to assume a natural resting posture. NMT 30 min prior to the injection of the test dose, determine the "control temperature" of each rabbit: this is the base for the determination of any temperature increase resulting from the injection of a test solution. In any one group of test rabbits, use only those rabbits whose control temperatures do not vary by more than 1° from each other, and do not use any rabbit with a temperature exceeding 39.8° .

Unless otherwise specified in the individual monograph, inject into an ear vein of each of three rabbits 10 mL of the test solution per kg of body weight, completing each injection within 10 min after start of administration. The test solution is *either* the product, constituted if necessary as directed in the labeling, *or* the material under test treated as directed in the individual monograph and injected in the dose specified therein. For pyrogen testing of devices or injection assemblies, use washings or rinsings of the surfaces that come in contact with the parenterally administered material or with the injection site or internal tissues of the patient. Assure that all test solutions are protected from contamination. Perform the injection after warming the test solution to a temperature of $37 \pm 2^\circ$. Record the temperature at 30-min intervals between 1 and 3 h subsequent to the injection.

TEST INTERPRETATION AND CONTINUATION

Consider any temperature decreases as zero rise. If no rabbit shows an individual rise in temperature of 0.5° or more above its respective control temperature, the product meets the requirements for the absence of pyrogens. If any rabbit shows an individual temperature rise of 0.5° or more, continue the test using five other rabbits. If NMT three of the eight rabbits show individual rises in temperature of 0.5° or more and if the sum of the eight individual maximum temperature rises does not exceed 3.3° , the material under examination meets the requirements for the absence of pyrogens.

RADIOACTIVE PHARMACEUTICALS

Test Dose for Preformulated, Ready-to-Use Products Labeled with Radioactivity

AGGREGATED ALBUMIN AND OTHER PARTICLE-CONTAINING PRODUCTS

For the rabbit pyrogen test, dilute the product with *Sodium Chloride Injection* to NLT 100 $\mu\text{Ci}/\text{mL}$, and inject a dose of 3 mL/kg of body weight into each rabbit.

OTHER PRODUCTS

Where physical half-life of radionuclide is greater than 1 day: Calculate the maximum volume of the product that might be injected into a human subject. This calculation takes into account the maximum recommended radioactive dose of the product, in μCi , and the radioactive assay, in $\mu\text{Ci}/\text{mL}$, of the product at its expiration date or time. Using this information, calculate the maximum volume dose per kg to a 70-kg human subject.

For the rabbit pyrogen test, inject a minimum of 10 times this dose per kg of body weight into each rabbit. If necessary, dilute with *Sodium Chloride Injection*. The total injected volume per rabbit is NLT 1 mL and NMT 10 mL of solution.

Where physical half-life of radionuclide is less than 1 day: For products labeled with radionuclides with a half-life of less than 1 day, the dosage calculations are identical to those described in the first paragraph in *Other Products*. These products may be released for distribution prior to completion of the rabbit pyrogen test, but such test must be initiated at NMT 36 h after release.

Test Dose for Pharmaceutical Constituents or Reagents to Be Labeled

The following test dose requirements pertain to reagents that are to be labeled or constituted prior to use by the direct addition of radioactive solutions such as *Sodium Pertechnetate Tc 99m Injection*, i.e., "cold kits".

Assume that the entire contents of the vial of nonradioactive reagent will be injected into a 70-kg human subject, or that $1/70$ of the total contents per kg will be injected. If the contents are dry, constitute with a measured volume of *Sodium Chloride Injection*.

For the rabbit pyrogen test, inject ($1/7$) of the vial contents per kg of body weight into each rabbit. The maximum dose per rabbit is the entire contents of a single vial. The total injected volume per rabbit is NLT 1 mL and NMT 10 mL of solution.

¹ United States Food and Drug Administration, Guidance for Industry. Pyrogen and Endotoxins Testing: Questions and Answers, June 2012.
<http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm310098.pdf>.

BRIEFING

(481) **Riboflavin Assay**, *USP* 38 page 326. This general test chapter is being revised as part of the *USP* monograph modernization effort. The following changes are proposed:

1. Convert the current existing method in the chapter from the classic format to the redesigned format to more closely align with the current *USP-NF* monograph style.
2. Consolidate all of the existing chromatographic procedures in the various monographs by migrating them to this chapter, which will serve as a central location for cross-references for all riboflavin assay procedures described in the individual monographs.
3. Add a new high-performance liquid chromatography (HPLC) assay, *Chromatographic Methods, Procedure 4*, using hydrophilic interaction liquid chromatography (HILIC) for the determination of riboflavin in the dietary supplements dosage forms.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

The following Briefing list includes monographs and/or chapters that both reference the General Chapter under revision and require revision to keep references to the General Chapter accurate. Other monographs and/or chapters may also be listed, even where the reference to the General Chapter remains unchanged, as additional notice to stakeholders where there is believed to be potential for the change in the general chapter itself to affect pass-fail determinations for particular monograph articles.

Chapter Dependencies (click to expand).

• Riboflavin Injection • Riboflavin Tablets

(FI: H. Dinh.)

Correspondence Number—C138545

Comment deadline: January 31, 2016

(481) RIBOFLAVIN ASSAY

ASSAY

Change to read:

•

▲ Chemical Methods, Procedure 1▲^{USP40}

The following procedure is suitable for preparations in which riboflavin is a constituent of a mixture of several ingredients. In using the procedure, keep the pH of solutions below 7.0, and protect the solutions from direct sunlight at all stages.

Standard riboflavin stock solution: To 50.0 mg of USP Riboflavin RS, previously dried and stored protected from light in a desiccator over phosphorus pentoxide, add about 300 mL of 0.02 N *Acetic Acid*, and heat the mixture on a steam bath with frequent agitation until the riboflavin has dissolved, then cool. To this solution add 0.02 N *Acetic Acid* to make 500 mL; then mix. Store the solution under toluene in a refrigerator.

Dilute an accurately measured portion of this solution by using 0.02 N *Acetic Acid* to a concentration of 10.0 µg/mL of the dried USP Riboflavin RS to obtain the *Standard riboflavin stock solution*. Store the solution under toluene in a refrigerator.

Standard solution: Dilute with water 10.0 mL of *Standard riboflavin stock solution* in a 100-mL volumetric flask to volume, and mix. Each mL represents 1.0 µg of USP Riboflavin RS. Prepare fresh *Standard solution* for each assay.

Sample solution: Place an amount of the material to be assayed in a flask of suitable size, and add a volume of 0.1 N *Hydrochloric Acid* equal in mL to NLT 10 times the dry weight of the material in grams, but the resulting solution will contain NMT 100 µg/mL of riboflavin. If the material is not readily soluble, comminute the material so that it may be evenly dispersed in the liquid. Agitate vigorously, and wash down the sides of the flask with 0.1 N *Hydrochloric Acid*.

Heat the mixture in an autoclave at 121°–123° for 30 min, and cool. If clumping occurs, agitate the mixture until the particles are evenly dispersed. Adjust the mixture, with vigorous agitation, to a pH of 6.0–6.5 with *Sodium Hydroxide* solution,¹ then add *Hydrochloric Acid* solution¹ immediately until no further precipitation occurs (usually at a pH of approximately 4.5, which is the isoelectric point of many of the proteins present).

Dilute the mixture with water to make a measured volume that contains about 0.11 µg of riboflavin in each mL, and filter through paper known not to adsorb riboflavin. To an aliquot of the filtrate add, with vigorous agitation, *Sodium Hydroxide* solution¹ to produce a pH of 6.6–6.8, dilute the solution with water to make a final measured volume that contains approximately 0.1 µg of riboflavin in each mL, and if cloudiness occurs, filter again.

Instrumental conditions

(See *Fluorescence Spectroscopy* (853).)

Mode: Fluorescence

Excitation wavelength: 444 nm

Emission wavelength: 530 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

To each of four or more tubes (or reaction vessels) add 10.0 mL of the *Sample solution*. To each of two or more of these tubes add 1.0 mL of the *Standard solution*, and mix; and to each of two or more of the remaining tubes add 1.0 mL of water, and mix. To each tube add 1.0 mL of *Glacial Acetic Acid*, mix, then add, with mixing, 0.50 mL of *Potassium Permanganate* solution (1 in 25), and allow to stand for 2 min. To each tube add, with mixing, 0.50 mL of *Hydrogen Peroxide Solution*, whereupon the permanganate color is destroyed within 10 s. Shake the tubes vigorously until excess oxygen is expelled. Remove any gas bubbles remaining on the sides of the tubes after foaming has ceased by tipping the tubes so that the solution flows slowly from end to end.

Measure the fluorescence of all tubes, designating the average reading from the tubes containing only the *Sample solution* as I_U , and designating the average from the tubes containing both the *Sample solution* and the *Standard solution* as I_S . Then to each of one or more tubes of each kind add, with mixing, 20 mg of *Sodium Hydrosulfite*, and within 5 s again measure the fluorescence, designating the average reading as I_B .

Calculate the quantity, in mg, of riboflavin ($C_{17}H_{20}N_4O_6$) in each mL of the *Sample solution* taken:

$$\text{Result} = [0.0001 \times (I_U - I_B)] / (I_S - I_U)$$

I_U = average reading of the tubes containing only the *Sample solution*

I_B = average reading of fluorescence of the tubes after adding 20 mg of sodium hydrofluorite

I_S = average reading of the tubes containing both the *Sample solution* and *Standard solution*

Calculate the quantity, in mg, of riboflavin ($C_{17}H_{20}N_4O_6$) in each Capsule or Tablet.

Add the following:

▲ ● Chemical Methods, Procedure 2

This procedure is suitable for the determination of riboflavin as a dietary ingredient or active pharmaceutical ingredient. [Note—Conduct the entire *Analysis* without exposure to direct sunlight.]

Standard solution: Transfer 50 mg of USP Riboflavin RS to a 1000-mL volumetric flask

containing 50 mL of water. Add 5 mL of *Acetic Acid* and sufficient water to make 800 mL. Heat on a steam bath, protected from light, with frequent agitation until dissolved. Cool to 25°, and dilute with water to volume. Dilute this solution with water to bring it within the operating sensitivity of the fluorometer used.

Sample solution: Transfer 50 mg of Riboflavin to a 1000-mL volumetric flask containing 50 mL of water. Add 5 mL of *Acetic Acid* and sufficient water to make 800 mL. Heat on a steam bath, protected from light, with frequent agitation until dissolved. Cool to 25°, and dilute with water to volume. Dilute this solution with water to bring it to the same concentration as that of the *Standard solution*.

Blank: Prepare as directed for the *Sample solution*, except omit the test specimen.

Instrumental conditions

(See *Fluorescence Spectroscopy* (853).)

Mode: Fluorescence

Excitation wavelength: 444 nm

Emission wavelength: 530 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Measure the fluorescence intensity of the *Standard solution*. Immediately after the reading, add to the solution 10 mg of *Sodium Hydrosulfite*, stirring with a glass rod until dissolved, and at once measure the fluorescence again. [Note—Depending on the final concentration of riboflavin in the solution, it may be necessary to increase the amount of *Sodium Hydrosulfite* to suppress the fluorescence activity completely.] The difference between the two readings represents the fluorescence intensity (I_S) due to the *Standard solution*. Similarly, measure the fluorescence intensity (I_U) due to the *Sample solution*. Perform the *Blank* determination, and make any necessary correction.

Calculate the percentage of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of Riboflavin taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorescence of the *Sample solution*

I_S = fluorescence of the *Standard solution*

C_S = concentration of USP Riboflavin RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of riboflavin the *Sample solution* ($\mu\text{g/mL}$)

▲USP40

Add the following:

▲The following liquid chromatographic procedures are provided for the determination of riboflavin as an active pharmaceutical ingredient, a dietary supplement ingredient, or a component in the dietary supplements or pharmaceutical dosage forms. Use the appropriate USP Reference Standards.

Throughout these procedures, protect solutions containing and derived from the test specimen and the Reference Standards from the atmosphere and light, preferably by the use of low-actinic glassware.▲USP40

Add the following:

▲ ● Chromatographic Methods, Procedure 1

This procedure can be used to determine riboflavin in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of riboflavin from the formulation by the *Diluent*, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: *Acetonitrile*, *Glacial Acetic Acid*, and water (5:1:94)

Mobile phase: A mixture of *Methanol*, *Glacial Acetic Acid*, and water (27:1:73) containing 140 mg of *Sodium 1-Hexanesulfonate* per 100 mL

Standard solution: Transfer 20 mg of USP Riboflavin RS to a 200-mL volumetric flask, and add 180 mL of *Diluent*. Immerse the flask in a hot water bath maintained at 65°–70° for 10 min with regular shaking, or using a vortex mixer, until all of the solid materials are dissolved. Chill rapidly in a cold water bath for 10 min to room temperature, and dilute with *Diluent* to volume.

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to 2.5 mg of riboflavin, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 5 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 5 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [Note—Use the filtrate within 3 h of filtration.]

Sample solution for Tablets: Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 2.5 mg of riboflavin, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 5 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 5 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [Note—Use the filtrate within 3 h of filtration.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; packing *L1*

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

Calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of riboflavin from the appropriate *Sample solution*

r_S = peak response of riboflavin from the *Standard solution*

C_S = concentration of USP Riboflavin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of riboflavin in the appropriate *Sample solution* (mg/mL)

▲USP40

Add the following:

▲ ● Chromatographic Methods, Procedure 2

This procedure can be used to determine riboflavin in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of riboflavin from the formulation by the *Extraction solvent*, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Extraction solvent: Transfer 1 mL of *Glacial Acetic Acid* and 2.5 g of *Edetate Disodium* to a 100-mL volumetric flask. Dissolve in and dilute with water to volume. Mix the resulting solution with *Methanol* (3:1).

Solution A: 6.8 g of *Sodium Acetate* per 1000 mL of water

Mobile phase: Prepare a mixture of *Solution A* and *Methanol* (13:7). Add 2 mL of *Triethylamine* per L of the mixture, and adjust with *Glacial Acetic Acid* to a pH of 5.2.

Standard stock solution: Transfer 20 mg of USP Riboflavin RS to a 200-mL volumetric

flask, and add 180 mL of *Extraction solvent*. Immerse the flask for 5 min in a water bath maintained at 65°–75°. Mix well, and repeat if necessary until dissolved. Chill rapidly in a cold water bath to room temperature, and dilute with *Extraction solvent* to volume.

Standard solution: Dilute 5.0 mL of the *Standard stock solution* with *Extraction solvent* to 25.0 mL.

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without loss of shell material, and transfer the contents to a beaker. Remove any contents adhering to the shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the net weight of the Capsule contents. Transfer a portion of the Capsule contents, equivalent to 2 mg of riboflavin, to a 200-mL volumetric flask. Add 100.0 mL of *Extraction solvent*, and mix for 20 min using a wrist-action shaker. Immerse the flask in a water bath maintained at 70°–75°, and heat for 20 min. Mix on a vortex mixer for 30 s, cool to room temperature, and filter. Use the clear filtrate.

Sample solution for Tablets: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to a nominal amount of 2 mg of riboflavin, to a 200-mL volumetric flask. Add 100.0 mL of *Extraction solvent*, and mix for 20 min using a wrist-action shaker. Immerse the flask in a water bath maintained at 70°–75°, and heat for 20 min. Mix on a vortex mixer for 30 s, cool to room temperature, and filter. Use the clear filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing *L1*

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

Calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of riboflavin from the appropriate *Sample solution*

r_S = peak response of riboflavin from the *Standard solution*

C_S = concentration of USP Riboflavin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of riboflavin in the appropriate *Sample solution* (mg/mL)

Add the following:**▲ ● Chromatographic Methods, Procedure 3**

This procedure can be used to determine riboflavin in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of riboflavin from the formulation by mixtures of organic solvents, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solutions*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: 25 mg/mL of *Eдетate Disodium* in water

Mobile phase: Transfer 0.4 mL of *Triethylamine*, 15.0 mL of *Glacial Acetic Acid*, and 350 mL of *Methanol* to a 2000-mL volumetric flask. Dilute with 0.008 M *Sodium 1-Hexanesulfonate* to volume.

Standard stock solution: 0.08 mg/mL of USP Riboflavin RS in *Diluent*, with heating if necessary

Standard solution for Capsules/Tablets: Transfer 5.0 mL of *Standard stock solution* to a stoppered 125-mL flask. Add 10.0 mL of a mixture of *Methanol* and *Glacial Acetic Acid* (9:1) and 30.0 mL of a mixture of *Methanol* and *Ethylene Glycol* (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.

Standard solution for Oral Solution: 8 µg/mL of USP Riboflavin RS in *Diluent*, diluted from the *Standard stock solution*

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to 0.4 mg of riboflavin, to a stoppered 125-mL flask. Add 10.0 mL of a mixture of *Methanol* and *Glacial Acetic Acid* (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.

Sample solution for Oral Solution: Equivalent to 8 µg/mL of riboflavin from oral solution in the *Diluent*

Sample solution for Tablets: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 0.4 mg of riboflavin, to a stoppered 125-mL flask. Add 10.0 mL of a mixture of *Methanol* and *Glacial Acetic Acid* (9:1) and 30.0 mL of a mixture of

Methanol and *Ethylene Glycol* (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 50°

Flow rate: 2 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: Appropriate *Standard solution* and appropriate *Sample solution*

For Capsules and Tablets, calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of riboflavin from the appropriate *Sample solution*

r_S = peak response of riboflavin from the *Standard solution* for Capsules/Tablets

C_S = concentration of USP Riboflavin RS in the *Standard solution* for Capsules/Tablets (mg/mL)

C_U = nominal concentration of riboflavin in the appropriate *Sample solution* (mg/mL)

For Oral Solution, calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of riboflavin from the *Sample solution* for Oral Solution

r_S = peak response of riboflavin from the *Standard solution* for Oral Solution

C_S = concentration of USP Riboflavin RS in the *Standard solution* for Oral Solution (mg/mL)

C_U = nominal concentration of riboflavin in the *Sample solution* for Oral Solution (mg/mL)

▲USP40

Add the following:

▲ ● Chromatographic Methods, Procedure 4

This procedure can be used to determine riboflavin in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*

- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is a newly added procedure as part of the USP monograph modernization efforts. The procedure uses hydrophilic interaction liquid chromatography (HILIC), and the sample preparation involves the extraction of riboflavin from the formulation by the *Diluent*, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: *Methanol, Glacial Acetic Acid, and water (50:1:49)*

Solution A: *50 mM Ammonium Formate; adjust to a pH of 9.0 with Ammonium Hydroxide.*

Solution B: *Acetonitrile*

Mobile phase: Gradient elution. See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	11	89
8	17	83
15	23	77
20	30	70
21	50	50
24	50	50
25	11	89
30	11	89

Standard solution: Transfer 20 mg of USP Riboflavin RS to a 200-mL volumetric flask, and add 160 mL of *Diluent*. Immerse the flask in a hot water bath maintained at 65°–70° for 10 min with regular shaking or using a vortex mixer, until all of the solid materials are dissolved. Chill rapidly in a cold water bath for 10 min to room temperature, and dilute with *Diluent* to volume.

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to 2.5 mg of riboflavin, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 68°, heat for 10 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 10 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate.

Sample solution for Tablets: Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 2.5 mg of riboflavin, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 10 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 10 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [Note—Use the filtrate within 3 h of filtration.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 267 nm

Column: 4.6-mm × 15-cm; 3.5 μm packing L68

Flow rate: 1.2 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

Calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of riboflavin from the appropriate *Sample solution*

r_S = peak response of riboflavin from the *Standard solution*

C_S = concentration of USP Riboflavin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of riboflavin in the appropriate *Sample solution* (mg/mL)

▲USP40

ADDITIONAL REQUIREMENTS

- **USP Reference Standards** (11)

USP Riboflavin RS

¹ The concentrations of the hydrochloric acid and sodium hydroxide solutions used are not stated in each instance, because these concentrations may be varied depending upon the amount of material taken for assay, volume of test solution, and buffering effect of material.

BRIEFING

(531) **Thiamine Assay**, *USP* 38 page 334. This general test chapter is being revised as part of the USP monograph modernization effort. The following changes are proposed:

1. Convert the currently existing method in the chapter from the classic format to the redesigned format to more closely align with current *USP-NF* monograph style.

2. Consolidate all of the existing chromatographic procedures in the various monographs by migrating them to this chapter, which will serve as a central location for cross-references for all thiamine assay procedures described in the individual monographs.
3. Add a new high-performance liquid chromatography (HPLC) assay, *Chromatographic Methods, Procedure 7*, using hydrophilic interaction liquid chromatography (HILIC) for the determination of thiamine in the dietary supplements dosage forms.

Additionally, minor editorial changes have been made to update the chapter to current *USP* style.

The following Briefing list includes monographs and/or chapters that both reference the General Chapter under revision and require revision to keep references to the General Chapter accurate. Other monographs and/or chapters may also be listed, even where the reference to the General Chapter remains unchanged, as additional notice to stakeholders where there is believed to be potential for the change in the general chapter itself to affect pass-fail determinations for particular monograph articles.

Chapter Dependencies (click to expand).

- Thiamine Hydrochloride Tablets

(FI: H. Dinh.)

Correspondence Number—C133758

Comment deadline: January 31, 2016

(531) THIAMINE ASSAY

ASSAY

Change to read:

- **Chemical Methods, Procedure 1**

▲ The following procedure is provided for the determination of thiamine as an ingredient of pharmacopeial preparations containing other active constituents. The procedure involves thiamine reacting with potassium ferricyanide and subsequently being determined by fluorescence detection. Throughout the procedure, protect solutions containing and derived from the test specimen and the Reference Standard from the atmosphere and light, preferably by the use of low-actinic glassware.

▲*USP40*

Potassium ferricyanide solution: Dissolve 1.0 g of *Potassium Ferricyanide* in water to make 100 mL. Prepare fresh on the day of use.

Oxidizing reagent: Mix 4.0 mL of *Potassium Ferricyanide* solution with a sufficient amount of 3.5 N *Sodium Hydroxide* to make 100 mL. Use this solution within 4 h.

Quinine sulfate stock solution: Dissolve 10 mg of quinine sulfate in 0.1 N *Sulfuric Acid* to make 1000 mL. Preserve this solution, protected from light, in a refrigerator.

Quinine sulfate standard solution: Dilute 0.1 N *Sulfuric Acid* with *Quinine sulfate stock solution* (39:1). This solution fluoresces to approximately the same degree as the thiochrome obtained from 1 µg of thiamine hydrochloride and is used to correct the fluorometer at frequent intervals for variation in sensitivity from reading to reading within an assay. Prepare this solution fresh on the day of use.

Standard thiamine hydrochloride stock solution: Transfer about 25 mg of USP Thiamine Hydrochloride RS, accurately weighed, to a 1000-mL volumetric flask. Dissolve the weighed *Standard* in about 300 mL of dilute alcohol solution (1 in 5) adjusted with 3 N *Hydrochloric Acid* to a pH of 4.0, and add the acidified, dilute alcohol to volume. Store in a refrigerator in a light-resistant container. Prepare this stock solution fresh each month.

Standard solution: Dilute a portion of *Standard thiamine hydrochloride stock solution* quantitatively and stepwise with 0.2 N *Hydrochloric Acid* to obtain the *Standard solution*, each mL of which represents 0.2 µg of USP Thiamine Hydrochloride RS.

Sample solution: Place in a suitable volumetric flask a sufficient amount of the material to be assayed, accurately weighed or measured by volume as directed, such that when diluted to volume with 0.2 N *Hydrochloric Acid*, the resulting solution will contain about 100 µg of thiamine hydrochloride (or mononitrate) per mL. If the sample is difficult to dissolve, the solution may be heated on a steam bath, and then cooled and diluted with the acid to volume. Dilute 5 mL of this solution, quantitatively and stepwise, using 0.2 N *Hydrochloric Acid*, to an estimated concentration of 0.2 µg of thiamine hydrochloride (or mononitrate) per mL.

Instrumental conditions

(See *Fluorescence Spectroscopy* (853).)

Mode: Fluorescence

Excitation wavelength: 365 nm

Emission wavelength: 435 nm

Analysis: To each of three or more tubes (or other suitable vessels) of about 40-mL capacity, pipet 5 mL of *Standard solution*. To each of two of these tubes, add rapidly (within 1 to 2 s), with mixing, 3.0 mL of *Oxidizing reagent*, and within 30 s add 20.0 mL of *Isobutyl Alcohol*, then mix vigorously for 90 s by shaking the capped tubes manually, or by bubbling a stream of air through the mixture. Prepare a blank in the remaining tube of the standard by substituting for the *Oxidizing reagent* with an equal volume of 3.5 N *Sodium Hydroxide* and proceed in the same manner. Into each of three or more similar tubes, pipet 5 mL of the *Sample solution*. Treat these tubes in the same manner as directed for the tubes containing the *Standard solution*. Into each of the six tubes, pipet 2 mL of *Dehydrated Alcohol*, swirl for a few seconds, allow the phases to separate, and decant or draw off about 10 mL of the clear, supernatant isobutyl alcohol solution into the standardized cells; then measure the fluorescence in a suitable fluorometer, having an input filter of narrow transmittance range with a maximum at about 365 nm and an output filter of narrow transmittance range with a maximum at about 435 nm. Calculate the quantity, in µg, of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in each 5 mL of the *Sample solution*:

$$\text{Result} = (A - b)/(S - d)$$

A = average fluorometer readings of the portions of the *Sample solution* treated with the *Oxidizing reagent*

b = reading for the blank of the *Sample solution*

S = average fluorometer readings of the portions of the *Standard solution* treated with the *Oxidizing reagent*

d = reading for the blank of the *Standard solution*

Calculate the quantity, in mg, of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the assay material on the basis of the aliquots taken. Where indicated, the quantity, in mg, of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) may be calculated by multiplying the quantity of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) found by 0.9706.

Add the following:

▲ The following liquid chromatographic procedures are provided for the determination of thiamine as an active pharmaceutical ingredient, a dietary supplement ingredient, or a component in dietary supplements or pharmaceutical dosage forms.

Throughout these procedures, protect solutions containing and derived from the test specimen and the Reference Standards from the atmosphere and light, preferably by the use of low-actinic glassware. ▲USP40

Add the following:

▲ ● **Chromatographic Methods, Procedure 1**

This procedure can be used to determine thiamine in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of thiamine from the formulation by the *Diluent*, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: *Acetonitrile*, *Glacial Acetic Acid*, and water (5:1:94)

Mobile phase: A mixture of *Methanol*, *Glacial Acetic Acid*, and water (27:1:73) containing 140 mg of *Sodium 1-Hexanesulfonate* per 100 mL

Standard solution: Transfer 20 mg of USP Thiamine Hydrochloride RS to a 200-mL volumetric flask, and add 180 mL of the *Diluent*. Immerse the flask in a hot water bath maintained at 65°–70° for 10 min with regular shaking or using a vortex mixer until all of the solid materials are dissolved. Chill rapidly in a cold water bath for 10 min to room temperature, and dilute with the *Diluent* to volume.

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to 2.5 mg of thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of the *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath

maintained at 65°–70°, heat for 5 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 5 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [Note—Use the filtrate within 3 h of filtration.]

Sample solution for Tablets: Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 2.5 mg of thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of the *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 5 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 5 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [Note—Use the filtrate within 3 h of filtration.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; packing *L1*

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

For products containing thiamine hydrochloride, calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*

r_S = peak response of thiamine from the *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of thiamine hydrochloride in the appropriate *Sample solution* (mg/mL)

For products containing thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*

r_S = peak response of thiamine from the *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of thiamine mononitrate in the appropriate *Sample solution* (mg/mL)

M_{r1} = molecular weight of thiamine mononitrate, 327.36

M_{r2} = molecular weight of thiamine hydrochloride, 337.27

▲USP40

Add the following:

▲ ● **Chromatographic Methods, Procedure 2**

This procedure can be used to determine thiamine in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of thiamine from the formulation by dilute hydrochloric acid, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Solution A: 1.88 g/L of *Sodium 1-Hexanesulfonate* in 0.1% *Phosphoric Acid*

Mobile phase: *Solution A* and *Acetonitrile* (46:9)

Standard stock solution: 0.1 mg/mL of USP Thiamine Hydrochloride RS in 0.2 N *Hydrochloric Acid*

Standard solution: 0.02 mg/mL of USP Thiamine Hydrochloride RS from *Standard stock solution* diluted with 0.2 N *Hydrochloric Acid*

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Mix a portion of the Capsule contents with a volume of 0.2 N *Hydrochloric Acid* to obtain a concentration of 0.02 mg/mL of thiamine. Shake the solution for 15 min with a wrist-action shaker, and heat to boiling for 30 min. Cool to room temperature, and filter. Use the clear filtrate.

Sample solution for Tablets: Weigh and finely powder NLT 20 Tablets. Mix a portion of the powder with a volume of 0.2 N *Hydrochloric Acid* to obtain a concentration of 0.02 mg/mL of thiamine. Shake for 15 min with a wrist-action shaker, and heat to boiling for 30 min. Cool to room temperature, and filter. Use the clear filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing *L1*

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

For products containing thiamine hydrochloride, calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*

r_S = peak response of thiamine from the *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of thiamine hydrochloride in the appropriate *Sample solution* (mg/mL)

For products containing thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*

r_S = peak response of thiamine from the *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of thiamine mononitrate in the appropriate *Sample solution* (mg/mL)

M_{r1} = molecular weight of thiamine mononitrate, 327.36

M_{r2} = molecular weight of thiamine hydrochloride, 337.27

▲USP40

Add the following:

▲ ● **Chromatographic Methods, Procedure 3**

This procedure can be used to determine thiamine in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Oral Solution*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Oral Solution*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*

- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Oral Solution*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of thiamine from the formulation by mixtures of organic solvents, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solutions*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: 25 mg/mL of *Edetate Disodium* in water

Mobile phase: Transfer 0.4 mL of *Triethylamine*, 15.0 mL of *Glacial Acetic Acid*, and 350 mL of *Methanol* to a 2000-mL volumetric flask. Dilute with 0.008 M *Sodium 1-Hexanesulfonate* to volume.

Standard stock solution: 0.24 mg/mL of USP Thiamine Hydrochloride RS in the *Diluent*, with heating if necessary

Standard solution for Capsules/Tablets: Transfer 5.0 mL of *Standard stock solution* to a stoppered 125-mL flask. Add 10.0 mL of a mixture of *Methanol* and *Glacial Acetic Acid* (9:1) and 30.0 mL of a mixture of *Methanol* and *Ethylene Glycol* (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, and discard the first few mL of the filtrate.

Standard solution for Oral Solution: 24 µg/mL of USP Thiamine Hydrochloride RS, diluted from the *Standard stock solution* with *Diluent*

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to 1.2 mg of thiamine hydrochloride, to a stoppered 125-mL flask. Add 10.0 mL of a mixture of *Methanol* and *Glacial Acetic Acid* (9:1) and 30.0 mL of a mixture of *Methanol* and *Ethylene Glycol* (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, and discard the first few mL of the filtrate.

Sample solution for Oral Solution: Equivalent to 24 µg/mL of thiamine hydrochloride or thiamine mononitrate from oral solution in the *Diluent*

Sample solution for Tablets: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 1.2 mg of thiamine, to a stoppered 125-mL flask. Add 10.0 mL of a mixture of *Methanol* and *Glacial Acetic Acid* (9:1) and 30.0 mL of a mixture of *Methanol* and *Ethylene Glycol* (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, and discard the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 50°

Flow rate: 2 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: Appropriate *Standard solution* and appropriate *Sample solution*

For products containing thiamine hydrochloride, calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*

r_S = peak response of thiamine from the appropriate *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the appropriate *Standard solution* (mg/mL)

C_U = nominal concentration of thiamine in the appropriate *Sample solution* (mg/mL)

For products containing thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*

r_S = peak response of thiamine from the appropriate *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the appropriate *Standard solution* (mg/mL)

C_U = nominal concentration of thiamine mononitrate in the appropriate *Sample solution* (mg/mL)

M_{r1} = molecular weight of thiamine mononitrate, 327.36

M_{r2} = molecular weight of thiamine hydrochloride, 337.27

▲USP40

Add the following:

▲ ● **Chromatographic Methods, Procedure 4**

This procedure can be used to determine thiamine in:

- An active pharmaceutical ingredient
- A dietary ingredient

This is the procedure that involves the dissolution of the sample directly into the *Mobile phase*. The procedure is applicable for the determination of thiamine hydrochloride or thiamine mononitrate as active pharmaceutical or dietary supplement ingredients.

Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Solution A: 0.005 M *Sodium 1-Octanesulfonate* in dilute *Glacial Acetic Acid* (1 in 100)

Solution B: *Methanol* and *Acetonitrile* (3:2)

Mobile phase: *Solution A* and *Solution B* (60:40)

Internal standard solution: 2% (v/v) of methylbenzoate in *Methanol*

Standard solution: Prepare a 1-mg/mL solution of USP Thiamine Hydrochloride RS in *Mobile phase*. Transfer 20.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. The *Standard solution* contains 400 µg/mL of thiamine hydrochloride.

Sample solution: Prepare a 2-mg/mL solution of thiamine hydrochloride or thiamine mononitrate in *Mobile phase*. Transfer 10.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing *L1*

Flow rate: 1 mL/min. [Note—The flow rate may be adjusted to obtain a retention time of about 12 min for thiamine.]

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between the thiamine and methylbenzoate peaks

Tailing factor: NMT 2.0 for the thiamine peak

Column efficiency: NLT 1500 theoretical plates for the thiamine

Relative standard deviation: NMT 2.0% for the ratios of thiamine peak response to the internal standard peak response

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of *Sample* taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = internal standard ratio (peak response of thiamine/peak response of the internal standard) from the *Sample solution*

R_S = internal standard ratio (peak response of thiamine/peak response of the internal standard) from the *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of thiamine hydrochloride in the *Sample solution* (mg/mL)

If the active pharmaceutical or dietary supplement ingredient is thiamine mononitrate,

calculate the percentage of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of *Sample* taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = internal standard ratio (peak response of thiamine/peak response of the internal standard) from the *Sample solution*

R_S = internal standard ratio (peak response of thiamine/peak response of the internal standard) from the *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of thiamine mononitrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of thiamine mononitrate, 327.36

M_{r2} = molecular weight of thiamine hydrochloride, 337.27

▲USP40

Add the following:

▲ ● Chromatographic Methods, Procedure 5

This procedure can be used to determine thiamine in:

- *Thiamine Hydrochloride Injection*
- *Thiamine Hydrochloride Oral Solution*
- *Thiamine Mononitrate Oral Solution*

This is the procedure that involves the dissolution of the sample directly into the *Mobile phase*. The procedure is applicable for the determination of thiamine hydrochloride or thiamine mononitrate as an active ingredient in the formulations such as those used in *Thiamine Hydrochloride Injection*, *Thiamine Hydrochloride Oral Solution*, and *Thiamine Mononitrate Oral Solution*.

Unless specified in the individual monographs, the *Standard solution*, *Internal standard solution*, and *Sample solution* are prepared as follows.

Mobile phase: *Methanol* and 0.04 M aqueous *Monobasic Potassium Phosphate* (45:55)

Internal standard solution: 100 µg/mL of methylparaben in *Mobile phase*

Standard stock solution: 500 µg/mL of USP Thiamine Hydrochloride RS in *Mobile phase*

Standard solution: Dilute a mixture of equal volumes of the *Standard stock solution* and *Internal standard solution* with *Mobile phase* to obtain a concentration of USP Thiamine Hydrochloride RS of about 50 µg/mL.

Sample stock solution: Equivalent to 500 µg/mL of thiamine hydrochloride or thiamine mononitrate in *Mobile phase* from an accurately measured volume of Oral Solution or Injection

Sample solution: Dilute a mixture of equal volumes of the *Internal standard solution* and *Sample stock solution* with *Mobile phase* to obtain a concentration of thiamine hydrochloride or thiamine mononitrate of about 50 µg/mL.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing *L1*

Flow rate: 1 mL/min

Injection volume: 25 μ L

System suitability

Sample: *Standard solution*. [Note—The relative retention times for thiamine and methylparaben are about 0.35 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6.0 between thiamine and methylparaben

Column efficiency: NLT 1500 theoretical plates for the thiamine peak

Relative standard deviation: NMT 2.0% for the ratios of thiamine peak response to the methylparaben peak response

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of *Sample* taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = internal standard ratio (peak response of thiamine/peak response of the methylparaben) from the *Sample solution*

R_S = internal standard ratio (peak response of thiamine/peak response of the methylparaben) from the *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of thiamine hydrochloride in the *Sample solution* (mg/mL)

If the products contain thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of *Sample* taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = internal standard ratio (peak response of thiamine/peak response of the internal standard) from the *Sample solution*

R_S = internal standard ratio (peak response of thiamine/peak response of the internal standard) from the *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of thiamine mononitrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of thiamine mononitrate, 327.36

M_{r2} = molecular weight of thiamine hydrochloride, 337.27

▲USP40

Add the following:

▲ ● **Chromatographic Methods, Procedure 6**

This procedure can be used to determine thiamine in the following:

- *Thiamine Hydrochloride Tablet*

This is the procedure that involves the dissolution of the sample directly into water. The procedure is applicable to the performance (dissolution) test for thiamine hydrochloride

tablets.

Unless specified in the individual monographs, the *Standard solution* and *Sample solution* are prepared as follows.

Medium: Water, 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Mobile phase: A mixture of *Methanol*, *Glacial Acetic Acid*, and water (27:1:73) containing 140 mg of *Sodium 1-Hexanesulfonate* per 100 mL

Standard solution: A known concentration of USP Thiamine Hydrochloride RS in *Medium* (water)

Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* (water) if necessary

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; packing *L1*

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V/L) \times 100$$

r_U = peak response of thiamine from the *Sample solution*

r_S = peak response of thiamine from the *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*

V = volume of *Medium*, 900 mL

L = labeled amount of thiamine hydrochloride (mg/Tablet)

▲USP40

Add the following:

▲ ● Chromatographic Methods, Procedure 7

This procedure can be used to determine thiamine in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Oral Solution*

- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Oral Solution*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Oral Solution*
- *Water-Soluble Vitamins with Minerals Tablets*

This is a newly added procedure as part of the USP monograph modernization efforts. The procedure uses hydrophilic interaction liquid chromatography (HILIC), and the sample preparation involves the extraction of thiamine from the formulation by the *Diluent*, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: *Methanol, Glacial Acetic Acid*, and water (50:1:49)

Solution A: 50 mM *Ammonium Formate*; adjust to a pH of 9.0 with *Ammonium Hydroxide*.

Solution B: *Acetonitrile*

Mobile phase: Gradient elution. See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	11	89
8	17	83
15	23	77
20	30	70
21	50	50
24	50	50
25	11	89
30	11	89

Standard solution: Transfer 20 mg of USP Thiamine Hydrochloride RS to a 200-mL volumetric flask, and add 160 mL of the *Diluent*. Immerse the flask in a hot water bath maintained at 65°–70° for 10 min with regular shaking or using a vortex mixer, until all of the solid materials are dissolved. Chill rapidly in a cold water bath for 10 min to room temperature, and dilute with the *Diluent* to volume.

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to 2.5 mg of thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex

mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 68°, heat for 10 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 10 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate.

Sample solution for Oral Solution: Equivalent to 0.1 mg/mL of thiamine hydrochloride in *Diluent*, from an accurately measured volume of Oral Solution. In an appropriate volumetric flask, dissolve the sample with the *Diluent* to about 80% of the total volume, immerse the flask in a water bath maintained at 68° for 10 min, chill rapidly in a cold water bath to room temperature, and dilute with the *Diluent*. Mix well, and filter a portion of the solution; finally, use the clear filtrate.

Sample solution for Tablets: Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 2.5 mg of thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of the *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 10 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 10 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [Note—Use the filtrate within 3 h of filtration.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 267 nm

Column: 4.6-mm × 15-cm; 3.5 μm packing L68

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

For products containing thiamine hydrochloride, calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*

r_S = peak response of thiamine from the *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of thiamine in the appropriate *Sample solution* (mg/mL)

For products containing thiamine mononitrate, calculate the percentage of the labeled

amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*

r_S = peak response of thiamine from the *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of thiamine mononitrate in the appropriate *Sample solution* (mg/mL)

M_{r1} = molecular weight of thiamine mononitrate, 327.36

M_{r2} = molecular weight of thiamine hydrochloride, 337.27

▲USP40

ADDITIONAL REQUIREMENTS

- **USP Reference Standards** <11>

USP Thiamine Hydrochloride RS

BRIEFING

<797> **Pharmaceutical Compounding—Sterile Preparations**, *USP 39* page 626. It is proposed to revise this chapter to improve clarity, respond to stakeholder input, and reflect new science. Major edits to the chapter include:

1. Reorganized existing chapter to group similar topics together, eliminate redundancies, and clarify requirements. Key procedural information is placed in boxes so that it can be easily referenced and followed.
2. Collapsed compounded sterile preparations (CSP) microbial risk categories from three to two and changed terminology. No sterile compounding is inherently “low risk” and preparation of all CSPs must be done carefully. Categories were renamed neutrally as Category 1 and 2 CSPs, which are distinguished primarily by the conditions under which they are made and the time within which they are used. Category 1 CSPs have a shorter beyond use date (BUD) and may be prepared in a segregated compounding area; Category 2 CSPs have a longer BUD and must be prepared in a cleanroom environment.
3. Removed specific information on handling of hazardous drugs and added references to *Hazardous Drugs—Handling in Healthcare Settings* <800>.
4. Introduced terminology for “in-use time” to refer to the time before which a conventionally manufactured product used to make a CSP must be used after it has been opened or punctured, or a CSP must be used after it has been opened or punctured.

Additionally, the chapter was revised to add requirements for maintaining master formulation and compounding records, provide guidance on use of isolators, and add guidance for sterility testing of CSP prepared in batch sizes of less than 40.

The proposed chapter is posted online at www.usp.org/usp-nf/notices/general-chapter-797-proposed-revision with line numbers. Please provide the line numbers corresponding to your comments when submitting comments to CompoundingSL@usp.org.

Additionally, minor editorial changes have been made to update the chapter to current *USP*

style.

The following Briefing list includes monographs and/or chapters that both reference the General Chapter under revision and require revision to keep references to the General Chapter accurate. Other monographs and/or chapters may also be listed, even where the reference to the General Chapter remains unchanged, as additional notice to stakeholders where there is believed to be potential for the change in the general chapter itself to affect pass-fail determinations for particular monograph articles.

Chapter Dependencies (click to expand).

7 LABELING	659 PACKAGING AND STORAGE REQUIREMENTS
* ()	* ()
795 PHARMACEUTICAL COMPOUNDING—	1046 CELLULAR AND TISSUE-BASED PRODUCTS
* ()	* ()
NONSTERILE PREPARATIONS	
1047 GENE THERAPY PRODUCTS	1066 PHYSICAL ENVIRONMENTS THAT PROMOTE
* ()	* ()
	SAFE MEDICATION USE
1151 PHARMACEUTICAL DOSAGE FORMS	1160 PHARMACEUTICAL CALCULATIONS IN
* ()	* ()
	PHARMACY PRACTICE
1163 QUALITY ASSURANCE IN	1197 GOOD DISTRIBUTION PRACTICES FOR
* ()	* ()
PHARMACEUTICAL COMPOUNDING	BULK PHARMACEUTICAL EXCIPIENTS
* Cisapride Compounded Injection, Veterinary	* Cyclosporine Compounded Ophthalmic Solution, Veterinary
* Hydrochloric Acid Injection	* Methylene Blue Injection, Veterinary
* Sodium Bromide Injection, Veterinary	* Voriconazole Compounded Ophthalmic Solution, Veterinary

(CMP: J. Sun.)

Correspondence Number—C163428

Comment deadline: January 31, 2016

(797) PHARMACEUTICAL COMPOUNDING—STERILE PREPARATIONS

Change to read:

INTRODUCTION

~~The objective of this chapter is to describe conditions and practices to prevent harm, including death, to patients that could result from (1) microbial contamination (nonsterility), (2)~~

excessive bacterial endotoxins, (3) variability in the intended strength of correct ingredients that exceeds either monograph limits for official articles (see "official" and "article" in the *General Notices and Requirements*) or 10% for nonofficial articles, (4) unintended chemical and physical contaminants, and (5) ingredients of inappropriate quality in compounded sterile preparations (CSPs). Contaminated CSPs are potentially most hazardous to patients when administered into body cavities, central nervous and vascular systems, eyes, and joints, and when used as baths for live organs and tissues. When CSPs contain excessive bacterial endotoxins (see *Bacterial Endotoxins Test* (85)), they are potentially most hazardous to patients when administered into the central nervous system.

Despite the extensive attention in this chapter to the provision, maintenance, and evaluation of air quality, the avoidance of direct or physical contact contamination is paramount. It is generally acknowledged that direct or physical contact of critical sites of CSPs with contaminants, especially microbial sources, poses the greatest probability of risk to patients. Therefore, compounding personnel must be meticulously conscientious in precluding contact contamination of CSPs both within and outside ISO Class 5 (see *Table 1*) areas.

To achieve the above five conditions and practices, this chapter provides minimum practice and quality standards for CSPs of drugs and nutrients based on current scientific information and best sterile compounding practices. The use of technologies, techniques, materials, and procedures other than those described in this chapter is not prohibited so long as they have been proven to be equivalent or superior with statistical significance to those described herein. The standards in this chapter do not pertain to the *clinical administration* of CSPs to patients via application, implantation, infusion, inhalation, injection, insertion, instillation, and irrigation, which are the routes of administration. Four specific categories of CSPs are described in this chapter: low risk level, medium risk level, and high risk level, and immediate use. Sterile compounding differs from nonsterile compounding (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)—

• (CN 1 May 2016)

) primarily by requiring the maintenance of sterility when compounding exclusively with sterile ingredients and components (i.e., with immediate use CSPs, low risk level CSPs, and medium risk level CSPs) and the achievement of sterility when compounding with nonsterile ingredients and components (i.e., with high risk level CSPs). Some differences between standards for sterile compounding in this chapter and those for nonsterile compounding in *Pharmaceutical Compounding—Nonsterile Preparations* (795) include, but are not limited to, ISO-classified air environments (see *Table 1*); personnel garbing and gloving; personnel training and testing in principles and practices of aseptic manipulations and sterilization; environmental quality specifications and monitoring; and disinfection of gloves and surfaces of ISO Class 5 (see *Table 1*) sources.

Table 1. ISO Classification of Particulate Matter in Room Air (limits are in particles of 0.5 μm and larger per cubic meter [current ISO] and cubic feet [former Federal Standard No. 209E, FS-209E])*

Class Name		Particle Count	
ISO Class	U.S. FS 209E	ISO, m ³	FS 209E, ft ³
3	Class 1	35.2	1
4	Class 10	352	10
5	Class 100	3,520	100
6	Class 1,000	35,200	1,000
7	Class 10,000	352,000	10,000
8	Class 100,000	3,520,000	100,000

*Adapted from former Federal Standard No. 209E, General Services Administration, Washington, DC, 20407 (September 11, 1992) and ISO 14644-1:1999, Cleanrooms and associated controlled environments—Part 1: Classification of air cleanliness. For example, 3,520 particles of 0.5 µm per m³ or larger (ISO Class 5) is equivalent to 100 particles per ft³ (Class 100) (1 m³ = 35.2 ft³).

The standards in this chapter are intended to apply to all persons who prepare CSPs and all places where CSPs are prepared (e.g., hospitals and other healthcare institutions, patient treatment clinics, pharmacies, physicians' practice facilities, and other locations and facilities in which CSPs are prepared, stored, and transported). Persons who perform sterile compounding include pharmacists, nurses, pharmacy technicians, and physicians. These terms recognize that most sterile compounding is performed by or under the supervision of pharmacists in pharmacies and also that this chapter applies to all healthcare personnel who prepare, store, and transport CSPs. For the purposes of this chapter, CSPs include any of the following:

1. Compounded biologics, diagnostics, drugs, nutrients, and radiopharmaceuticals, including but not limited to the following dosage forms that must be sterile when they are administered to patients: aqueous bronchial and nasal inhalations, baths and soaks for live organs and tissues, injections (e.g., colloidal dispersions, emulsions, solutions, suspensions), irrigations for wounds and body cavities, ophthalmic drops and ointments, and tissue implants.
2. Manufactured sterile products that are either prepared strictly according to the instructions appearing in manufacturers' approved labeling (product package inserts) or prepared differently than published in such labeling. [Note—The FDA states that "Compounding does not include mixing, reconstituting, or similar acts that are performed in accordance with the directions contained in approved labeling provided by the product's manufacturer and other manufacturer directions consistent with that labeling" [21 USC 321 (k) and (m)]. However, the FDA approved labeling (product package insert) rarely describes environmental quality (e.g., ISO Class air designation, exposure durations to non-ISO classified air, personnel garbing and gloving, and other aseptic precautions by which sterile products are to be prepared for administration). Beyond-use exposure and storage dates or times (see *General Notices and Requirements and Pharmaceutical Compounding—Nonsterile Preparations* (795)) for sterile products that have been either opened or prepared for administration are not specified in all package inserts for all sterile products. Furthermore, when such durations are specified, they may refer to chemical stability and not necessarily to microbiological purity or safety.]

ORGANIZATION OF THIS CHAPTER

The sections in this chapter are organized to facilitate the practitioner's understanding of the fundamental accuracy and quality practices for preparing CSPs. They provide a foundation for the development and implementation of essential procedures for the safe preparation of low-risk, medium-risk, and high-risk level CSPs and immediate-use CSPs, which are classified according to the potential for microbial, chemical, and physical contamination. The chapter is divided into the following main sections:

- Responsibility of Compounding Personnel
- CSP Microbial Contamination Risk Levels
- Personnel Training and Evaluation in Aseptic Manipulation Skills
- Immediate-Use CSPs
- Single-Dose and Multiple-Dose Containers
- Hazardous Drugs as CSPs
- Radiopharmaceuticals as CSPs
- Allergen Extracts as CSPs
- Verification of Compounding Accuracy and Sterility
- Environmental Quality and Control
- Suggested Standard Operating Procedures (SOPs)
- Elements of Quality Control
- Verification of Automated Compounding Devices (ACDs) for Parenteral Nutrition Compounding
- Finished Preparation Release Checks and Tests
- Storage and Beyond-Use Dating
- Maintaining Sterility, Purity, and Stability of Dispensed and Distributed CSPs
- Patient or Caregiver Training
- Patient Monitoring and Adverse Events Reporting
- Quality Assurance (QA) Program
- Abbreviations and Acronyms
- Glossary
- Appendices I–V

The requirements and recommendations in this chapter are summarized in *Appendix I*. A list of abbreviations and acronyms is included at the end of the main text, before the *Appendices*.

All personnel who prepare CSPs shall be responsible for understanding these fundamental practices and precautions, for developing and implementing appropriate procedures, and for continually evaluating these procedures and the quality of final CSPs to prevent harm.

RESPONSIBILITY OF COMPOUNDING PERSONNEL

Compounding personnel are responsible for ensuring that CSPs are accurately identified, measured, diluted, and mixed and are correctly purified, sterilized, packaged, sealed, labeled, stored, dispensed, and distributed. These performance responsibilities include maintaining appropriate cleanliness conditions and providing labeling and supplementary instructions for the proper clinical administration of CSPs.

Compounding supervisors shall ensure, through either direct measurement or appropriate information sources, that specific CSPs maintain their labeled strength within monograph limits for *USP* articles, or within 10% if not specified, until their BUDs. All CSPs are prepared in a manner that maintains sterility and minimizes the introduction of particulate matter.

~~A written quality assurance procedure includes the following in-process checks that are applied, as appropriate, to specific CSPs: accuracy and precision of measuring and weighing; the requirement for sterility; methods of sterilization and purification; safe limits and ranges for strength of ingredients, bacterial endotoxins, and particulate matter; pH; labeling accuracy and completeness; BUD assignment; and packaging and storage requirements. The dispenser shall, when appropriate and practicable, obtain and evaluate results of testing for identity, strength, purity, and sterility before a CSP is dispensed. Qualified licensed healthcare professionals who supervise compounding and dispensing of CSPs shall ensure that the following objectives are achieved:~~

- ~~1. Compounding personnel are adequately skilled, educated, instructed, and trained to correctly perform and document the following activities in their sterile compounding duties:~~
 - ~~a. perform antiseptic hand cleansing and disinfection of nonsterile compounding surfaces;~~
 - ~~b. select and appropriately don protective garb;~~
 - ~~c. maintain or achieve sterility of CSPs in ISO Class 5 (see *Table 1*) PEC devices and protect personnel and compounding environments from contamination by radioactive, cytotoxic, and chemotoxic drugs (see *Hazardous Drugs as CSPs* and *Radiopharmaceuticals as CSPs*);~~
 - ~~d. identify, weigh, and measure ingredients; and~~
 - ~~e. manipulate sterile products aseptically, sterilize high-risk level CSPs, and label and quality inspect CSPs.~~
- ~~2. Ingredients have their correct identity, quality, and purity.~~
- ~~3. Opened or partially used packages of ingredients for subsequent use in CSPs are properly stored under restricted access conditions in the compounding facility. Such packages cannot be used when visual inspection detects unauthorized breaks in the container, closure, and seal; when the contents do not possess the expected appearance, aroma, and texture; when the contents do not pass identification tests specified by the compounding facility; and when either the BUD or expiration date has been exceeded.~~
- ~~4. Water-containing CSPs that are nonsterile during any phase of the compounding procedure are sterilized within 6 hours after completing the preparation in order to minimize the generation of bacterial endotoxins.~~
- ~~5. Sterilization methods achieve sterility of CSPs while maintaining the labeled strength of active ingredients and the physical integrity of packaging.~~
- ~~6. Measuring, mixing, sterilizing, and purifying devices are clean, appropriately accurate, and effective for their intended use.~~
- ~~7. Potential harm from added substances and differences in rate and extent of bioavailability of active ingredients for other than oral route of administration are carefully evaluated before such CSPs are dispensed and administered.~~
- ~~8. Packaging selected for CSPs is appropriate to preserve the sterility and strength until the BUD.~~
- ~~9. While being used, the compounding environment maintains the sterility or the presterilization purity, whichever is appropriate, of the CSP.~~
- ~~10. Labels on CSPs list the names and amounts or concentrations of active ingredients, and the labels or labeling of injections (see *Preservation, Packaging, Storage, and Labeling in the General Notices and Requirements*) list the names and amounts or concentrations~~

of all ingredients (see *Labeling (7)* (CN-1 May 2016)). Before being dispensed or administered, the clarity of solutions is visually confirmed; also, the identity and amounts of ingredients, procedures to prepare and sterilize CSPs, and specific release criteria are reviewed to ensure their accuracy and completeness.

11. BUDs are assigned on the basis of direct testing or extrapolation from reliable literature sources and other documentation (see *Stability Criteria and Beyond-Use Dating* under *Pharmaceutical Compounding—Nonsterile Preparations (795)*).
12. Procedures for measuring, mixing, dilution, purification, sterilization, packaging, and labeling conform to the correct sequence and quality established for the specified CSP.
13. Deficiencies in compounding, labeling, packaging, and quality testing and inspection can be rapidly identified and corrected.
14. When time and personnel availability so permit, compounding manipulations and procedures are separated from postcompounding quality inspection and review before CSPs are dispensed.

This chapter emphasizes the need to maintain high standards for the quality and control of processes, components, and environments and for the skill and knowledge of personnel who prepare CSPs. The rigor of in-process quality control checks and of postcompounding quality inspection and testing increases with the potential hazard of the route of administration. For example, nonsterility, excessive bacterial endotoxin contamination, large errors in strength of correct ingredients, and incorrect ingredients in CSPs are potentially more dangerous to patients when the CSPs are administered into the vascular and central nervous systems than when administered by most other routes.

CSP MICROBIAL CONTAMINATION RISK LEVELS

The three contamination categories for CSPs described in this section are assigned primarily according to the potential for microbial contamination during the compounding of low-risk level CSPs and medium-risk level CSPs or the potential for not sterilizing high-risk level CSPs, any of which would subject patients to risk of harm, including death. High-risk level CSPs must be sterilized before being administered to patients. The appropriate risk level—low, medium, or high—is assigned according to the corresponding probability of contaminating a CSP with (1) microbial contamination (e.g., microbial organisms, spores, endotoxins) and (2) chemical and physical contamination (e.g., foreign chemicals, physical matter). Potential sources of contamination include, but are not limited to, solid and liquid matter from compounding personnel and objects; nonsterile components employed and incorporated before terminal sterilization; inappropriate conditions within the restricted compounding environment; prolonged presterilization procedures with aqueous preparations; and nonsterile dosage forms used to compound CSPs.

The characteristics described below for low-, medium-, and high-risk level CSPs are intended as a guide to the breadth and depth of care necessary in compounding, but they are neither exhaustive nor prescriptive. The licensed healthcare professionals who supervise compounding are responsible for determining the procedural and environmental quality practices and attributes that are necessary for the risk level they assign to specific CSPs.

These risk levels apply to the quality of CSPs immediately after the final aseptic mixing or filling or immediately after the final sterilization, unless precluded by the specific characteristics of

the preparation. Upon subsequent storage and shipping of freshly finished CSPs, an increase in the risks of chemical degradation of ingredients, contamination from physical damage to packaging, and permeability of plastic and elastomeric packaging is expected. In such cases, compounding personnel are responsible for considering the potential additional risks to the integrity of CSPs when assigning BUDs. The pre-administration storage duration and temperature limits specified in the following subsections apply in the absence of direct sterility testing results that justify different limits for specific CSPs.

Low-Risk Level CSPs

CSPs compounded under all the following conditions are at a low risk of contamination.

Low-Risk Conditions—

1. The CSPs are compounded with aseptic manipulations entirely within ISO Class 5 (see *Table 1*) or better air quality using only sterile ingredients, products, components, and devices.
2. The compounding involves only transfer, measuring, and mixing manipulations using not more than three commercially manufactured packages of sterile products and not more than two entries into any one sterile container or package (e.g., bag, vial) of sterile product or administration container/device to prepare the CSP.
3. Manipulations are limited to aseptically opening ampuls, penetrating disinfected stoppers on vials with sterile needles and syringes, and transferring sterile liquids in sterile syringes to sterile administration devices, package containers of other sterile products, and containers for storage and dispensing.
4. For a low risk level preparation, in the absence of passing a sterility test (see *Sterility Tests (71)*), the storage periods cannot exceed the following time periods: before administration, the CSPs are properly stored and are exposed for not more than 48 hours at controlled room temperature (see *General Notices and Requirements*), for not more than 14 days at a cold temperature (see *General Notices and Requirements*), and for 45 days in solid frozen state between -25° and -10° .

Examples of Low-Risk Compounding—

1. Single volume transfers of sterile dosage forms from ampuls, bottles, bags, and vials using sterile syringes with sterile needles, other administration devices, and other sterile containers. The solution content of ampuls should be passed through a sterile filter to remove any particles.
2. Simple aseptic measuring and transferring with not more than three packages of manufactured sterile products, including an infusion or diluent solution to compound drug admixtures and nutritional solutions.

Low-Risk Level CSPs with 12-Hour or Less BUD—If the PEC is a CAI or CACI that does not meet the requirements described in *Placement of Primary Engineering Controls* or is a laminar airflow workbench (LAFW) or a biological safety cabinet (BSC) that cannot be located within an ISO-Class 7 (see *Table 1*) buffer area, then only low risk level nonhazardous and radiopharmaceutical CSPs pursuant to a physician's order for a specific patient may be prepared, and administration of such CSPs shall commence within 12 hours of preparation or as recommended in the manufacturers' package insert, whichever is less. Low risk level CSPs with

a 12-hour or less BUD shall meet all of the following four criteria:

1. PECs (LAFWs, BSCs, CAIs, CACIs,) shall be certified and maintain ISO Class 5 (see *Table 1*) as described in *Facility Design and Environmental Controls* for exposure of critical sites and shall be in a segregated compounding area restricted to sterile compounding activities that minimize the risk of CSP contamination.
2. The segregated compounding area shall not be in a location that has unsealed windows or doors that connect to the outdoors or high traffic flow, or that is adjacent to construction sites, warehouses, or food preparation. Note that this list is not intended to be all inclusive.
3. Personnel shall follow the procedures described in *Personnel Cleansing and Garbing and Additional Personnel Requirements* prior to compounding. Sinks should not be located adjacent to the ISO Class 5 (see *Table 1*) PEC. Sinks should be separated from the immediate area of the ISO Class 5 (see *Table 1*) PEC device.
4. The specifications in *Cleaning and Disinfecting the Sterile Compounding Areas, Personnel Training and Competency Evaluation of Garbing, Aseptic Work Practices and Cleaning/Disinfection Procedures*, and *Viable and Nonviable Environmental Sampling (ES) Testing* shall be followed as described in the chapter.

Compounding personnel must recognize that the absence of an ISO Class 7 (see *Table 1*) buffer area environment in a general uncontrolled environment increases the potential of microbial contamination, and administration durations of microbially contaminated CSPs exceeding a few hours increase the potential for clinically significant microbial colonization, and thus for patient harm, especially in critically ill or immunocompromised patients.

Quality Assurance—Quality assurance practices include, but are not limited to the following:

1. Routine disinfection and air quality testing of the direct compounding environment to minimize microbial surface contamination and maintain ISO Class 5 (see *Table 1*) air quality.
2. Visual confirmation that compounding personnel are properly donning and wearing appropriate items and types of protective garments, including eye protection and face masks.
3. Review of all orders and packages of ingredients to ensure that the correct identity and amounts of ingredients were compounded.
4. Visual inspection of CSPs to ensure the absence of particulate matter in solutions, the absence of leakage from vials and bags, and the accuracy and thoroughness of labeling.

Media-Fill Test Procedure—This test or an equivalent test is performed at least annually by each person authorized to compound in a low-risk level environment under conditions that closely simulate the most challenging or stressful conditions encountered during compounding of low-risk level CSPs. Once begun, this test is completed without interruption. *Example of test procedure:* within an ISO Class 5 (see *Table 1*) air quality environment, three sets of four 5-mL aliquots of sterile Soybean-Casein Digest Medium (also known as trypticase soy broth or trypticase soy agar [TSA]) are transferred with the same sterile 10-mL syringe and vented needle combination into separate sealed, empty, sterile 30-mL clear vials (i.e., four 5-mL aliquots into each of three 30-mL vials). Sterile adhesive seals are aseptically affixed to the rubber closures on the three filled vials, then the vials are incubated at 20° to 25° or at 30° to 35° for a minimum of 14 days. If two temperatures are used for incubation of media-filled

~~samples, then these filled containers should be incubated for at least 7 days at each temperature (see *Microbiological Control and Monitoring of Aseptic Processing Environments* (1116)). Inspect for microbial growth over 14 days as described in *Personnel Training and Competency Evaluation of Garbing, Aseptic Work Practices and Cleaning/Disinfection Procedures*.~~

Medium-Risk Level CSPs

~~When CSPs are compounded aseptically under *Low-Risk Conditions* and one or more of the following conditions exists, such CSPs are at a medium risk of contamination.~~

Medium-Risk Conditions—

- ~~1. Multiple individual or small doses of sterile products are combined or pooled to prepare a CSP that will be administered either to multiple patients or to one patient on multiple occasions.~~
- ~~2. The compounding process includes complex aseptic manipulations other than the single-volume transfer.~~
- ~~3. The compounding process requires unusually long duration, such as that required to complete dissolution or homogeneous mixing.~~
- ~~4. For a medium-risk preparation, in the absence of passing a sterility test (see *Sterility Tests* (71)), the storage periods cannot exceed the following time periods: before administration, the CSPs are properly stored and are exposed for not more than 30 hours at controlled room temperature (see *General Notices and Requirements*), for not more than 9 days at a cold temperature (see *General Notices and Requirements*), and for 45 days in solid frozen state between -25° and -10° .~~

Examples of Medium-Risk Compounding—

- ~~1. Compounding of total parenteral nutrition fluids using manual or automated devices during which there are multiple injections, detachments, and attachments of nutrient source products to the device or machine to deliver all nutritional components to a final sterile container.~~
- ~~2. Filling of reservoirs of injection and infusion devices with more than three sterile drug products and evacuation of air from those reservoirs before the filled device is dispensed.~~
- ~~3. Transfer of volumes from multiple ampuls or vials into one or more final sterile containers.~~

Quality Assurance—~~Quality assurance procedures for medium-risk level CSPs include all those for low-risk level CSPs, as well as a more challenging media-fill test passed annually or more frequently.~~

Media-Fill Test Procedure—~~This test or an equivalent test is performed at least annually under conditions that closely simulate the most challenging or stressful conditions encountered during compounding. Once begun, this test is completed without interruption. *Example of test procedure:* within an ISO Class 5 (see *Table 1*) air quality environment, six 100-mL aliquots of sterile Soybean Casein Digest Medium are aseptically transferred by gravity through separate tubing sets into separate evacuated sterile containers. The six containers are then arranged as three pairs, and a sterile 10-mL syringe and 18-gauge needle combination is used to exchange two 5-mL aliquots of medium from one container to the other container in the pair. For~~

example, after a 5-mL aliquot from the first container is added to the second container in the pair, the second container is agitated for 10 seconds, then a 5-mL aliquot is removed and returned to the first container in the pair. The first container is then agitated for 10 seconds, and the next 5-mL aliquot is transferred from it back to the second container in the pair. Following the two 5-mL aliquot exchanges in each pair of containers, a 5-mL aliquot of medium from each container is aseptically injected into a sealed, empty, sterile 10-mL clear vial, using a sterile 10-mL syringe and vented needle. Sterile adhesive seals are aseptically affixed to the rubber closures on the three filled vials, then the vials are incubated at 20° to 25° or at 30° to 35° for a minimum of 14 days. If two temperatures are used for incubation of media-filled samples, then these filled containers should be incubated for at least 7 days at each temperature (see *Microbiological Control and Monitoring of Aseptic Processing Environments* (1116)). Inspect for microbial growth over 14 days as described in *Personnel Training and Competency Evaluation of Garbing, Aseptic Work Practices and Cleaning/Disinfection Procedures*.

High-Risk Level CSPs

CSPs compounded under any of the following conditions are either contaminated or at a high risk to become contaminated:

High-Risk Conditions—

1. Nonsterile ingredients, including manufactured products not intended for sterile routes of administration (e.g., oral), are incorporated or a nonsterile device is employed before terminal sterilization.
2. Any of the following are exposed to air quality worse than ISO Class 5 (see *Table 1*) for more than 1 hour (see *Immediate-Use CSPs*):
 - sterile contents of commercially manufactured products,
 - CSPs that lack effective antimicrobial preservatives, and
 - sterile surfaces of devices and containers for the preparation, transfer, sterilization, and packaging of CSPs.
3. Compounding personnel are improperly garbed and gloved (see *Personnel Cleansing and Use of Barrier Protective Equipment*).
4. Nonsterile water-containing preparations are stored for more than 6 hours before being sterilized.
5. It is assumed, and not verified by examination of labeling and documentation from suppliers or by direct determination, that the chemical purity and content strength of ingredients meet their original or compendial specifications in unopened or in opened packages of bulk ingredients (see *Ingredient Selection under Pharmaceutical Compounding—Nonsterile Preparations* (795)).

For a sterilized high-risk level preparation, in the absence of passing a sterility test, the storage periods cannot exceed the following time periods: before administration, the CSPs are properly stored and are exposed for not more than 24 hours at controlled room temperature (see *General Notices and Requirements*), for not more than 3 days at a cold temperature (see *General Notices and Requirements*), and for 45 days in solid frozen state between –25° and –10°. [Note—Sterility tests for autoclaved CSPs are not required unless they are prepared in batches of more than 25 units.]

All nonsterile measuring, mixing, and purifying devices are rinsed thoroughly with sterile,

pyrogen-free water, and then thoroughly drained or dried immediately before use for high-risk compounding. All high-risk level CSP solutions subjected to terminal sterilization are prefiltered by passing through a filter with a nominal pore size not larger than 1.2 µm preceding or during filling into their final containers to remove particulate matter. Sterilization of high-risk level CSPs by filtration shall be performed with a sterile 0.2 µm or 0.22 µm nominal pore size filter entirely within an ISO Class 5 (see *Table 1*) or superior air quality environment.

Examples of High-Risk Conditions—

1. Dissolving nonsterile bulk drug and nutrient powders to make solutions that will be terminally sterilized.
2. Exposing the sterile ingredients and components used to prepare and package CSPs to room air quality worse than ISO Class 5 (see *Table 1*) for more than 1 hour (see *Immediate-Use CSPs*).
3. Measuring and mixing sterile ingredients in nonsterile devices before sterilization is performed.
4. Assuming, without appropriate evidence or direct determination, that packages of bulk ingredients contain at least 95% by weight of their active chemical moiety and have not been contaminated or adulterated between uses.

Quality Assurance—Quality assurance procedures for high-risk level CSPs include all those for low-risk level CSPs. In addition, a media-fill test that represents high-risk level compounding is performed semiannually by each person authorized to compound high-risk level CSPs.

Media-Fill Test Procedure for CSPs Sterilized by Filtration—This test or an equivalent test is performed under conditions that closely simulate the most challenging or stressful conditions encountered when compounding high-risk level CSPs. Once begun, this test is completed without interruption. *Example of test procedure* (in the following sequence):

1. Dissolve 3 g of nonsterile commercially available Soybean Casein Digest Medium in 100 mL of nonbacteriostatic water to make a 3% nonsterile solution.
2. Draw 25 mL of the medium into each of three 30 mL sterile syringes. Transfer 5 mL from each syringe into separate sterile 10 mL vials. These vials are the positive controls to generate exponential microbial growth, which is indicated by visible turbidity upon incubation.
3. Under aseptic conditions and using aseptic techniques, affix a sterile 0.2 µm or 0.22 µm nominal pore size filter unit and a 20-gauge needle to each syringe. Inject the next 10 mL from each syringe into three separate 10 mL sterile vials. Repeat the process for three more vials. Label all vials, affix sterile adhesive seals to the closure of the nine vials, and incubate them at 20° to 25° or at 30° to 35° for a minimum of 14 days. If two temperatures are used for incubation of media-filled samples, then these filled containers should be incubated for at least 7 days at each temperature (see *Microbiological Control and Monitoring of Aseptic Processing Environments (1116)*). Inspect for microbial growth over 14 days as described in *Personnel Training and Competency Evaluation of Garbing, Aseptic Work Practices and Cleaning/Disinfection Procedures*.

PERSONNEL TRAINING AND EVALUATION IN ASEPTIC MANIPULATION SKILLS

Personnel who prepare CSPs shall be trained conscientiously and skillfully by expert personnel and through audio-video instructional sources and professional publications in the theoretical principles and practical skills of aseptic manipulations and in achieving and maintaining ISO Class 5 (see *Table 1*) environmental conditions before they begin to prepare CSPs. Compounding personnel shall perform didactic review and pass written and media-fill testing of aseptic manipulative skills initially, at least annually thereafter for low- and medium-risk level compounding, and semiannually for high-risk level compounding. Compounding personnel who fail written tests or whose media-fill test vials result in gross microbial colonization shall be immediately re-instructed and re-evaluated by expert compounding personnel to ensure correction of all aseptic practice deficiencies.

Media-Fill Challenge Testing—The skill of personnel to aseptically prepare CSPs may be evaluated using sterile fluid bacterial culture media-fill verification¹ (i.e., sterile bacterial culture medium transfer via a sterile syringe and needle). Media-fill testing is used to assess the quality of the aseptic skill of compounding personnel. Media-fill tests represent the most challenging or stressful conditions actually encountered by the personnel being evaluated when they prepare particular risk level CSPs and when sterilizing high-risk level CSPs. Media-fill challenge tests that simulate high-risk level compounding are also used to verify the capability of the compounding environment and process to produce a sterile preparation.

Commercially available sterile fluid culture media, such as Soybean-Casein Digest Medium (see *Sterility Tests* (71)), shall be able to promote exponential colonization of bacteria that are most likely to be transmitted to CSPs from the compounding personnel and environment. Media-filled vials are generally incubated at 20° to 25° or at 30° to 35° for a minimum of 14 days. If two temperatures are used for incubation of media-filled samples, then these filled containers should be incubated for at least 7 days at each temperature (see *Microbiological Control and Monitoring of Aseptic Processing Environments* (1116)). Failure is indicated by visible turbidity in the medium on or before 14 days.

IMMEDIATE-USE CSPS

The immediate-use provision is intended only for those situations where there is a need for emergency or immediate patient administration of a CSP. Such situations may include cardiopulmonary resuscitation, emergency room treatment, preparation of diagnostic agents, or critical therapy where the preparation of the CSP under conditions described for *Low-Risk Level CSPs* subjects the patient to additional risk due to delays in therapy. Immediate-use CSPs are not intended for storage for anticipated needs or batch compounding. Preparations that are medium-risk level and high-risk level CSPs shall not be prepared as immediate-use CSPs.

Immediate-use CSPs are exempt from the requirements described for *Low-Risk Level CSPs* only when all of the following criteria are met:

1. The compounding process involves simple transfer of not more than three commercially manufactured packages of sterile nonhazardous products or diagnostic radiopharmaceutical products from the manufacturers' original containers and not more than two entries into any one container or package (e.g., bag, vial) of sterile infusion solution or administration container/device. For example, anti-neoplastics shall not be prepared as immediate-use CSPs because they are hazardous drugs.
2. Unless required for the preparation, the compounding procedure is a continuous process

- not to exceed 1 hour.
3. During preparation, aseptic technique is followed and, if not immediately administered, the finished CSP is under continuous supervision to minimize the potential for contact with nonsterile surfaces, introduction of particulate matter or biological fluids, mix-ups with other CSPs, and direct contact of outside surfaces.
 4. Administration begins not later than 1 hour following the start of the preparation of the CSP.
 5. Unless immediately and completely administered by the person who prepared it or immediate and complete administration is witnessed by the preparer, the CSP shall bear a label listing patient identification information, the names and amounts of all ingredients, the name or initials of the person who prepared the CSP, and the exact 1-hour BUD and time.
 6. If administration has not begun within 1 hour following the start of preparing the CSP, the CSP shall be promptly, properly, and safely discarded.

Compounding in worse than ISO Class 5 (see *Table 1*) conditions increases the likelihood of microbial contamination, and administration durations of microbially contaminated CSPs exceeding a few hours increase the potential for clinically significant microbial colonization and thus for patient harm, especially in critically ill or immunocompromised patients.

SINGLE-DOSE AND MULTIPLE-DOSE CONTAINERS

Opened or needle-punctured single-dose containers, such as bags, bottles, syringes, and vials of sterile products and CSPs shall be used within 1 hour if opened in worse than ISO Class 5 (see *Table 1*) air quality (see *Immediate-Use CSPs*), and any remaining contents must be discarded. Single-dose vials exposed to ISO Class 5 (see *Table 1*) or cleaner air may be used up to 6 hours after initial needle puncture. Opened single-dose ampuls shall not be stored for any time period. Multiple-dose containers (e.g., vials) are formulated for removal of portions on multiple occasions because they usually contain antimicrobial preservatives. The BUD after initially entering or opening (e.g., needle-punctured) multiple-dose containers is 28 days (see *Antimicrobial Effectiveness Testing* (51)) unless otherwise specified by the manufacturer.

HAZARDOUS DRUGS AS CSPS

Although the potential therapeutic benefits of compounded sterile hazardous drug preparations generally outweigh the risks of their adverse effects in ill patients, exposed healthcare workers risk similar adverse effects with no therapeutic benefit. Occupational exposure to hazardous drugs can result in (1) acute effects, such as skin rashes; (2) chronic effects, including adverse reproductive events; and (3) possibly cancer (see Appendix A of NIOSH Publication no. 2004-165).

Hazardous drugs shall be prepared for administration only under conditions that protect the healthcare workers and other personnel in the preparation and storage areas. Hazardous drugs shall be stored separately from other inventory in a manner to prevent contamination and personnel exposure. Many hazardous drugs have sufficient vapor pressures that allow volatilization at room temperature; thus storage is preferably within a containment area such as a negative pressure room. The storage area should have sufficient general exhaust ventilation, at least 12 air changes per hour (ACPH)² to dilute and remove any airborne contaminants.

Hazardous drugs shall be handled with caution at all times using appropriate chemotherapy

~~gloves during receiving, distribution, stocking, inventorying, preparation for administration, and disposal. Hazardous drugs shall be prepared in an ISO Class 5 (see *Table 1*) environment with protective engineering controls in place and following aseptic practices specified for the appropriate contamination risk levels defined in this chapter. Access shall be limited to areas where drugs are stored and prepared to protect persons not involved in drug preparation.~~

~~All hazardous drugs shall be prepared in a BSC³ or a CACI that meets or exceeds the standards for CACI in this chapter. The ISO Class 5 (see *Table 1*) BSC or CACI shall be placed in an ISO Class 7 (see *Table 1*) area that is physically separated (i.e., a different area from other preparation areas) and optimally has not less than 0.01-inch water column negative pressure to adjacent positive pressure ISO Class 7 (see *Table 1*) or better ante-areas, thus providing inward airflow to contain any airborne drug. A pressure indicator shall be installed that can be readily monitored for correct room pressurization. The BSC and CACI optimally should be 100% vented to the outside air through HEPA filtration.~~

~~If a CACI that meets the requirements of this chapter is used outside of a buffer area, the compounding area shall maintain a minimum negative pressure of 0.01-inch water column and have a minimum of 12 ACPHs.~~

~~When closed-system vial transfer devices (CSTDs) (i.e., vial transfer systems that allow no venting or exposure of hazardous substance to the environment) are used, they shall be used within the ISO Class 5 (see *Table 1*) environment of a BSC or CACI. The use of a CSTD is preferred because of their inherent closed-system process. In facilities that prepare a low volume of hazardous drugs, the use of two tiers of containment (e.g., CSTD within a BSC or CACI that is located in a non-negative pressure room) is acceptable.~~

~~Appropriate personnel protective equipment (PPE) shall be worn when compounding in a BSC or CACI and when using CSTD devices. PPE should include gowns, face masks, eye protection, hair covers, shoe covers or dedicated shoes, double gloving with sterile chemo-type gloves, and compliance with manufacturers' recommendations when using a CACI.~~

~~All personnel who compound hazardous drugs shall be fully trained in the storage, handling, and disposal of these drugs. This training shall occur prior to preparing or handling hazardous CSPs, and its effectiveness shall be verified by testing specific hazardous drugs preparation techniques. Such verification shall be documented for each person at least annually. This training shall include didactic overview of hazardous drugs, including mutagenic, teratogenic, and carcinogenic properties, and it shall include ongoing training for each new hazardous drug that enters the marketplace. Compounding personnel of reproductive capability shall confirm in writing that they understand the risks of handling hazardous drugs. The training shall include at least the following: (1) safe aseptic manipulation practices; (2) negative pressure techniques when utilizing a BSC or CACI; (3) correct use of CSTD devices; (4) containment, cleanup, and disposal procedures for breakages and spills; and (5) treatment of personnel contact and inhalation exposure.~~

~~*note—Because standards of assay and unacceptable quantities of contamination of each drug have not been established in the literature, the following paragraph is a recommendation only. Future standards will be adopted as these assay methods are developed and proven.*~~

~~In order to ensure containment, especially in operations preparing large volumes of hazardous drugs, environmental sampling to detect uncontained hazardous drugs should be performed~~

routinely (e.g., initially as a benchmark and at least every 6 months or more often as needed to verify containment). This sampling should include surface wipe sampling of the working area of BSCs and CACIs; counter tops where finished preparations are placed; areas adjacent to BSCs and CACIs, including the floor directly under the working area; and patient administration areas. Common marker hazardous drugs that can be assayed include cyclophosphamide, ifosfamide, methotrexate, and fluorouracil. If any measurable contamination (cyclophosphamide levels greater than 1.00 ng per cm² have been found to cause human uptake) is found by any of these quality assurance procedures, practitioners shall make the decision to identify, document, and contain the cause of contamination. Such action may include retraining, thorough cleaning (utilizing high pH soap and water), and improving engineering controls. Examples of improving engineering controls are (1) venting BSCs or CACIs 100% to the outside, (2) implementing a CSTD, or (3) re-assessing types of BSCs or CACIs.

Disposal of all hazardous drug wastes shall comply with all applicable federal and state regulations. All personnel who perform routine custodial waste removal and cleaning activities in storage and preparation areas for hazardous drugs shall be trained in appropriate procedures to protect themselves and prevent contamination.

RADIOPHARMACEUTICALS AS CSPS

In the case of production of radiopharmaceuticals for positron emission tomography (PET), general test chapter *Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses* (823) supersedes this chapter. Upon release of a PET radiopharmaceutical as a finished drug product from a production facility, the further handling, manipulation, or use of the product will be considered compounding, and the content of this section and chapter is applicable.

For the purposes of this chapter, radiopharmaceuticals compounded from sterile components in closed sterile containers and with a volume of 100 mL or less for a single dose injection or not more than 30 mL taken from a multiple dose container (see *• Packaging and Storage Requirements* (659) *•* (CN 1 May 2016) *•*) shall be designated as, and conform to, the standards for *Low-Risk Level CSPs*.

These radiopharmaceuticals shall be compounded using appropriately shielded vials and syringes in a properly functioning and certified ISO Class 5 (see *Table 1*) PEC located in an ISO Class 8 (see *Table 1*) or cleaner air environment to permit compliance with special handling, shielding, and negative air flow requirements.

Radiopharmaceutical vials designed for multi-use, compounded with technetium-99m, exposed to ISO Class 5 (see *Table 1*) environment, and punctured by needles with no direct contact contamination may be used up to the time indicated by manufacturers' recommendations. Storage and transport of properly shielded vials of radiopharmaceutical CSPs may occur in a limited access ambient environment without a specific ISO class designation.

Technetium-99m/molybdenum-99 generator systems shall be stored and eluted (operated) under conditions recommended by manufacturers and applicable state and federal regulations. Such generator systems shall be eluted in an ISO Class 8 (see *Table 1*) or cleaner air environment to permit special handling, shielding, and air flow requirements. To limit acute and chronic radiation exposure of inspecting personnel to a level that is as low as reasonably achievable (ALARA), direct visual inspection of radiopharmaceutical CSPs containing high

~~concentrations of doses of radioactivity shall be conducted in accordance with ALARA.~~

~~Radiopharmaceuticals prepared as *Low Risk Level CSPs with 12 Hour or Less BUD* shall be prepared in a segregated compounding area. A line of demarcation defining the segregated compounding area shall be established. Materials and garb exposed in a patient care and treatment area shall not cross a line of demarcation into the segregated compounding area.~~

ALLERGEN EXTRACTS AS CSPS

~~Allergen extracts as CSPs are single dose and multiple dose *intra*dermal or *subcutaneous injections* that are prepared by specially trained physicians and personnel under their direct supervision. Allergen extracts as CSPs are not subject to the personnel, environmental, and storage requirements for all *CSP Microbial Contamination Risk Levels* in this chapter only when all of the following criteria are met:~~

- ~~1. The compounding process involves simple transfer via sterile needles and syringes of commercial sterile allergen products and appropriate sterile added substances (e.g., glycerin, phenol in sodium chloride injection).~~
- ~~2. All allergen extracts as CSPs shall contain appropriate substances in effective concentrations to prevent the growth of microorganisms. Nonpreserved allergen extracts shall comply with the appropriate CSP risk level requirements in the chapter.~~
- ~~3. Before beginning compounding activities, personnel perform a thorough hand cleansing procedure by removing debris from under fingernails using a nail cleaner under running warm water followed by vigorous hand and arm washing to the elbows for at least 30 seconds with either nonantimicrobial or antimicrobial soap and water.~~
- ~~4. Compounding personnel don hair covers, facial hair covers, gowns, and face masks.~~
- ~~5. Compounding personnel perform antiseptic hand cleansing with an alcohol-based surgical hand scrub with persistent activity.~~
- ~~6. Compounding personnel don powder-free sterile gloves that are compatible with sterile 70% isopropyl alcohol (IPA) before beginning compounding manipulations.~~
- ~~7. Compounding personnel disinfect their gloves intermittently with sterile 70% IPA when preparing multiple allergen extracts as CSPs.~~
- ~~8. Ampul necks and vial stoppers on packages of manufactured sterile ingredients are disinfected by careful wiping with sterile 70% IPA swabs to ensure that the critical sites are wet for at least 10 seconds and allowed to dry before they are used to compound allergen extracts as CSPs.~~
- ~~9. The aseptic compounding manipulations minimize direct contact contamination (e.g., from glove fingertips, blood, nasal and oral secretions, shed skin and cosmetics, other nonsterile materials) of critical sites (e.g., needles, opened ampuls, vial stoppers).~~
- ~~10. The label of each multiple-dose vial (MDV) of allergen extracts as CSPs lists the name of one specific patient and a BUD and storage temperature range that is assigned based on manufacturers' recommendations or peer-reviewed publications.~~
- ~~11. Single-dose allergen extracts as CSPs shall not be stored for subsequent additional use.~~

~~Personnel who compound allergen extracts as CSPs must be aware of greater potential risk of microbial and foreign material contamination when allergen extracts as CSPs are compounded in compliance with the foregoing criteria instead of the more rigorous standards in this chapter for *CSP Microbial Contamination Risk Levels*. Although contaminated allergen extracts as CSPs can pose health risks to patients when they are injected *intra*dermally or *subcutaneously*, these~~

risks are substantially greater if the extract is inadvertently injected *intravenously*.

VERIFICATION OF COMPOUNDING ACCURACY AND STERILITY

The compounding procedures and sterilization methods for CSPs correspond to correctly designed and verified written documentation in the compounding facility. Verification requires planned testing, monitoring, and documentation to demonstrate adherence to environmental quality requirements, personnel practices, and procedures critical to achieving and maintaining sterility, accuracy, and purity of finished CSPs. For example, sterility testing (see *Test for Sterility of the Product To Be Examined under Sterility Tests* (71)) may be applied to specimens of low and medium risk level CSPs, and standard self-contained biological indicators (BI) shall be added to nondispensable specimens of high risk level CSPs before terminal sterilization for subsequent evaluation to determine whether the sterilization cycle was adequate (see *Biological Indicators for Sterilization* (1035)). Packaged and labeled CSPs shall be visually inspected for physical integrity and expected appearance, including final fill amount. The accuracy of identities, concentrations, amounts, and purities of ingredients in CSPs shall be confirmed by reviewing labels on packages, observing and documenting correct measurements with approved and correctly standardized devices, and reviewing information in labeling and certificates of analysis provided by suppliers. When the correct identity, purity, strength, and sterility of ingredients and components of CSPs cannot be confirmed (in cases of, for example, unlabeled syringes, opened ampuls, punctured stoppers of vials and bags, containers of ingredients with incomplete labeling), such ingredients and components shall be discarded immediately.

Some individual ingredients, such as bulk drug substances, are not labeled with expiration dates when they are stable indefinitely in their commercial packages under their labeled storage conditions. However, despite retaining full chemical stability, such ingredients may gain or lose moisture during storage and use. Changes in moisture content may require testing (see *Loss on Drying* (731)) to determine the correct amount to weigh for accurate content of active chemical moieties in CSPs (see *Pharmaceutical Calculations in Prescription Compounding* (1160)).

Although not required, a quantitative stability indicating chemical assay is recommended to ensure compounding accuracy of CSPs, especially those that contain drug ingredients with a narrow therapeutic plasma concentration range.

Sterilization Methods

The licensed healthcare professionals who supervise compounding shall be responsible for determining that the selected sterilization method (see *Methods of Sterilization under Sterilization and Sterility Assurance of Compendial Articles* (1211)) both sterilizes and maintains the strength, purity, quality, and packaging integrity of CSPs. The selected sterilization process is obtained from experience and appropriate information sources (e.g., see *Sterilization and Sterility Assurance of Compendial Articles* (1211)) and, preferably, verified wherever possible to achieve sterility in the particular CSPs. General guidelines for matching CSPs and components to appropriate sterilization methods include the following:

1. CSPs have been ascertained to remain physically and chemically stable when subjected to the selected sterilization method.
2. Glass and metal devices may be covered tightly with aluminum foil, then exposed to dry

heat in an oven at a mean temperature of 250° for 30 minutes to achieve sterility and depyrogenation (see *Dry Heat Sterilization* under *Sterilization and Sterility Assurance of Compendial Articles* (1211) and *Bacterial Endotoxins Test* (85)). Such items are either used immediately or stored until use in an environment suitable for compounding *Low-Risk Level CSPs* and *Medium-Risk Level CSPs*.

3. Personnel ascertain from appropriate information sources that the sterile microporous membrane filter used to sterilize CSP solutions, during either compounding or administration, is chemically and physically compatible with the CSP.

sterilization of high-risk level csps by filtration

Commercially available sterile filters shall be approved for human use applications in sterilizing pharmaceutical fluids. Sterile filters used to sterilize CSPs shall be pyrogen free and have a nominal pore size of 0.2 or 0.22 µm. They shall be certified by the manufacturer to retain at least 10⁷ microorganisms of a strain of *Brevundimonas (Pseudomonas) diminuta* on each square centimeter of upstream filter surface area under conditions similar to those in which the CSPs will be sterilized (see *High-Risk Conditions* in *High-Risk Level CSPs*).

The compounding supervisor shall ensure, directly or from appropriate documentation, that the filters are chemically and physically stable at the pressure and temperature conditions to be used, that they have enough capacity to filter the required volumes, and that they will achieve sterility and maintain prefiltration pharmaceutical quality, including strength of ingredients of the specific CSP. The filter dimensions and liquid material to be sterile filtered shall permit the sterilization process to be completed rapidly, without the replacement of the filter during the process. When CSPs are known to contain excessive particulate matter, a prefilter of larger nominal pore size membrane is placed upstream from the sterilizing filter to remove gross particulate contaminants in order to maximize the efficiency of the sterilizing filter.

Filter units used to sterilize CSPs shall also be subjected to manufacturers' recommended integrity test, such as the bubble point test.

Compounding personnel shall ascertain that selected filters will achieve sterilization of the particular CSPs being sterilized. Large deviations from usual or expected chemical and physical properties of CSPs (e.g., water miscible alcohols) may cause undetectable damage to filter integrity and shrinkage of microorganisms to sizes smaller than filter nominal pore size.

sterilization of high-risk level csps by steam

The process of thermal sterilization employing saturated steam under pressure, or autoclaving, is the preferred method to terminally sterilize aqueous preparations that have been verified to maintain their full chemical and physical stability under the conditions employed (see *Steam Sterilization* under *Sterilization and Sterility Assurance of Compendial Articles* (1211)). To achieve sterility, all materials are to be exposed to steam at 121° under a pressure of about 1 atmosphere or 15 psi for the duration verified by testing to achieve sterility of the items, which is usually 20 to 60 minutes for CSPs. An allowance shall be made for the time required for the material to reach 121° before the sterilization exposure duration is timed.

Not directly exposing items to pressurized steam may result in survival of microbial organisms and spores. Before their sterilization, plastic, glass, and metal devices are tightly wrapped in low-particle-shedding paper or fabrics or sealed in envelopes that prevent poststerilization microbial penetration. Immediately before filling ampuls and vials that will be steam sterilized,

solutions are passed through a filter having a nominal pore size not larger than 1.2 µm for removal of particulate matter. Sealed containers shall be able to generate steam internally; thus, stoppered and crimped empty vials shall contain a small amount of moisture to generate steam.

The description of steam sterilization conditions and duration for specific CSPs shall be included in written documentation in the compounding facility. The effectiveness of steam sterilization shall be verified using appropriate BIs of *Bacillus stearothermophilus* (see *Biological Indicators* (1035)) and other confirmation methods such as temperature sensing devices (see *Sterilization and Sterility Assurance of Compendial Articles* (1211) and *Sterility Tests* (71)).

~~sterilization of high-risk level csp's by dry heat~~

Dry heat sterilization is usually done as a batch process in an oven designed for sterilization. Heated filtered air shall be evenly distributed throughout the chamber by a blower device. The oven should be equipped with a system for controlling temperature and exposure period. Sterilization by dry heat requires higher temperatures and longer exposure times than does sterilization by steam. Dry heat shall be used only for those materials that cannot be sterilized by steam, when either the moisture would damage the material or the material is impermeable. During sterilization, sufficient space shall be left between materials to allow for good circulation of the hot air. The description of dry heat sterilization conditions and duration for specific CSPs shall be included in written documentation in the compounding facility. The effectiveness of dry heat sterilization shall be verified using appropriate BIs of *Bacillus subtilis* (see *Biological Indicators* (1035)) and other confirmation methods such as temperature sensing devices (see *Sterilization and Sterility Assurance of Compendial Articles* (1211) and *Sterility Tests* (71)). [Note—Dry heat sterilization may be performed at a lower temperature than may be effective for depyrogenation].

Depyrogenation by Dry Heat

Dry heat depyrogenation shall be used to render glassware or containers such as vials free from pyrogens as well as viable microbes. A typical cycle would be 30 minutes at 250°. The description of the dry heat depyrogenation cycle and duration for specific load items shall be included in written documentation in the compounding facility. The effectiveness of the dry heat depyrogenation cycle shall be verified using endotoxin challenge vials (ECVs). The bacterial endotoxin test should be performed on the ECVs to verify that the cycle is capable of achieving a 3-log reduction in endotoxin (see *Sterilization and Sterility Assurance of Compendial Articles* (1211) and *Bacterial Endotoxins Test* (85)).

ENVIRONMENTAL QUALITY AND CONTROL

Achieving and maintaining sterility and overall freedom from contamination of a CSP is dependent on the quality status of the components incorporated, the process utilized, personnel performance, and the environmental conditions under which the process is performed. The standards required for the environmental conditions depend on the amount of exposure of the CSP to the immediate environment anticipated during processing. The quality and control of environmental conditions for each risk level of operation are explained in this section. In addition, operations using nonsterile components require the use of a method of preparation designed to produce a sterile preparation.

Exposure of Critical Sites

Maintaining the sterility and cleanliness (i.e., freedom from sterile foreign materials) of critical sites is a primary safeguard for CSPs. Critical sites are locations that include any component or fluid pathway surfaces (e.g., vial septa, injection ports, beakers) or openings (e.g., opened ampuls, needle hubs) exposed and at risk of direct contact with air (e.g., ambient room or HEPA filtered), moisture (e.g., oral and mucosal secretions), or touch contamination. The risk of, or potential for, critical sites to be contaminated with microorganisms and foreign matter increases with increasing exposed area of the critical sites, the density or concentration of contaminants, and exposure duration to worse than ISO Class 5 (see *Table 1*) air. Examples include an opened ampul or vial stopper on a 10-mL or larger vial or an injection port on a package of intravenous solution having an area larger than the point of a needle or the tip of a syringe.

The nature of a critical site also affects the risk of contamination. The relatively rough, permeable surface of an elastomeric closure retains microorganisms and other contaminants after swabbing with a sterile 70% IPA pad more readily than does the smoother glass surface of the neck of an ampul. Therefore, the surface disinfection can be expected to be more effective for an ampul.

Protection of critical sites by precluding physical contact and airborne contamination shall be given the highest priority in sterile compounding practice. Airborne contaminants, especially those generated by sterile compounding personnel, are much more likely to reach critical sites than are contaminants that are adhering to the floor or other surfaces below the work level. Furthermore, large and high density particles that are generated and introduced by compounding manipulations and personnel have the potential to settle on critical sites even when those critical sites are exposed within ISO Class 5 (see *Table 1*) air.

ISO Class 5 Air Sources, Buffer Areas, and Ante-Areas

The most common sources of ISO Class 5 (see *Table 1*) air quality for exposure of critical sites are horizontal and vertical LAFWs, CAIs, and CACIs. A clean room (see *Microbiological Control and Monitoring of Aseptic Processing Environments* (1116)) is a compounding environment that is supplied with HEPA or HEPA filtered air that meets ISO Class 7 (see *Table 1*), the access to which is limited to personnel trained and authorized to perform sterile compounding and facility cleaning. A buffer area is an area that provides at least ISO Class 7 (see *Table 1*) air quality.

Figure 1 is a conceptual representation of the placement of an ISO Class 5 (see *Table 1*) PEC in a segregated compounding area used for low risk level CSPs with 12-hour or less BUD. This plan depicts the most critical operation area located within the PEC in a designated area (see definition of *Segregated Compounding Area*) separated from activities not essential to the preparation of CSPs. Placement of devices (e.g., computers, printers) and objects (e.g., carts, cabinets) that are not essential to compounding in the segregated area should be restricted or limited, depending on their effect on air quality in the ISO Class 5 (see *Table 1*) PEC.

Conceptual representation of USP Chapter <797> facility requirements

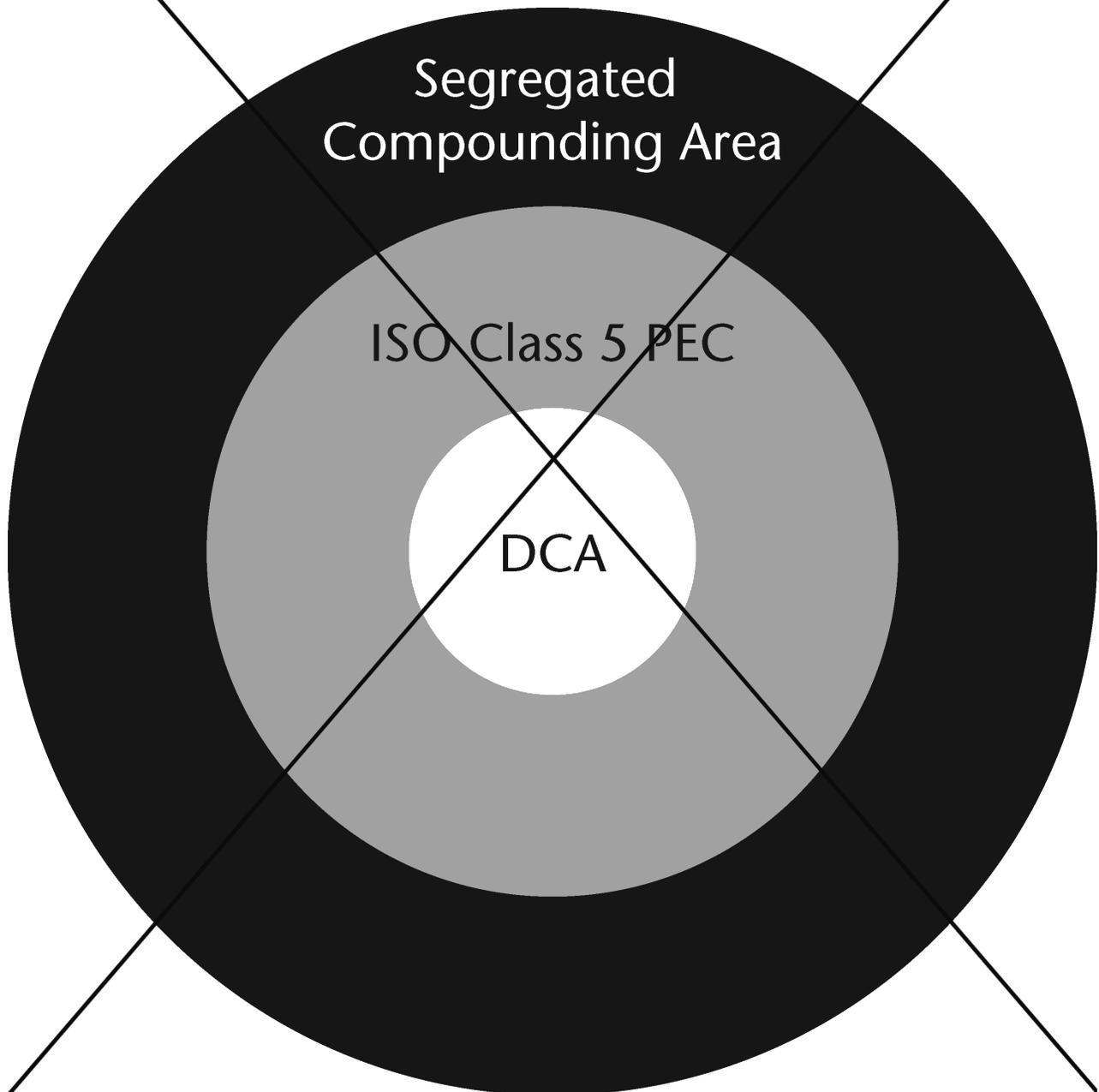


Figure 1. Conceptual representation of the placement of an ISO Class 5 PEC in a segregated compounding area used for low-risk level CSPs with 12-hour or less-BUD.

Figure 2 is a conceptual representation of the arrangement of a facility for preparation of CSPs categorized as low-, medium-, and high-risk level. The quality of the environmental air increases with movement from the outer boundary to the direct compounding area (DCA). Placement of devices in ante-areas and buffer areas is dictated by their effect on the designated environmental quality of atmospheres and surfaces, which shall be verified by monitoring (see *Viable and Nonviable Environmental Sampling (ES) Testing*). It is the

responsibility of each compounding facility to ensure that each source of ISO Class 5 (see *Table 1*) environment for exposure of critical sites and sterilization by filtration is properly located, operated, maintained, monitored, and verified.

Conceptual representation of USP Chapter <797> facility requirements

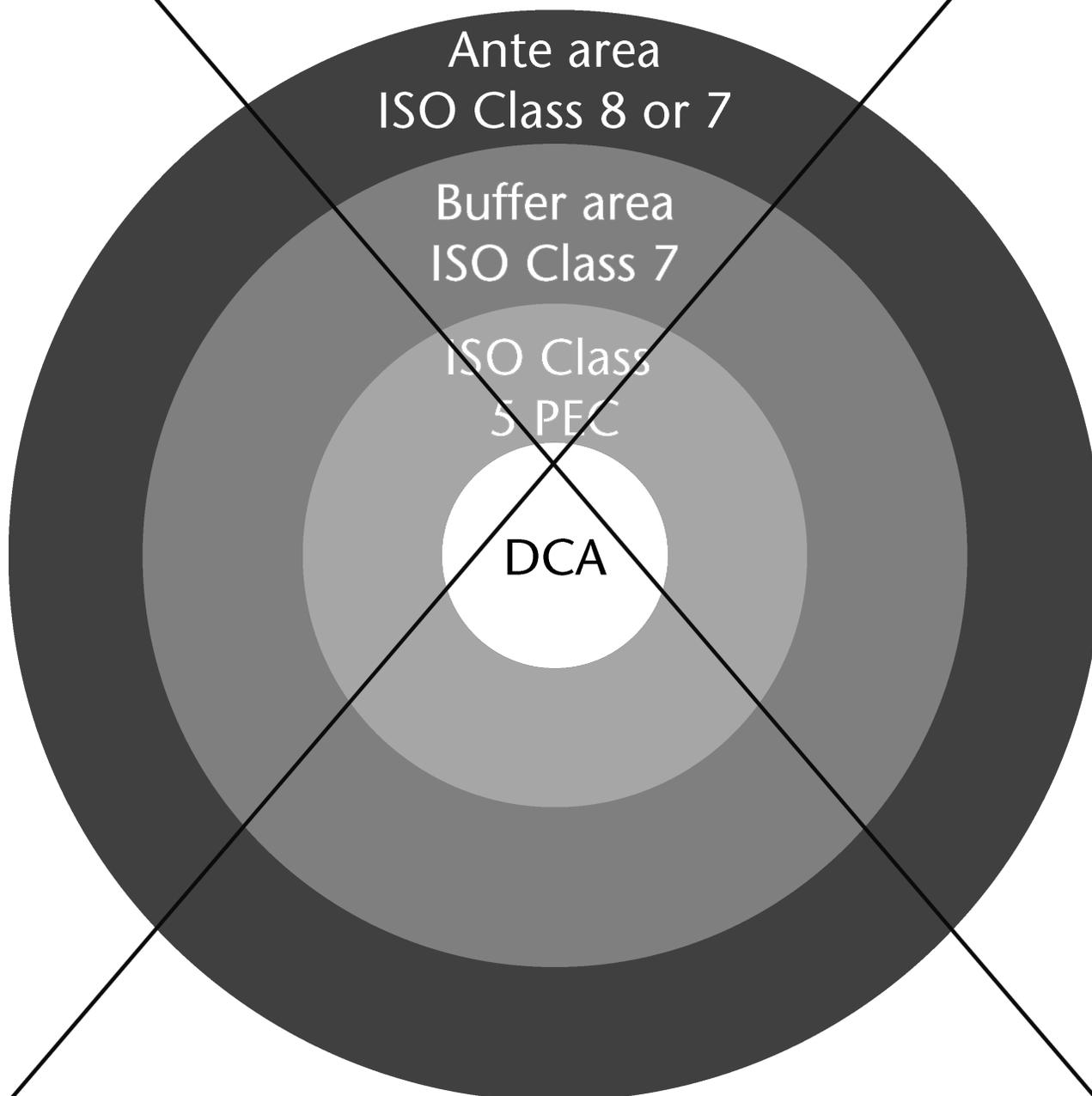


Figure 2. Conceptual representation of the arrangement of a facility for preparation of CSPs categorized as low, medium, and high risk level.

Placement of devices (e.g., computers, printers) and objects (e.g., carts, cabinets) that are not essential to compounding in buffer areas is dictated by their effect on the required environmental quality of air atmospheres and surfaces, which shall be verified by monitoring

~~(see *Viable and Nonviable Environmental Sampling (ES) Testing*). It is the responsibility of each compounding facility to ensure that each source of ISO Class 5 (see *Table 1*) environment for exposure of critical sites and sterilization by filtration is properly located, operated, maintained, monitored, and verified.~~

Facility Design and Environmental Controls

~~Compounding facilities are physically designed and environmentally controlled to minimize airborne contamination from contacting critical sites. These facilities shall also provide a comfortable and well-lighted working environment, which typically includes a temperature of 20° or cooler, to maintain comfortable conditions for compounding personnel to perform flawlessly when attired in the required aseptic compounding garb. PECs typically include, but are not limited to, LAFWs, BSCs, CAIs, and CACIs, which provide an ISO Class 5 (see *Table 1*) environment for the exposure of critical sites. PECs shall maintain ISO Class 5 (see *Table 1*) or better conditions for 0.5-µm particles (dynamic operating conditions) while compounding CSPs. Secondary engineering controls such as buffer areas and ante-areas generally serve as a core for the location of the PEC. Buffer areas are designed to maintain at least ISO Class 7 (see *Table 1*) conditions for 0.5-µm particles under dynamic conditions and ISO Class 8 (see *Table 1*) conditions for 0.5-µm and larger particles under dynamic conditions for the ante-areas. Airborne contamination control is achieved in the PEC through the use of HEPA filters. The airflow in the PEC shall be unidirectional (laminar flow), and because of the particle collection efficiency of the filter, the "first air" at the face of the filter is, for the purposes of aseptic compounding, free from airborne particulate contamination. HEPA-filtered air shall be supplied in critical areas (ISO Class 5, see *Table 1*) at a velocity sufficient to sweep particles away from the compounding area and maintain unidirectional airflow during operations. Proper design and control prevents turbulence and stagnant air in the critical area. In-situ air pattern analysis via smoke studies shall be conducted at the critical area to demonstrate unidirectional airflow and sweeping action over and away from the product under dynamic conditions.~~

~~The principles of HEPA-filtered unidirectional airflow in the work environment shall be understood and practiced in the compounding process in order to achieve the desired environmental conditions. Policies and procedures for maintaining and working within the PEC area shall be written and followed. The policies and procedures will be determined by the scope and risk levels of the aseptic compounding activities utilized during the preparation of the CSPs. The CSP work environment is designed to have the cleanest work surfaces (PEC) located in a buffer area. The buffer area shall maintain at least ISO Class 7 (see *Table 1*) conditions for 0.5-µm and larger particles under dynamic operating conditions. The room shall be segregated from surrounding, unclassified spaces to reduce the risk of contaminants being blown, dragged, or otherwise introduced into the filtered unidirectional airflow environment, and this segregation shall be continuously monitored. For rooms providing a physical separation through the use of walls, doors, and pass-throughs, a minimum differential positive pressure of 0.02 to 0.05-inch water column is required. For buffer areas not physically separated from the ante-areas, the principle of displacement airflow shall be employed. This concept utilizes a low pressure differential, high airflow principle. Using displacement airflow typically requires an air velocity of 40 ft per minute or more from the buffer area across the line of demarcation into the ante-area.~~

~~The displacement concept shall not be used for high-risk compounding.⁴ The PEC shall be placed within a buffer area in such a manner as to avoid conditions that could adversely affect their operation. For example, strong air currents from opened doors, personnel traffic, or air~~

streams from the HVAC systems can disrupt the unidirectional airflow in open-faced workbenches. The operators may also create disruptions in airflow by their own movements and by the placement of objects onto the work surface. The PEC shall be placed out of the traffic flow and in a manner to avoid disruption from the HVAC system and room cross-drafts. Room air exchanges are typically expressed as ACPHs. Adequate HEPA-filtered airflow supplied to the buffer area and ante-area is required to maintain cleanliness classification during operational activity through the number of ACPHs. Factors that should be considered when determining air-change requirements include number of personnel working in the room and compounding processes that generate particulates, as well as temperature effects. An ISO Class 7 (see *Table 1*) buffer area and ante-area supplied with HEPA-filtered air shall receive an ACPH of not less than 30. The PEC is a good augmentation to generating air changes in the air supply of an area but cannot be the sole source of HEPA-filtered air. If the area has an ISO Class 5 (see *Table 1*) recirculating device, a minimum of 15 ACPHs through the area supply HEPA filters is adequate, providing the combined ACPH is not less than 30. More air changes may be required, depending on the number of personnel and processes. HEPA-filtered supply air shall be introduced at the ceiling, and returns should be mounted low on the wall, creating a general top-down dilution of area air with HEPA-filtered make-up air. Ceiling-mounted returns are not recommended. All HEPA filters should be efficiency tested using the most penetrating particle size and should be leak tested at the factory and then leak tested again in situ after installation.⁵

Activities and tasks carried out within the buffer area shall be limited to only those necessary when working within a controlled environment. Only the furniture, equipment, supplies, and other material required for the compounding activities to be performed shall be brought into the area, and they shall be nonpermeable, nonshedding, cleanable, and resistant to disinfectants. Whenever such items are brought into the area, they shall first be cleaned and disinfected. Whenever possible, equipment and other items used in the buffer area shall not be taken out of the area except for calibration, servicing, or other activities associated with the proper maintenance of the item.

The surfaces of ceilings, walls, floors, fixtures, shelving, counters, and cabinets in the buffer area shall be smooth, impervious, free from cracks and crevices, and nonshedding, thereby promoting cleanability and minimizing spaces in which microorganisms and other contaminants may accumulate. The surfaces shall be resistant to damage by disinfectant agents. Junctionures of ceilings to walls shall be coved or caulked to avoid cracks and crevices where dirt can accumulate. If ceilings consist of inlaid panels, the panels shall be impregnated with a polymer to render them impervious and hydrophobic, and they shall be caulked around each perimeter to seal them to the support frame. Walls may be constructed of flexible material (e.g., heavy gauge polymer), panels locked together and sealed, or of epoxy-coated gypsum board. Preferably, floors are overlaid with wide sheet vinyl flooring with heat-welded seams and coving to the sidewall. Dust-collecting overhangs, such as ceiling utility pipes, and ledges, such as windowsills, should be avoided. The exterior lens surface of ceiling lighting fixtures should be smooth, mounted flush, and sealed. Any other penetrations through the ceiling or walls shall be sealed. The buffer area shall not contain sources of water (sinks) or floor drains. Work surfaces shall be constructed of smooth, impervious materials, such as stainless steel or molded plastic, so that they are easily cleaned and disinfected. Carts should be of stainless steel wire, nonporous plastic, or sheet metal construction with good quality, cleanable casters to promote mobility. Storage shelving, counters, and cabinets shall be smooth, impervious, free from cracks

and crevices, nonshedding, cleanable, and disinfectable; their number, design, and manner of installation shall promote effective cleaning and disinfection.

Placement of Primary Engineering Controls

PECs (LAFWs, BSCs, CAIs, and CACIs) shall be located within a restricted access ISO Class 7 (see *Table 1*) buffer area (see *Figure 1*), with the following CAI/CACI exceptions below:

- Only authorized personnel and materials required for compounding and cleaning shall be permitted in the buffer area.
- Presterilization procedures for high-risk level CSPs, such as weighing and mixing, shall be completed in no worse than an ISO Class 8 (see *Table 1*) environment.
- PECs shall be located out of traffic patterns and away from room air currents that could disrupt the intended airflow patterns.

CAIs and CACIs shall be placed in an ISO Class 7 (see *Table 1*) buffer area *unless* they meet all of the following conditions:

- The isolator shall provide isolation from the room and maintain ISO Class 5 (see *Table 1*) during dynamic operating conditions, including transferring ingredients, components, and devices into and out of the isolator and during preparation of CSPs.
- Particle counts sampled approximately 6 to 12 inches upstream of the critical exposure site shall maintain ISO Class 5 (see *Table 1*) levels during compounding operations.
- Not more than 3520 particles (0.5 μm and larger) per m^3 shall be counted during material transfer, with the particle counter probe located as near to the transfer door as possible without obstructing the transfer.⁶

It is incumbent on the compounding personnel to obtain documentation from the manufacturer that the CAI/CACI will meet this standard when located in environments where the background particle counts exceed ISO Class 8 (see *Table 1*) for 0.5 μm and larger particles. When isolators are used for sterile compounding, the recovery time to achieve ISO Class 5 (see *Table 1*) air quality shall be documented and internal procedures developed to ensure that adequate recovery time is allowed after material transfer before and during compounding operations.

If the PEC is a CAI or CACI that does not meet the requirements above or is a LAFW or BSC that cannot be located within an ISO Class 7 (see *Table 1*) buffer area, then only low-risk level nonhazardous and radiopharmaceutical CSPs pursuant to a physician order for a specific patient may be prepared, and administration of the CSP shall commence within 12 hours of preparation or as recommended in the manufacturer's package insert, whichever is less.

Viable and Nonviable Environmental Sampling (ES) Testing

The ES program should provide information to staff and leadership to demonstrate that the PEC is maintaining an environment within the compounding area that consistently ensures acceptably low viable and nonviable particle levels. The compounding area includes the ISO Class 5 (see *Table 1*) PEC (LAFWs, BSCs, CAIs, and CACIs), buffer areas, ante-areas, and segregated compounding areas.

Environmental sampling shall occur as part a comprehensive quality management program and shall occur minimally under any of the following conditions:

- as part of the commissioning and certification of new facilities and equipment;
- following any servicing of facilities and equipment;
- as part of the re-certification of facilities and equipment (i.e., every 6 months);

- in response to identified problems with end products or staff technique; or
- in response to issues with CSPs, observed compounding personnel work practices, or patient-related infections (where the CSP is being considered as a potential source of the infection).

ENVIRONMENTAL NONVIABLE PARTICLE TESTING PROGRAM

A program to sample nonviable airborne particles differs from that for viable particles in that it is intended to directly measure the performance of the engineering controls used to create the various levels of air cleanliness, for example, ISO Class 5, 7, or 8 (see *Table 1*).

Engineering Control Performance Verification—PECs (LAFWs, BSCs, CAIs, and CACIs) and secondary engineering controls (buffer and ante areas) are essential components of the overall contamination control strategy for aseptic compounding. As such, it is imperative that they perform as designed and that the resulting levels of contamination be within acceptable limits. Certification procedures such as those outlined in *Certification Guide for Sterile Compounding Facilities (CAG-003-2006)*⁷ shall be performed by a qualified individual no less than every 6 months and whenever the device or room is relocated or altered or major service to the facility is performed.

Total Particle Counts—Certification that each ISO classified area, for example, ISO Class 5, 7, and 8 (see *Table 1*), is within established guidelines shall be performed no less than every 6 months and whenever the LAFW, BSC, CAI, or CACI is relocated or the physical structure of the buffer area or ante area has been altered. Testing shall be performed by qualified operators using current, state-of-the-art electronic equipment with results of the following:

- ISO Class 5: not more than 3520 particles 0.5 μm and larger size per cubic meter of air for any LAFW, BSC, CAI, and CACI;
- ISO Class 7: not more than 352,000 particles of 0.5 μm size and larger per cubic meter of air for any buffer area;
- ISO Class 8: not more than 3,520,000 particles of 0.5 μm size and larger per cubic meter of air for any ante area.

All certification records shall be maintained and reviewed by supervising personnel or other designated employees to ensure that the controlled environments comply with the proper air cleanliness, room pressures, and ACPHs.

PRESSURE DIFFERENTIAL MONITORING

A pressure gauge or velocity meter shall be installed to monitor the pressure differential or airflow between the buffer area and the ante area and between the ante area and the general environment outside the compounding area. The results shall be reviewed and documented on a log at least every work shift (minimum frequency shall be at least daily) or by a continuous recording device. The pressure between the ISO Class 7 (see *Table 1*) and the general pharmacy area shall not be less than 5 Pa (0.02 inch water column). In facilities where low- and medium-risk level CSPs are prepared, differential airflow shall maintain a minimum velocity of 0.2 meters per second (40 feet per minute) between buffer area and ante area.

ENVIRONMENTAL VIABLE AIRBORNE PARTICLE TESTING PROGRAM

The risk of contaminating a CSP prepared under low-risk level and medium-risk level conditions is highly dependent on proper hand hygiene and garbing practices, compounding personnel aseptic technique, and the presence of surface contamination, assuming that all work is

performed in a certified and properly functioning ISO Class 5 (see *Table 1*) PEC and secondary engineering controls, ISO Class 7 (see *Table 1*) buffer area, and ISO Class 8 (see *Table 1*) ante-area. High-risk level CSPs pose the greatest threat to patients because compounding personnel are tasked with the requirement of processing nonsterile components and devices in order to achieve sterility.

A sampling program in conjunction with an observational audit is designed to evaluate the competency of compounding personnel work practices, allowing for the implementation of corrective actions on an ongoing basis (see *Personnel Training and Competency Evaluation of Garbing, Aseptic Work Practices and Cleaning/Disinfection Procedures*).

Sampling Plan—An appropriate environmental sampling plan shall be developed for airborne viable particles based on a risk assessment of compounding activities performed.

Selected sampling sites shall include locations within each ISO Class 5 (see *Table 1*) environment and in the ISO Class 7 and 8 (see *Table 1*) areas and in the segregated compounding areas at greatest risk of contamination (e.g., work areas near the ISO Class 5 [see *Table 1*] environment, counters near doors, pass-through boxes). The plan shall include sample location, method of collection, frequency of sampling, volume of air sampled, and time of day as related to activity in the compounding area and action levels.

Review of the data generated during a sampling event may detect elevated amounts of airborne microbial bioburden; such changes may be indicative of adverse changes within the environment. It is recommended that compounding personnel refer to *Microbiological Control and Monitoring of Aseptic Processing Environments* (1116) and the CDC's "Guidelines for Environmental Infection Control in Healthcare Facilities, 2003" for more information.

Growth Medium—A general microbiological growth medium such as Soybean Casein Digest Medium shall be used to support the growth of bacteria. Malt extract agar or some other media that supports the growth of fungi shall be used in high-risk level compounding environments. Media used for surface sampling must be supplemented with additives to neutralize the effects of disinfecting agents (e.g., TSA with lecithin and polysorbate 80).

Viable Air Sampling—Evaluation of airborne microorganisms using volumetric collection methods in the controlled air environments (LAFWs, CAIs, clean room or buffer areas, and ante-areas) shall be performed by properly trained individuals for all compounding risk levels.

Impaction shall be the preferred method of volumetric air sampling. Use of settling plates for qualitative air sampling may not be able to determine adequately the quality of air in the controlled environment. The settling of particles by gravity onto culture plates depends on the particle size and may be influenced by air movement. Consequently, the number of colony-forming units (cfu) on a settling plate may not always relate to the concentrations of viable particles in the sampled environment.

For low-, medium-, and high-risk level compounding, air sampling shall be performed at locations that are prone to contamination during compounding activities and during other activities such as staging, labeling, gowning, and cleaning. Locations shall include zones of air backwash turbulence within LAFW and other areas where air backwash turbulence may enter the compounding area (doorways, in and around ISO Class 5 [see *Table 1*] PEC and environments). Consideration should be given to the overall effect the chosen sampling method will have on the unidirectional airflow within a compounding environment.

For low-risk level CSPs with 12-hour or less BUD prepared in a PEC (LAFWs, BSCs, CAIs) that maintains an ISO Class 5 (see *Table 1*), air sampling shall be performed at locations inside the ISO Class 5 (see *Table 1*) environment and other areas that are in close proximity to the ISO Class 5 (see *Table 1*) environment during the certification of the PEC.

Air Sampling Devices—There are a number of manufacturers of electronic air sampling equipment. It is important that personnel refer to the manufacturer's recommended procedures when using the equipment to perform volumetric air sampling procedures. The instructions in the manufacturer's user's manual for verification and use of electric air samplers that actively collect volumes of air for evaluation must be followed. A sufficient volume of air (400 to 1000 liters) shall be tested at each location in order to maximize sensitivity. The volumetric air sampling devices need to be serviced and calibrated as recommended by the manufacturer.

It is recommended that compounding personnel also refer to *Methodology and Instrumentation for Quantitation of Viable Airborne Microorganisms* under *Microbiological Control and Monitoring of Aseptic Processing Environments* (1116), which provides more information on the use of volumetric air samplers and volume of air that should be sampled to detect environmental bioburden excursions.

Air Sampling Frequency and Process—Air sampling shall be performed at least semiannually (i.e., every 6 months) as part of the re-certification of facilities and equipment. If compounding occurs in multiple locations within an institution (e.g., main pharmacy, satellites), environmental sampling is required for each individual compounding area. A sufficient volume of air shall be sampled and the manufacturer's guidelines for use of the electronic air sampling equipment followed. Any facility construction or equipment servicing may require that air sampling be performed during these events.

Incubation Period—At the end of the designated sampling or exposure period for air sampling activities, the microbial growth media plates are recovered and their covers secured (e.g., taped), and they are inverted and incubated at a temperature and for a time period conducive to multiplication of microorganisms. TSA should be incubated at 30° to 35° for 48 to 72 hours. Malt extract agar or other suitable fungal media should be incubated at 26° to 30° for 5 to 7 days. The number of discrete colonies of microorganisms are counted and reported as cfu and documented on an environmental sampling form. Counts from air sampling need to be transformed into cfu per cubic meter of air and evaluated for adverse trends.

Action Levels, Documentation, and Data Evaluation—The value of viable microbial sampling of the air in the compounding environment is realized when the data are used to identify and correct an unacceptable situation. Sampling data shall be collected and reviewed on a periodic basis as a means of evaluating the overall control of the compounding environment. If an activity consistently shows elevated levels of microbial growth, competent microbiology personnel shall be consulted.

Any cfu count that exceeds its respective action level (see *Table 2*) should prompt a re-evaluation of the adequacy of personnel work practices, cleaning procedures, operational procedures, and air filtration efficiency within the aseptic compounding location. An investigation into the source of the contamination shall be conducted. Sources could include HVAC systems, damaged HEPA filters, and changes in personnel garbing or work practices. The source of the problem shall be eliminated, the affected area cleaned, and resampling performed. Counts of cfu are to be used as an approximate measure of the environmental microbial

bioburden. Action levels are determined on the basis of cfu data gathered at each sampling location and trended over time. The numbers in *Table 2* should be used only as guidelines. Regardless of the number of cfu identified in the pharmacy, further corrective actions will be dictated by the identification of microorganisms recovered (at least the genus level) by an appropriate credentialed laboratory of any microbial bioburden captured as a cfu using an impaction air sampler. Highly pathogenic microorganisms (e.g., Gram-negative rods, coagulase positive staphylococcus, molds and yeasts) can be potentially fatal to patients receiving CSPs and must be immediately remedied, regardless of cfu count, with the assistance of a competent microbiologist, infection control professional, or industrial hygienist.

Table 2. Recommended Action Levels for Microbial Contamination*

†(cfu per cubic meter [1000 liters] of air per plate)

Classification	Air Sample†
ISO Class 5	> 1
ISO Class 7	> 10
ISO Class 8 or worse	> 100
* -Guidance for Industry -Sterile Drug Products Produced by Aseptic Processing -Current Good Manufacturing Practice -US HHS, FDA -September 2004.	

Additional Personnel Requirements

Food, drinks, and materials exposed in patient care and treatment areas shall not enter ante-areas, buffer areas, or segregated compounding areas where components and ingredients of CSPs are present. When compounding activities require the manipulation of a patient's blood-derived or other biological material (e.g., radiolabeling a patient's or donor's white blood cells), the manipulations shall be clearly separated from routine material handling procedures and equipment used in CSP preparation activities, and they shall be controlled by specific SOPs in order to avoid any cross-contamination. Packaged compounding supplies and components, such as needles, syringes, tubing sets, and small and large volume parenterals, should be uncartoned and wiped down with a disinfectant that does not leave a residue (e.g., sterile 70% IPA), when possible in an ante-area of ISO Class 8 (see *Table 1*) air quality, before being passed into the buffer areas. Personnel hand hygiene and garbing procedures are also performed in the ante-area, which may contain a sink that enables hands-free use with a closed system of soap dispensing to minimize the risk of extrinsic contamination. There shall be some demarcation designation that separates the ante-area from the buffer area. Adequate provision for performing antiseptic hand cleansing using an alcohol-based surgical hand scrub with persistent activity followed by the donning of sterile gloves should be provided after entry into the buffer area.

Cleaning and Disinfecting the Compounding Area

Environmental contact is a major source of microbial contamination of CSPs. Consequently, scrupulous attention to cleaning and disinfecting the sterile compounding areas is required to minimize this as a source of CSP contamination.

The cleaning and disinfecting practices and frequencies in this section apply to ISO Class 5 (see *Table 1*) compounding areas for exposure of critical sites as well as buffer areas, ante-areas, and segregated compounding areas. Compounding personnel are responsible for ensuring that the frequency of cleaning is in accordance with the requirements stated in *Table 3* and

determining the cleaning and disinfecting products to be used (see *Appendix II*). Any organizational or institutional policies regarding disinfectant selection should be considered by compounding personnel. All cleaning and disinfecting practices and policies for the compounding of CSPs shall be included in written SOPs and shall be followed by all compounding personnel.

Table 3. Minimum Frequency of Cleaning and Disinfecting Compounding Areas

Site	Minimum Frequency
ISO Class 5 (see <i>Table 1</i>) Primary Engineering Control (e.g., LAFW, BSC, CAI, CACI)	At the beginning of each shift, before each batch, not longer than 30 minutes following the previous surface disinfection when ongoing compounding activities are occurring, after spills, and when surface contamination is known or suspected
Counters and easily cleanable work surfaces	Daily
Floors	Daily
Walls	Monthly
Ceilings	Monthly
Storage shelving	Monthly

The selection and use of disinfectants in healthcare facilities is guided by several properties, such as microbicidal activity, inactivation by organic matter, residue, and shelf life (see *Appendix II*). In general, highly toxic disinfectants, such as glutaraldehyde, are not used on housekeeping surfaces (e.g., floors, countertops). Many disinfectants registered by the EPA are one-step disinfectants. This means that the disinfectant has been formulated to be effective in the presence of light to moderate soiling without a pre-cleaning step.

Surfaces in LAFWs, BSCs, CAIs, and CACIs, which are intimate to the exposure of critical sites, require disinfecting more frequently than do housekeeping surfaces such as walls and ceilings. Disinfecting sterile compounding areas shall occur on a regular basis at the intervals noted in *Table 3* when spills occur, when the surfaces are visibly soiled, and when microbial contamination is known to have been or is suspected of having been introduced into the compounding areas.

When the surface to be disinfected has heavy soiling, a cleaning step is recommended prior to the application of the disinfectant. Trained compounding personnel are responsible for developing, implementing, and practicing the procedures for cleaning and disinfecting the DCAs written in the SOPs. Cleaning and disinfecting shall occur before compounding is performed. Items shall be removed from all areas to be cleaned, and surfaces shall be cleaned by removing loose material and residue from spills; for example, water-soluble solid residues are removed with sterile water (for injection or irrigation) and low-shedding wipes. This shall be followed by wiping with a residue-free disinfecting agent such as sterile 70% IPA, which is allowed to dry before compounding begins.

Cleaning and disinfecting surfaces in the LAFWs, BSCs, CAIs, and CACIs are the most critical practices before the preparation of CSPs. Consequently, such surfaces shall be cleaned and disinfected frequently, including at the beginning of each work shift, before each batch preparation is started, every 30 minutes during continuous compounding periods of individual CSPs, when there are spills, and when surface contamination is known or suspected from procedural breaches.

~~Work surfaces in the ISO Class 7 (see Table 1) buffer areas and ISO Class 8 (see Table 1) ante areas as well as segregated compounding areas shall be cleaned and disinfected at least daily, and dust and debris shall be removed when necessary from storage sites for compounding ingredients and supplies using a method that does not degrade the ISO Class 7 or 8 (see Table 1) air quality (see *Disinfectants and Antiseptics* (1072)).~~

~~Floors in the buffer or clean area, ante area, and segregated compounding area are cleaned by mopping with a cleaning and disinfecting agent once daily at a time when no aseptic operations are in progress. Mopping shall be performed by trained personnel using approved agents and procedures described in the written SOPs. It is incumbent on compounding personnel to ensure that such cleaning is performed properly. In the buffer or clean area, ante area, and segregated compounding area, walls, ceilings, and shelving shall be cleaned and disinfected monthly. Cleaning and disinfecting agents are to be used with careful consideration of compatibilities, effectiveness, and inappropriate or toxic residues (see *Appendix II*). Their schedules of use and methods of application shall be in accordance with written SOPs and followed by custodial or compounding personnel.~~

~~All cleaning materials, such as wipers, sponges, and mops, shall be nonshedding, preferably composed of synthetic micro fibers, and dedicated to use in the buffer or clean area, ante area, and segregated compounding areas and shall not be removed from these areas except for disposal. Floor mops may be used in both the buffer or clean area and ante area, but only in that order. Ideally, all cleaning tools are discarded after one use by collection in suitable plastic bags and removed with minimal agitation. If cleaning materials (e.g., mops) are reused, procedures shall be developed (based on manufacturers' recommendations) that ensure that the effectiveness of the cleaning device is maintained and that repeated use does not add to the bioburden of the area being cleaned.~~

~~Supplies and equipment removed from shipping cartons shall be wiped with a suitable disinfecting agent (e.g., sterile 70% IPA) delivered from a spray bottle or other suitable delivery method. After the disinfectant is sprayed or wiped on a surface to be disinfected, the disinfectant shall be allowed to dry, during which time the item shall not be used for compounding purposes.~~

~~Wiping with small sterile 70% IPA swabs that are commercially available in individual foil-sealed packages (or a comparable method) is preferred for disinfecting entry points on bags and vials, allowing the IPA to dry before piercing stoppers with sterile needles and breaking necks of ampuls. The surface of the sterile 70% IPA swabs used for disinfecting entry points of sterile packages and devices shall not contact any other object before contacting the surface of the entry point. Sterile 70% IPA wetted gauze pads or other particle-generating material shall not be used to disinfect the sterile entry points of packages and devices.~~

~~When sterile supplies are received in sealed pouches designed to keep them sterile until opening, the sterile supplies may be removed from the covering pouches as the supplies are introduced into the ISO Class 5 (see Table 1) PEC (LAFW, BSC, CAI, CACI) without the need to disinfect the individual sterile supply items. No shipping or other external cartons may be taken into the buffer or clean area or segregated compounding area.~~

Personnel Cleansing and Garbing

~~The careful cleansing of hands and arms and the correct donning of PPE by compounding personnel constitute the first major step in preventing microbial contamination in CSPs.~~

~~Personnel shall also be thoroughly competent and highly motivated to perform flawless aseptic manipulations with ingredients, devices, and components of CSPs. Squamous cells are normally shed from the human body at a rate of 10^6 or more per hour, and those skin particles are laden with microorganisms.^{8,9} When individuals are experiencing rashes, sunburn, weeping sores, conjunctivitis, active respiratory infection, as well as when they wear cosmetics, they shed these particles at even higher rates. Particles shed from compounding personnel pose an increased risk of microbial contamination of critical sites of CSPs. Therefore, compounding personnel with such conditions as mentioned above shall be excluded from working in ISO Class 5 (see *Table 1*) and ISO Class 7 (see *Table 1*) compounding areas until their conditions are remedied.~~

~~Before entering the buffer area or segregated compounding area (see *Low-Risk Level CSPs with 12-Hour or Less BUD*), compounding personnel shall remove personal outer garments (e.g., bandannas, coats, hats, jackets, scarves, sweaters, vests); all cosmetics, because they shed flakes and particles; and all hand, wrist, and other visible jewelry or piercings (e.g., earrings, lip or eyebrow piercings) that can interfere with the effectiveness of PPE (e.g., fit of gloves and cuffs of sleeves). The wearing of artificial nails or extenders is prohibited while working in the sterile compounding environment. Natural nails shall be kept neat and trimmed.~~

~~Personnel shall don the following PPE in an order that proceeds from those activities considered the dirtiest to those considered the cleanest. Garbing activities considered the dirtiest include donning of dedicated shoes or shoe covers, head and facial hair covers (e.g., beard covers in addition to face masks), and face masks/eye shields. Eye shields are optional unless working with irritants such as germicidal disinfecting agents or when preparing hazardous drugs.~~

~~After donning dedicated shoes or shoe covers, head and facial hair covers, and face masks, a hand-cleansing procedure shall be performed by removing debris from underneath fingernails using a nail cleaner under running warm water followed by vigorous hand washing. Hands and forearms shall be washed to the elbows for at least 30 seconds with soap (either nonantimicrobial or antimicrobial) and water while in the ante-area. The use of antimicrobial scrub brushes is not recommended because they can cause skin irritation and skin damage. Hands and forearms to the elbows will be completely dried using either lint-free disposable towels or an electronic hand dryer. After completion of hand washing, a nonshedding gown with sleeves that fit snugly around the wrists and enclosed at the neck is donned. Gowns designated for buffer area use shall be worn, and preferably they should be disposable. If reusable gowns are worn, they should be laundered appropriately for buffer area use.~~

~~Once inside the buffer area or segregated compounding area (see *Low-Risk Level CSPs with 12-Hour or Less BUD*), and prior to donning sterile powder-free gloves, antiseptic hand cleansing shall be performed using a waterless alcohol-based surgical hand scrub with persistent activity¹⁰ following manufacturers' recommendations. Hands are allowed to dry thoroughly before donning sterile gloves.~~

~~Sterile gloves shall be the last item donned before compounding begins. Gloves become contaminated when they contact nonsterile surfaces during compounding activities. Disinfection of contaminated gloved hands may be accomplished by wiping or rubbing sterile 70% IPA to all contact surface areas of the gloves and letting the gloved hands dry thoroughly. Only use gloves that have been tested for compatibility with alcohol disinfection by the manufacturer. Routine application of sterile 70% IPA shall occur throughout the compounding process and whenever nonsterile surfaces (e.g. vials, counter tops, chairs, carts) are touched. Gloves on~~

~~hands shall also be routinely inspected for holes, punctures, or tears and replaced immediately if such are detected. Antiseptic hand cleansing shall be performed as indicated above. Compounding personnel shall be trained and evaluated in the avoidance of touching critical sites.~~

~~When compounding personnel exit the compounding area during a work shift, the exterior gown may be removed and retained in the compounding area if not visibly soiled, to be re-donned during that same work shift only. However, shoe covers, hair and facial hair covers, face masks/eye shields, and gloves shall be replaced with new ones before re-entering the compounding area, and proper hand hygiene shall be performed.~~

~~During high-risk compounding activities that precede terminal sterilization, such as weighing and mixing of nonsterile ingredients, compounding personnel shall be garbed and gloved the same as when performing compounding in an ISO Class 5 (see *Table 1*) environment. Properly garbed and gloved compounding personnel who are exposed to air quality that is either known or suspected to be worse than ISO Class 7 (see *Table 1*) shall re-garb PPE along with washing their hands properly, performing antiseptic hand cleansing with a waterless alcohol-based surgical hand scrub, and donning sterile gloves upon re-entering the ISO Class 7 (see *Table 1*) buffer area. When CAIs and CACIs are the source of the ISO Class 5 (see *Table 1*) environment, the garbing and gloving requirements for compounding personnel should be as described above, unless the isolator manufacturer can provide written documentation based on validated environmental testing that any component(s) of PPE or personnel cleansing are not required.~~

Personnel Training and Competency Evaluation of Garbing, Aseptic Work Practices, and Cleaning/Disinfection Procedures

~~Personnel who prepare CSPs shall be trained conscientiously and skillfully by expert personnel and through multimedia instructional sources and professional publications in the theoretical principles and practical skills of garbing procedures, aseptic work practices, achieving and maintaining ISO Class 5 (see *Table 1*) environmental conditions, and cleaning and disinfection procedures. This training shall be completed and documented before any compounding personnel begin to prepare CSPs. Compounding personnel shall complete didactic training, pass written competence assessments, undergo skill assessment using observational audit tools, and media fill testing (see *Appendices III-V*).~~

~~Media fill testing of aseptic work skills shall be performed initially before beginning to prepare CSPs and at least annually thereafter for low and medium risk level compounding and semiannually for high-risk level compounding.~~

~~Compounding personnel who fail written tests or observational audits or whose media fill test vials have one or more units showing visible microbial contamination shall be re-instructed and re-evaluated by expert compounding personnel to ensure correction of all aseptic work practice deficiencies. Compounding personnel shall pass all evaluations prior to resuming compounding of sterile preparations. In addition to didactic evaluation and aseptic media fill, compounding personnel must demonstrate proficiency of proper hand hygiene, garbing, and consistent cleaning procedures.~~

~~In the event that cleaning and disinfecting procedures are also performed by other support personnel (e.g., institutional environmental services, housekeeping), thorough training of proper hand hygiene, garbing, and cleaning and disinfection procedures shall be done by a qualified~~

aseptic compounding expert. After completion of training, support personnel shall routinely undergo performance evaluation of proper hand hygiene, garbing, and all applicable cleaning and disinfecting procedures conducted by a qualified aseptic compounding expert.

COMPETENCY EVALUATION OF GARBING AND ASEPTIC WORK PRACTICE

The risk of contaminating a CSP prepared under low-risk level and medium-risk level conditions is highly dependent on proper hand hygiene and garbing practices, compounding personnel aseptic technique, and the presence of surface contamination, assuming that all work is performed in a certified and properly functioning ISO Class 5 (see *Table 1*) PEC and secondary engineering controls, ISO Class 7 (see *Table 1*) buffer area, and ISO Class 8 (see *Table 1*) ante-area. High-risk level CSPs pose the greatest threat to patients because compounding personnel are tasked with the requirement of processing nonsterile components and devices in order to achieve sterility. Compounding personnel shall be evaluated initially prior to beginning compounding CSPs and whenever an aseptic media fill is performed using a form such as the *Sample Form for Assessing Hand Hygiene and Garbing Related Practices of Compounding Personnel* (see *Appendix III*) and the personnel glove fingertip sampling procedures indicated below.

Aseptic Work Practice Assessment and Evaluation via Personnel Glove Fingertip

Sampling—Sampling of compounding personnel glove fingertips shall be performed for all CSP risk level compounding because direct touch contamination is the most likely source of introducing microorganisms into CSPs prepared by humans. Glove fingertip sampling shall be used to evaluate the competency of personnel in performing hand hygiene and garbing procedures in addition to educating compounding personnel on proper work practices, which include frequent and repeated glove disinfection using sterile 70% IPA during actual compounding of CSPs. All personnel shall demonstrate competency in proper hand hygiene and garbing procedures and in aseptic work practices (e.g., disinfection of component surfaces, routine disinfection of gloved hands).

Sterile contact agar plates shall be used to sample the gloved fingertips of compounding personnel after garbing in order to assess garbing competency and after completing the media-fill preparation (without applying sterile 70% IPA) in order to assess the adequacy of aseptic work practices prior to being initially allowed to prepare CSPs for human use and for more experienced personnel to maintain their qualifications to prepare CSPs for human use.

Garbing And Gloving Competency Evaluation—Compounding personnel shall be visually observed during the process of performing hand hygiene and garbing procedures (see *Personnel Cleansing and Garbing under Personnel Training and Evaluation in Aseptic Manipulation Skills* above). The visual observation shall be documented on a form such as the *Sample Form for Assessing Hand Hygiene and Garbing Related Practices of Compounding Personnel* (see *Appendix III*) and maintained to provide a permanent record and long-term assessment of personnel competency.

Gloved Fingertip Sampling—All compounding personnel shall successfully complete an initial competency evaluation and gloved fingertip/thumb sampling procedure (zero cfu) no less than three times before initially being allowed to compound CSPs for human use. Immediately after the compounding employee completes the hand hygiene and garbing procedure (e.g., donning of sterile gloves prior to any disinfection with sterile 70% IPA), the evaluator will collect a gloved fingertip and thumb sample from both hands of the compounding employee onto appropriate agar plates by lightly pressing each fingertip into the agar. The plates will be

incubated for the appropriate incubation period and at the appropriate temperature (see *Incubation Period*). After completing the initial gowning and gloving competency evaluation, re-evaluation of all compounding personnel for this competency shall occur at least annually for personnel who compound low and medium risk level CSPs and semi-annually for personnel who compound high risk level CSPs using one or more sample collections during any media-fill test procedure before they are allowed to continue compounding CSPs for human use.

Immediately prior to sampling, gloves shall not be disinfected with sterile 70% IPA. Disinfecting gloves immediately before sampling will provide false negative results. Plates filled with nutrient agar with neutralizing agents such as lecithin and polysorbate 80 added shall be used when sampling personnel fingertips. Personnel shall "touch" the agar with the fingertips of both hands in separate plates in a manner to create a slight impression in the agar. The sampled gloves shall be immediately discarded and proper hand hygiene performed after sampling. The nutrient agar plates shall be incubated as stated below (see *Incubation Period*). Results should be reported separately as number of cfu per employee per hand (left hand, right hand). The cfu action level for gloved hands will be based on the total number of cfu on both gloves, not per hand.

Incubation Period—At the end of the designated sampling period for compounding personnel competency assessment activities (surface or personnel), the agar plates are recovered and covers secured and they are inverted and incubated at a temperature and for a time period conducive to multiplication of microorganisms. TSA with lecithin and polysorbate 80 shall be incubated at 30° to 35° for 48 to 72 hours.

Aseptic Manipulation Competency Evaluation—After successful completion of an initial Hand Hygiene and Garbing Competency Evaluation, all compounding personnel shall have their aseptic technique and related practice competency evaluated initially during the *Media-Fill Test Procedure* and subsequent annual or semi-annual *Media-Fill Test Procedures*. Records of these evaluations will be maintained using a form such as the *Sample Form for Assessing Aseptic Technique and Related Practices of Compounding Personnel* (see *Appendix IV*) and maintained to provide a permanent record of and long-term assessment of personnel competency.

Media-Fill Test Procedure—The skill of personnel to aseptically prepare CSPs shall be evaluated using sterile fluid bacterial culture media-fill verification, (i.e., sterile bacterial culture medium transfer via a sterile syringe and needle). Media-fill testing is used to assess the quality of the aseptic skill of compounding personnel. Media-fill tests shall represent the most challenging or stressful conditions actually encountered by the personnel being evaluated when they prepare low and medium risk level CSPs and when sterilizing high risk level CSPs. Media-fill challenge tests are also used to verify the capability of the compounding environment and processes to produce sterile preparations.

A commercially available sterile fluid culture media, such as Soybean Casein Digest Medium (see *Sterility Tests* (71)), that is able to promote exponential colonization of bacteria that are most likely to be transmitted to CSPs from the compounding personnel and environment is commonly used. For high risk level CSPs nonsterile commercially available Soybean Casein Digest Medium may be used to make a 3% solution. Normal processing steps, including filter sterilization, shall be mimicked. Media-filled vials shall be incubated at 20° to 25° or at 30° to 35° for a minimum of 14 days. If two temperatures are used for incubation of media-filled samples, then these filled containers should be incubated for at least 7 days at each temperature (see

Microbiological Control and Monitoring of Aseptic Processing Environments (1116)). Failure is indicated by visible turbidity in any one of the media fill units on or before 14 days. Other methodologies recommended by a competent microbiologist to enhance recovery time and sensitivity to detect microbial contamination may be considered (see *CSP Microbial Contamination Risk Levels* for examples of media fill procedures).

SURFACE CLEANING AND DISINFECTION SAMPLING AND ASSESSMENT

Surface sampling is an important component of the maintenance of a suitable microbially controlled environment for compounding CSPs, especially since transfer of microbial contamination from improperly disinfected work surfaces via inadvertent touch contact by compounding personnel can be a potential source of contamination into CSPs. It is useful for evaluating facility and work surface cleaning and disinfecting procedures and employee competency in work practices such as disinfection of component/vial surface cleaning. Surface sampling shall be performed in all ISO classified areas on a periodic basis. Sampling can be accomplished using contact plates or swabs, and it shall be done at the conclusion of compounding. Locations to be sampled shall be defined in a sample plan or on a form. The size of the plate to be used for each sampled location usually ranges from 24 to 30 cm². Contact plates are filled with general solid agar growth medium and neutralizing agents above the rim of the plate, and they are used for sampling regular or flat surfaces. Swabs may be used for sampling irregular surfaces, especially for equipment (see *Microbiological Control and Monitoring of Aseptic Processing Environments (1116)*).

Cleaning and Disinfecting Competency Evaluation—Compounding personnel and other personnel responsible for cleaning shall be visually observed during the process of performing cleaning and disinfecting procedures, during initial personnel training on cleaning procedures, during changes in cleaning staff, and at the completion of any media fill test procedure (see *Cleaning and Disinfecting of Compounding Areas*).

The visual observation shall be documented using a form such as the *Sample Form for Assessing Cleaning and Disinfection Procedures* (see *Appendix V*) and maintained to provide a permanent record and long-term assessment of personnel competency.

Surface Collection Methods—To sample surfaces using a contact plate, gently touch the sample area with the agar surface and roll the plate across the surface to be sampled. The contact plate will leave a growth media residue behind; therefore, immediately after sampling with the contact plate, the sampled area shall be thoroughly wiped with a nonshedding wipe soaked in sterile 70% IPA.

If an area is sampled via the swab method, collection of the sample is processed by using appropriate procedures that will result in the surface location equivalent to that of a contact plate. After swabbing the surface to be sampled, swabs are placed in an appropriate diluent; an aliquot is planted on or in the specified nutrient agar. Results should be reported as cfu per unit of surface area.

Action Levels, Documentation, and Data Evaluation

The value of viable microbial monitoring of gloved fingertips and surfaces of components and the compounding environment are realized when the data are used to identify and correct an unacceptable work practice. Sampling data shall be collected and reviewed on a routine basis

as a means of evaluating the overall control of the compounding environment. If an activity consistently shows elevated levels of microbial growth, competent microbiology personnel shall be consulted.

Any cfu count that exceeds its respective action level (see *Table 4*) should prompt a re-evaluation of the adequacy of personnel work practices, cleaning procedures, operational procedures, and air filtration efficiency within the aseptic compounding location. An investigation into the source of the contamination shall be conducted. Sources could include HVAC systems, damaged HEPA filters, and changes in personnel garbing or working practices. The source of the problem shall be eliminated, the affected area cleaned, and resampling performed.

When gloved fingertip sample results exceed action levels after proper incubation, a review of hand hygiene and garbing procedures as well as glove and surface disinfection procedures and work practices shall be performed and documented. Employee training may be required to correct the source of the problem.

Counts of cfu are to be used as an approximate measure of the environmental microbial bioburden. Action levels are determined on the basis of cfu data gathered at each sampling location and trended over time. The numbers in *Table 4* should be used only as guidelines. Regardless of the number of cfu identified in the compounding facility, further corrective actions will be dictated by the identification of microorganisms recovered (at least the genus level) by an appropriate credentialed laboratory of any microbial bioburden captured as a cfu using an impaction air sampler. Highly pathogenic microorganisms (e.g., Gram-negative rods, coagulase positive staphylococcus, molds and yeasts) can be potentially fatal to patients receiving CSPs and shall be immediately remedied, regardless of cfu count, with the assistance of a competent microbiologist, infection control professional, or industrial hygienist.

Table 4. Recommended Action Levels for Microbial Contamination*

Classification	Fingertip Sample	Surface Sample (Contact Plate) (cfu per plate)
ISO Class 5	>3	>3
ISO Class 7	N/A	>5
ISO Class 8 or worse	N/A	>100
* Pharmaceutical Inspection Co-operation Scheme (PIC/S) Guide to Good Manufacturing Practice for Medicinal Products Annexes PE 009-6, 5 April 2007.		

SUGGESTED STANDARD OPERATING PROCEDURES (SOPS)

The compounding facility shall have written, properly approved SOPs designed to ensure the quality of the environment in which a CSP is prepared. The following procedures are recommended:

1. Access to the buffer area is restricted to qualified personnel with specific responsibilities or assigned tasks in the compounding area.
2. All cartoned supplies are decontaminated in the area by removing them from shipping cartons and wiping or spraying them with a nonresidue-generating disinfecting agent while they are being transferred to a clean and properly disinfected cart or other conveyance for introduction into the buffer area. Manufacturers' directions or published

- data for minimum contact time will be followed. Individual pouched sterile supplies need not be wiped because the pouches can be removed as these sterile supplies are introduced into the buffer area.
3. Supplies that are required frequently or otherwise needed close at hand but not necessarily needed for the scheduled operations of the shift are decontaminated and stored on shelving in the ante-area.
 4. Carts used to bring supplies from the storeroom cannot be rolled beyond the demarcation line in the ante-area, and carts used in the buffer area cannot be rolled outward beyond the demarcation line unless cleaned and disinfected before returning.
 5. Generally, supplies required for the scheduled operations of the shift are wiped down with an appropriate disinfecting agent and brought into the buffer area, preferably on one or more movable carts. Supplies that are required for back-up or general support of operations may be stored on the designated shelving in the buffer area, but excessive amounts of supplies are to be avoided.
 6. Nonessential objects that shed particles shall not be brought into the buffer area, including pencils, cardboard cartons, paper towels, and cotton items (e.g., gauze pads).
 7. Essential paper-related products (e.g., paper syringe overwraps, work records contained in a protective sleeve) shall be wiped down with an appropriate disinfecting agent prior to being brought into the buffer area.
 8. Traffic flow in and out of the buffer area shall be minimized.
 9. Personnel preparing to enter the buffer area shall remove all personal outer garments, cosmetics (because they shed flakes and particles), and all hand, wrist, and other visible jewelry or piercings that can interfere with the effectiveness of PPE.
 10. Personnel entering the ante-area shall don attire as described in *Personnel Cleansing and Garbing* and *Personnel Training and Competency Evaluation of Garbing, Aseptic Work Practices and Cleaning/Disinfection Procedures*.
 11. Personnel shall then thoroughly wash hands and forearms to the elbow with soap and water for at least 30 seconds. An air dryer or disposable nonshedding towels are used to dry hands and forearms after washing.
 12. Personnel entering the buffer area shall perform antiseptic hand cleansing prior to donning sterile gloves using a waterless alcohol-based surgical hand scrub with persistent activity.
 13. Chewing gum, drinks, candy, or food items shall not be brought into the buffer area or ante-area. Materials exposed in patient care and treatment areas shall never be introduced into areas where components and ingredients for CSPs are present.
 14. At the beginning of each compounding activity session, and whenever liquids are spilled, the surfaces of the direct compounding environment are first cleaned with USP Purified Water to remove water-soluble residues. Immediately thereafter, the same surfaces are disinfected with a nonresidue-generating agent using a nonlinting wipe.
 15. Primary engineering controls shall be operated continuously during compounding activity. When the blower is turned off and before other personnel enter to perform compounding activities, only one person shall enter the buffer area for the purposes of turning on the blower (for at least 30 minutes) and disinfecting the work surfaces.
 16. Traffic in the area of the DCA is minimized and controlled.
 17. Supplies used in the DCA for the planned procedures are accumulated and then decontaminated by wiping or spraying the outer surface with sterile 70% IPA or removing the outer wrap at the edge of the DCA as the item is introduced into the aseptic work area.

18. All supply items are arranged in the DCA so as to reduce clutter and provide maximum efficiency and order for the flow of work.
19. After proper introduction into the DCA of supply items required for and limited to the assigned operations, they are so arranged that a clear, uninterrupted path of HEPA-filtered air will bathe all critical sites at all times during the planned procedures. That is, no objects may be placed between the first air from HEPA filters and an exposed critical site.
20. All procedures are performed in a manner designed to minimize the risk of touch contamination. Gloves are disinfected with adequate frequency with an approved disinfectant such as sterile 70% IPA.
21. All rubber stoppers of vials and bottles and the necks of ampuls are disinfected by wiping with sterile 70% IPA and waiting for at least 10 seconds before they are used to prepare CSPs.
22. After the preparation of every CSP, the contents of the container are thoroughly mixed and then inspected for the presence of particulate matter, evidence of incompatibility, or other defects.
23. After procedures are completed, used syringes, bottles, vials, and other supplies are removed, but with a minimum of exit and re-entry into the DCA so as to minimize the risk of introducing contamination into the aseptic workspace.

ELEMENTS OF QUALITY CONTROL

A written description of specific training and performance evaluation program for individuals involved in the use of aseptic techniques for the preparation of sterile products shall be developed for each site. This program equips personnel with the appropriate knowledge and trains them in the required skills necessary to perform the assigned tasks. Each person assigned to the aseptic area in the preparation of sterile products shall successfully complete specialized training in aseptic techniques and aseptic area practices prior to preparing CSPs (see *Personnel Training and Evaluation in Aseptic Manipulation Skills* and *Personnel Training and Competency Evaluation of Garbing, Aseptic Work Practices and Cleaning/Disinfection Procedures*).

Ingredients and Devices

Compounding personnel ascertain that ingredients for CSPs are of the correct identity and appropriate quality using the following information: vendor labels, labeling, certificates of analysis, direct chemical analysis, and knowledge of compounding facility storage conditions:

STERILE INGREDIENTS AND DEVICES

Commercially available sterile drug products, sterile ready-to-use containers, and devices are examples of sterile components. A written procedure for unit-by-unit physical inspection preparatory to use is followed to ensure that these components are sterile, free from defects, and otherwise suitable for their intended use.

NONSTERILE INGREDIENTS AND DEVICES

If any nonsterile components, including containers and ingredients, are used to make a CSP, such CSPs must be high risk. Nonsterile active ingredients and added substances or excipients for CSPs should preferably be official *USP* or *NF* articles. When nonofficial ingredients are used, they shall be accompanied by certificates of analysis from their suppliers to aid compounding

personnel in judging the identity, quality, and purity in relation to the intended use in a particular CSP. Physical inspection of a package of ingredients is necessary in order to detect breaks in the container, looseness in the cap or closure, and deviation from the expected appearance, aroma, and texture of the contents.

Bulk or unformulated drug substances and added substances or excipients shall be stored in tightly closed containers under temperature, humidity, and lighting conditions that are either indicated in official monographs or approved by suppliers. The date of receipt by the compounding facility shall be clearly and indelibly marked on each package of ingredient. After receipt by the compounding facility, packages of ingredients that lack a supplier's expiration date cannot be used after 1 year unless either appropriate inspection or testing indicates that the ingredient has retained its purity and quality for use in CSPs.

Careful consideration and evaluation of nonsterile ingredient sources is especially warranted when the CSP will be administered into the vascular system, central nervous system, or eyes.

Upon receipt of each lot of the bulk drug substance or excipient used for CSPs, the individual compounding the preparation performs a visual inspection of the lot for evidence of deterioration, other types of unacceptable quality, and wrong identification. For bulk drug substances or excipients, visual inspection is performed on a routine basis as described in the written protocol.

Equipment

It is necessary that equipment, apparatus, and devices used to compound a CSP be consistently capable of operating properly and within acceptable tolerance limits. Written procedures outlining required equipment calibration, annual maintenance, monitoring for proper function, and controlled procedures for use of the equipment and specified time frames for these activities are established and followed. Routine maintenance and frequencies shall be outlined in these SOPs. Results from the equipment calibration, annual maintenance reports, and routine maintenance are kept on file for the lifetime of the equipment. Personnel are prepared through an appropriate combination of specific training and experience to operate or manipulate any piece of equipment, apparatus, or device they may use when preparing CSPs. Training includes gaining the ability to determine whether any item of equipment is operating properly or is malfunctioning.

VERIFICATION OF AUTOMATED COMPOUNDING DEVICES (ACDs) FOR PARENTERAL NUTRITION COMPOUNDING

ACDs for the preparation of parenteral nutrition admixtures are widely used by pharmacists in hospitals and other healthcare settings. They are designed to streamline the labor-intensive processes involved in the compounding of these multiple-component formulations by automatically delivering the individual nutritional components in a predetermined sequence under computerized control. Parenteral nutrition admixtures often contain 20 or more individual additives representing as many as 50 or more individual components (e.g., 15 to 20 crystalline amino acids, dextrose monohydrate, and lipids; 10 to 12 electrolyte salts; 5 to 7 trace minerals; and 12 vitamins). Thus, ACDs can provide improved accuracy and precision of the compounding process over the traditional manual compounding methods.

Accuracy

The accuracy of an ACD can be determined in various ways to ensure that the correct quantities of nutrients, electrolytes, or other nutritional components are delivered to the final infusion container. Initially, the ACD is tested for its volume and weight accuracy. For volume accuracy, a suitable volume of Sterile Water for Injection, USP, which represents a typical additive volume (e.g., 40 mL for small volume range of 1 to 100 mL, 300 mL for large volume range of 100 to 1000 mL), is programmed into the ACD and delivered to the appropriate volumetric container. The compounding personnel should then consult *Volumetric Apparatus* (31) for appropriate parameters to assess the volumetric performance of the ACD. For gravimetric accuracy, the balance used in conjunction with the ACD is tested using various weight sizes that represent the amounts typically used to deliver the various additives. Compounding personnel should consult *Balances* (41) (CN-1 May 2016) for acceptable tolerances of the weights used. In addition, the same volume of *Sterile Water for Injection* used to assess volumetric accuracy is then weighed on the balance used in conjunction with the ACD. For example, if 40 mL of water was used in the volumetric assessment, its corresponding weight should be about 40 g (assuming the relative density of water is 1.0). In addition, during the use of the ACD, certain additives, such as potassium chloride (corrected for density differences), can also be tested in the same manner as with an in-process test.

Finally, additional tests of accuracy may be employed that determine the content of certain ingredients in the final volume of the parenteral nutrition admixture. Generally, pharmacy departments do not have the capability to routinely perform chemical analyses such as analyses of dextrose or electrolyte concentrations. Consequently, hospital or institutional laboratories may be called upon to perform these quality assurance tests. However, the methods in such laboratories are often designed for biological, not pharmaceutical, systems. Thus, their testing procedures shall be verified to meet the *USP* requirements stated in the individual monograph for the component being tested. For example, under *Dextrose Injection*, the following is stated: It contains not less than 95.0% and not more than 105.0% of the labeled amount of $C_6H_{12}O_6 \cdot H_2O$. The hospital or institutional chemistry laboratories must validate their methods to apply to this range and correct for their typical measurement of anhydrous dextrose versus dextrose monohydrate. Similar ranges and issues exist, for example, for injections of calcium gluconate, magnesium sulfate, and potassium chloride. The critical point is the use of *USP* references and possible laboratory procedural differences.

Precision

The intermediate precision of the ACD can be determined on the basis of the day-to-day variations in performance of the accuracy measures. Thus, compounding personnel shall keep a daily record of the above-described accuracy assessments and review the results over time. This review shall occur at least at weekly intervals to avoid potentially clinically significant cumulative errors over time. This is especially true for additives with a narrow therapeutic index, such as potassium chloride.

FINISHED PREPARATION RELEASE CHECKS AND TESTS

The following quality metrics shall be performed for all CSPs before they are dispensed or administered:

Inspection of Solution Dosage Forms and Review of Compounding Procedures

All CSPs that are intended to be solutions shall be visually examined for the presence of

~~particulate matter and not administered or dispensed when such matter is observed. The prescription orders, written compounding procedure, preparation records, and expended materials used to make CSPs at all contamination risk levels are inspected for accuracy of correct identities and amounts of ingredients, aseptic mixing and sterilization, packaging, labeling, and expected physical appearance before they are administered or dispensed.~~

PHYSICAL INSPECTION

~~Finished CSPs are individually inspected in accordance with written procedures after compounding. If not distributed promptly, these CSPs are individually inspected just prior to leaving the storage area. Those CSPs that are not immediately distributed are stored in an appropriate location as described in the written procedures. Immediately after compounding, and as a condition of release, each CSP unit, where possible, should be inspected against lighted white or black background or both for evidence of visible particulates or other foreign matter. Prerelease inspection also includes container closure integrity and any other apparent visual defect. CSPs with observed defects should be immediately discarded or marked and segregated from acceptable products in a manner that prevents their administration. When CSPs are not distributed promptly after preparation, a predistribution inspection is conducted to ensure that a CSP with defects, such as precipitation, cloudiness, and leakage, which may develop between the time of release and the time of distribution, is not released.~~

Compounding Accuracy Checks

~~Written procedures for double-checking compounding accuracy shall be followed for every CSP during preparation and immediately prior to release. The double-check system should meet state regulations and include label accuracy and accuracy of the addition of all drug products or ingredients used to prepare the finished product and their volumes or quantities. The used additive containers and, for those additives for which the entire container was not expended, the syringes used to measure the additive should be quarantined with the final products until the final product check is completed. Compounding personnel shall visually confirm that ingredients measured in syringes match the written order being compounded. Preferably, a person other than the compounder can verify that correct volumes of correct ingredients were measured to make each CSP. For example, compounding personnel would pull the syringe plunger back to the volume measured.~~

~~When practical, the accuracy of measurements is confirmed by weighing a volume of the measured fluid, then calculating that volume by dividing the weight by the accurate value of the density, or specific gravity, of the measured fluid. Correct density or specific gravity values programmed in ACDs, which measure by weight using the quotient of the programmed volume divided by the density or specific gravity, shall be confirmed to be accurate before and after delivering volumes of the liquids assigned to each channel or port. These volume accuracy checks and the following additional safety and accuracy checks in this section shall be included in the SOP manual of the CSP facility.~~

Sterility Testing

~~All high-risk level CSPs that are prepared in groups of more than 25 identical individual single-dose packages (e.g., ampuls, bags, syringes, vials) or in multiple-dose vials (MDVs) for administration to multiple patients or that are exposed longer than 12 hours at 2° to 8° and longer than 6 hours at warmer than 8° before they are sterilized shall meet the sterility test~~

~~(see *Sterility Tests* (71)) before they are dispensed or administered. The *Membrane Filtration* method is the method of choice where feasible (e.g., components are compatible with the membrane). A method not described in the *USP* may be used if verification results demonstrate that the alternative is at least as effective and reliable as the *USP Membrane Filtration* method or the *USP Direct Inoculation of the Culture Medium* method where the *Membrane Filtration* method is not feasible.~~

~~When high-risk level CSPs are dispensed before receiving the results of their sterility tests, there shall be a written procedure requiring daily observation of the incubating test specimens and immediate recall of the dispensed CSPs when there is any evidence of microbial growth in the test specimens. In addition, the patient and the physician of the patient to whom a potentially contaminated CSP was administered are notified of the potential risk. Positive sterility test results should prompt a rapid and systematic investigation of aseptic technique, environmental control, and other sterility assurance controls to identify sources of contamination and correct problems in the methods or processes.~~

Bacterial Endotoxin (Pyrogen) Testing

~~All high-risk level CSPs, except those for inhalation and ophthalmic administration, that are prepared in groups of more than 25 identical individual single-dose packages (e.g., ampuls, bags, syringes, vials) or in MDVs for administration to multiple patients or that are exposed longer than 12 hours at 2° to 8° and longer than 6 hours at warmer than 8° before they are sterilized shall be tested to ensure that they do not contain excessive bacterial endotoxins (see *Bacterial Endotoxins Test* (85) and *Pyrogen Test* (151)). In the absence of a bacterial endotoxins limit in the official monograph or other CSP formula source, the CSP shall not exceed the amount of USP Endotoxin Units (per hour per kilogram of body weight or square meters of body surface area) specified in *Bacterial Endotoxins Test* (85) referenced above for the appropriate route of administration.~~

Identity and Strength Verification of Ingredients

~~Compounding facilities shall have at least the following written procedures for verifying the correct identity and quality of CSPs before they are dispensed and administered:~~

- ~~1. That labels of CSPs bear correct names and amounts or concentrations of ingredients, the total volume, the BUD, the appropriate route(s) of administration, the storage conditions, and other information for safe use.~~
- ~~2. That there are correct identities, purities, and amounts of ingredients by comparing the original written order with the written compounding record for the CSP.~~
- ~~3. That correct fill volumes in CSPs and correct quantities of filled units of the CSPs were obtained. When the strength of finished CSPs cannot be confirmed to be accurate, based on the above three inspections, the CSPs shall be assayed by methods that are specific for the active ingredients.~~

STORAGE AND BEYOND-USE DATING

~~BUDs for compounded preparations are usually assigned on the basis of professional experience, which should include careful interpretation of appropriate information sources for the same or similar formulations (see *Stability Criteria and Beyond-Use Dating* under *Pharmaceutical*~~

Compounding—Nonsterile Preparations (795). BUDs for CSPs are rarely based on preparation-specific chemical assay results, which are used with the Arrhenius equation to determine expiration dates (see *General Notices and Requirements*) for manufactured products. The majority of CSPs are aqueous solutions in which hydrolysis of dissolved ingredients is the most common chemical degradation reaction. The extent of hydrolysis and other heat-catalyzed degradation reactions at any particular time point in the life of a CSP represents the thermodynamic sum of exposure temperatures and durations. Such lifetime stability exposure is represented in the mean kinetic temperature calculation (see *Pharmaceutical Calculations in Prescription Compounding (1160)*). Drug hydrolysis rates increase exponentially with arithmetic temperature increase; thus, exposure of a beta-lactam antibiotic solution for 1 day at controlled room temperature (see *General Notices and Requirements*) will have an equivalent effect on the extent of hydrolysis of approximately 3 to 5 days in cold temperatures (see *General Notices and Requirements*).

Personnel who prepare, dispense, and administer CSPs shall store them strictly in accordance with the conditions stated on the label of ingredient products and finished CSPs. When CSPs are known to have been exposed to temperatures warmer than the warmest labeled limit or to temperatures exceeding 40° (see *General Notices and Requirements*) for more than 4 hours, such CSPs should be discarded unless direct assay data or appropriate documentation confirms their continued stability.

Determining Beyond-Use Dates

BUDs and expiration dates are not the same (see *General Notices and Requirements*). Expiration dates for the chemical and physical stability of manufactured sterile products are determined from results of rigorous analytical and performance testing, and they are specific for a particular formulation in its container and at stated exposure conditions of illumination and temperature. When CSPs deviate from conditions in the approved labeling of manufactured products contained in CSPs, compounding personnel may consult the manufacturer of particular products for advice on assigning BUDs based on chemical and physical stability parameters. BUDs for CSPs that are prepared strictly in accordance with manufacturers' product labeling shall be those specified in that labeling or from appropriate literature sources or direct testing. BUDs for CSPs that lack justification from either appropriate literature sources or by direct testing evidence shall be assigned as described in *Stability Criteria and Beyond-Use Dating under Pharmaceutical Compounding—Nonsterile Preparations (795)*.

In addition, compounding personnel may refer to applicable publications to obtain relevant stability, compatibility, and degradation information regarding the drug or its congeners. When assigning a beyond-use date, compounding personnel should consult and apply drug-specific and general stability documentation and literature where available, and they should consider the nature of the drug and its degradation mechanism, the container in which it is packaged, the expected storage conditions, and the intended duration of therapy (see *Expiration Date and Beyond-Use Date under Labeling in the General Notices and Requirements*). Stability information must be carefully interpreted in relation to the actual compounded formulation and conditions for storage and use. Predictions based on other evidence, such as publications, charts, and tables, would result in theoretical BUDs. Theoretically predicted beyond-use dating introduces varying degrees of assumptions and, hence, a likelihood of error or at least inaccuracy. The degree of error or inaccuracy would be dependent on the extent of differences

between the CSPs' characteristics (e.g., composition, concentration of ingredients, fill volume, container type and material) and the characteristics of the products from which stability data or information is to be extrapolated. The greater the doubt of the accuracy of theoretically predicted beyond use dating, the greater the need to determine dating periods experimentally. Theoretically predicted beyond use dating periods should be carefully considered for CSPs prepared from nonsterile bulk active ingredients having therapeutic activity, especially where these CSPs are expected to be compounded routinely. When CSPs will be distributed to and administered in residential locations other than healthcare facilities, the effect of potentially uncontrolled and unmonitored temperature conditions shall be considered when assigning BUDs. It must be ascertained that CSPs will not be exposed to warm temperatures (see *General Notices and Requirements*) unless the compounding facility has evidence to justify stability of CSPs during such exposure.

It should be recognized that the truly valid evidence of stability for predicting beyond use dating can be obtained only through product specific experimental studies. Semiquantitative procedures such as thin layer chromatography (TLC) may be acceptable for many CSPs. However, quantitative stability indicating assays such as high performance liquid chromatographic (HPLC) assays would be more appropriate for certain CSPs. Examples include CSPs with a narrow therapeutic index, where close monitoring or dose titration is required to ensure therapeutic effectiveness and to avoid toxicity; where a theoretically established beyond use dating period is supported by only marginal evidence; or where a significant margin of safety cannot be verified for the proposed beyond use dating period. In short, because beyond use dating periods established from product specific data acquired from the appropriate instrumental analyses are clearly more reliable than those predicted theoretically, the former approach is strongly urged to support dating periods exceeding 30 days.

To ensure consistent practices in determining and assigning BUDs, the compounding facility should have written policies and procedures governing the determination of the BUDs for all compounded products. When attempting to predict a theoretical BUD, a compounded or an admixed preparation should be considered as a unique system that has physical and chemical properties and stability characteristics that differ from its components. For example, antioxidant, buffering, or antimicrobial properties of a sterile vial for injection (SVI) might be lost upon its dilution, with the potential of seriously compromising the chemical stability of the SVI's active ingredient or the physical or microbiological stability of the SVI formulation in general. Thus, the properties stabilized in the SVI formulation usually cannot be expected to be carried over to the compounded or admixed preparation. Preparation specific, experimentally determined stability data evaluation protocols are preferable to published stability information.

Compounding personnel who assign BUDs to CSPs when lacking direct chemical assay results must critically interpret and evaluate the most appropriate available information sources to determine a conservative and safe BUD. The SOP manual of the compounding facility and each specific CSP formula record shall describe the general basis used to assign the BUD and storage conditions.

When manufactured MDVs (see *Multiple Dose Container* under *Preservation, Packaging, Storage, and Labeling* in the *General Notices and Requirements*) of sterile ingredients are used in CSPs, the stoppers of the MDVs are inspected for physical integrity and disinfected by wiping with a sterile 70% IPA swab before each penetration with a sterile withdrawal device. When contaminants or abnormal properties are suspected or observed in MDVs, such MDVs shall be discarded. The BUD after initially entering or opening (e.g., needle puncturing) multiple dose

containers is 28 days (see *Antimicrobial Effectiveness Testing* (51)) unless otherwise specified by the manufacturer.

Proprietary Bag and Vial Systems

The sterility storage and stability beyond use times for attached and activated (where activated is defined as allowing contact of the previously separate diluent and drug contents) container pairs of drug products for intravascular administration (e.g., ADD Vantage[®], Mini Bag Plus[®]) shall be applied as indicated by the manufacturer. In other words, follow manufacturers' instructions for handling and storing ADD Vantage[®], Mini Bag Plus[®], Add A Vial[®], Add Ease[®] products, and any others.

Monitoring Controlled Storage Areas

To ensure that product potency is retained through the manufacturer's labeled expiration date, compounding personnel shall monitor the drug storage areas within the compounding facility. Controlled temperature areas in compounding facilities include controlled room temperature, 20° to 25° with mean kinetic temperature 25°; controlled cold temperature, 2° to 8° with mean kinetic temperature 8°; cold temperature, 2° to 8°; freezing temperature, -25° and -10° (see *General Notices and Requirements*) if needed to achieve freezing, and the media specific temperature range for microbial culture media. A controlled temperature area shall be monitored at least once daily and the results documented on a temperature log. Additionally, compounding personnel shall note the storage temperature when placing the product into or removing the product from the storage unit in order to monitor any temperature aberrations. Suitable temperature recording devices may include a calibrated continuous recording device or a National Institute of Standards and Technology (NIST) calibrated thermometer that has adequate accuracy and sensitivity for the intended purpose, and it shall be properly calibrated at suitable intervals. If the compounding facility uses a continuous temperature recording device, compounding personnel shall verify at least once daily that the recording device itself is functioning properly.

The temperature sensing mechanisms shall be suitably placed in the controlled temperature storage space to reflect accurately its true temperature. In addition, the compounding facility shall adhere to appropriate procedures of all controlled storage spaces to ensure that such spaces are not subject to significantly prolonged temperature fluctuations as may occur, for example, by leaving a refrigerator door open too long.

MAINTAINING STERILITY, PURITY, AND STABILITY OF DISPENSED AND DISTRIBUTED CSPS

This section summarizes the responsibilities of compounding facilities for maintaining quality and control of CSPs that are dispensed and administered within their parent healthcare organizations.

Compounding personnel shall ensure proper storage and security of CSPs prepared by or dispensed from the compounding facility until either their BUDs are reached or they are administered to patients. In fulfilling this general responsibility, the compounding facility is responsible for the proper packaging, handling, transport, and storage of CSPs prepared by or dispensed from it, including the appropriate education, training, and supervision of compounding personnel assigned to these functions. The compounding facility should assist in the education

and training of noncompounding personnel responsible for carrying out any aspect of these functions.

Establishing, maintaining, and ensuring compliance with comprehensive written policies and procedures encompassing these responsibilities is a further responsibility of the compounding facility. Where noncompounding personnel are assigned tasks involving any of these responsibilities, the policies and procedures encompassing those tasks should be developed by compounding supervisors. The quality and control activities related to distribution of CSPs are summarized in the following five subsections. Activities or concerns that should be addressed as the compounding facility fulfills these responsibilities are as follows.

Packaging, Handling, and Transport

Inappropriate processes or techniques involved with packaging, handling, and transport can adversely affect quality and package integrity of CSPs. Although compounding personnel routinely perform many of the tasks associated with these functions, some tasks, such as transport, handling, and placement into storage, may be fulfilled by noncompounding personnel who are not under the direct administrative control of the compounding facility. Under these circumstances, appropriate SOPs shall be established by the compounding facility with the involvement of other departments or services whose personnel are responsible for carrying out those CSP-related functions for which the compounding facility has a direct interest. The performance of the noncompounding personnel is monitored for compliance to established policies and procedures.

The critical requirements that are unique to CSPs and that are necessary to ensure CSP quality and packaging integrity shall be addressed in SOPs. For example, techniques should be specified to prevent the depression of syringe plungers or dislodging of syringe tips during handling and transport. Additionally, disconnection of system components (e.g., where CSPs are dispensed with administration sets attached to them) shall be prevented through the BUD of the CSP. Foam padding or inserts are particularly useful where CSPs are transported by pneumatic tube systems. Regardless of the methods used, the compounding facility must evaluate their effectiveness and the reliability of the intended protection. Evaluation should be continuous—for example, through a surveillance system, including a system of problem reporting to the compounding facility.

Inappropriate transport and handling can adversely affect the quality of certain CSPs having unique stability concerns. For example, the physical shaking that might occur during pneumatic tube transport or undue exposure to heat or light must be addressed on a preparation-specific basis. Alternative transport modes or special packaging measures might be needed for the proper assurance of quality of these CSPs. The use of tamper-evident closures and seals on CSP ports can add an additional measure of security to ensure product integrity regardless of the transport method used.

Chemotoxic and other hazardous CSPs require safeguards to maintain the integrity of the CSP and to minimize the exposure potential of these products to the environment and to personnel who may come in contact with them. Transportation by pneumatic tube should be discouraged because of potential breakage and contamination. Special requirements associated with the packaging, transport, and handling of these agents include the prevention of accidental exposures or spills and the training of personnel in the event of an exposure or spill. Examples of special requirements of these agents also include exposure-reducing strategies such as the

~~use of Luer lock syringes and connections, syringe caps, the capping of container ports, sealed plastic bags, impact-resistant containers, and cautionary labeling.~~

Use and Storage

~~The compounding facility is responsible for ensuring that CSPs in the patient care setting maintain their quality until administered. The immediate labeling of the CSP container will display prominently and understandably the requirements for proper storage and expiration dating. Delivery and patient care setting personnel shall be properly trained to deliver the CSP to the appropriate storage location. Outdated and unused CSPs shall be returned to the compounding facility for disposition.~~

~~SOPs must exist to ensure that storage conditions in the patient care setting are suitable for the CSP-specific storage requirements. Procedures include daily monitoring and documentation of drug storage refrigerators to ensure temperatures between 2° and 8° and the monthly inspection of all drug storage locations by compounding personnel. Inspections shall confirm compliance with appropriate storage conditions, separation of drugs and food, proper use of MDVs, and the avoidance of using single-dose products as MDVs. CSPs, as well as all other drug products, shall be stored in the patient care area in such a way as to secure them from unauthorized personnel, visitors, and patients.~~

Readying for Administration

~~Procedures essential for generally ensuring quality, especially sterility assurance, when readying a CSP for its subsequent administration include proper hand washing, aseptic technique, site care, and change of administration sets. Additional procedures may also be essential for certain CSPs, devices, or techniques. Examples where such special procedures are needed include in-line filtration, the operation of automated infusion control devices, and the replenishment of CSPs into the reservoirs of implantable or portable infusion pumps. When CSPs are likely to be exposed to warmer than 30° for more than 1 hour during their administration to patients, the maintenance of their sterility and stability should be confirmed from either relevant and reliable sources or direct testing.~~

Redispensed CSPs

~~The compounding facility shall have the sole authority to determine when unopened, returned CSPs may be redispensed. Returned CSPs may be redispensed only when personnel responsible for sterile compounding can ensure that such CSPs are sterile, pure, and stable (contain labeled strength of ingredients). The following may provide such assurance: the CSPs were maintained under continuous refrigeration and protected from light, if required, and no evidence of tampering or any readying for use outside the compounding facility exists. Assignment of new storage times and BUDs that exceed the original dates for returned CSPs is permitted only when there is supporting evidence from sterility testing and quantitative assay of ingredients. Thus, initial preparation and thaw times should be documented and reliable measures should have been taken to prevent and detect tampering. Compliance with all procedures associated with maintaining product quality is essential. The CSPs shall not be redispensed if there is not adequate assurance that preparation quality and packaging integrity (including the connections of devices, where applicable) were continuously maintained between the time the CSPs left and the time they were returned. Additionally, CSPs shall not be redispensed if redispensing cannot be supported by the originally assigned BUD.~~

Education and Training

The assurance of CSPs' quality and packaging integrity is highly dependent on the proper adherence of all personnel to the pertinent SOPs. Compounding personnel shall design, implement, and maintain a formal education, training, and competency assessment program that encompasses all the functions and tasks addressed in the foregoing sections and all personnel to whom such functions and tasks are assigned. This program includes the assessment and documentation of procedural breaches, administration mishaps, side effects, allergic reactions, and complications associated with dosage or administration, such as extravasation. This program should be coordinated with the institution's adverse events and incident reporting programs.

Packing and Transporting CSPs

The following sections describe how to maintain sterility and stability of CSPs until they are delivered to patient care locations for administration:

packing cspsfor transit

When CSPs are distributed to locations outside the premises in which they are compounded, compounding personnel select packing containers and materials that are expected to maintain physical integrity, sterility, and stability of CSPs during transit. Packing is selected that simultaneously protects CSPs from damage, leakage, contamination, and degradation, and protects personnel who transport packed CSPs from harm. The SOP manual of the compounding facility specifically describes appropriate packing containers and insulating and stuffing materials, based on information from product specifications, vendors, and experience of compounding personnel. Written instructions that clearly explain how to safely open containers of packed CSPs are provided to patients and other recipients.

transit of csps

Compounding facilities that ship CSPs to locations outside their own premises shall select modes of transport that are expected to deliver properly packed CSPs in undamaged, sterile, and stable condition to recipients.

Compounding personnel should ascertain that temperatures of CSPs during transit by the selected mode will not exceed the warmest temperature specified on the storage temperature range on CSP labels. It is recommended that compounding personnel communicate directly with the couriers to learn shipping durations and exposure conditions that CSPs may encounter.

Compounding personnel shall include specific handling and exposure instructions on the exteriors of containers packed with CSPs to be transported and obtain reasonable assurance of compliance therewith from transporters. Compounding personnel shall periodically review the delivery performance of couriers to ascertain that CSPs are being efficiently and properly transported.

Storage in Locations Outside Compounding Facilities

Compounding facilities that ship CSPs to patients and other recipients outside their own premises shall ascertain or provide, whichever is appropriate, the following assurances:

1. Labels and accessory labeling for CSPs include clearly readable BUDs, storage instructions, and disposal instructions for out-of-date units.

2. ~~Each patient or other recipient is able to store the CSPs properly, including the use of a properly functioning refrigerator and freezer if CSPs are labeled for such storage.~~

PATIENT OR CAREGIVER TRAINING

~~A formal training program is provided as a means to ensure understanding and compliance with the many special and complex responsibilities placed on the patient or caregiver for the storage, handling, and administration of CSPs. The instructional objectives for the training program include all home care responsibilities expected of the patient or caregiver and is specified in terms of patient or caregiver competencies.~~

~~Upon the conclusion of the training program, the patient or caregiver should, correctly and consistently, be able to do the following:~~

1. ~~Describe the therapy involved, including the disease or condition for which the CSPs are prescribed, goals of therapy, expected therapeutic outcome, and potential side effects of the CSPs.~~
2. ~~Inspect all drug products, CSPs, devices, equipment, and supplies on receipt to ensure that proper temperatures were maintained during transport and that goods received show no evidence of deterioration or defects.~~
3. ~~Handle, store, and monitor all drug products, CSPs, and related supplies and equipment in the home, including all special requirements related to same.~~
4. ~~Visually inspect all drug products, CSPs, devices, and other items the patient or caregiver is required to use immediately prior to administration in a manner to ensure that all items are acceptable for use. For example, CSPs must be free from leakage, container cracks, particulates, precipitate, haziness, discoloration, or other deviations from the normal expected appearance, and the immediate packages of sterile devices must be completely sealed, with no evidence of loss of package integrity.~~
5. ~~Check labels immediately prior to administration to ensure the right drug, dose, patient, and time of administration.~~
6. ~~Clean the in-home preparation area, scrub hands, use proper aseptic technique, and manipulate all containers, equipment, apparatus, devices, and supplies used in conjunction with administration.~~
7. ~~Employ all techniques and precautions associated with CSP administration; for example, preparing supplies and equipment, handling of devices, priming the tubing, and discontinuing an infusion.~~
8. ~~Care for catheters, change dressings, and maintain site patency as indicated.~~
9. ~~Monitor for and detect occurrences of therapeutic complications such as infection, phlebitis, electrolyte imbalance, and catheter misplacement.~~
10. ~~Respond immediately to emergency or critical situations such as catheter breakage or displacement, tubing disconnection, clot formation, flow blockage, and equipment malfunction.~~
11. ~~Know when to seek and how to obtain professional emergency services or professional advice.~~
12. ~~Handle, contain, and dispose of wastes, such as needles, syringes, devices, biohazardous spills or residuals, and infectious substances.~~

~~Training programs include a hands-on demonstration and practice with actual items that the patient or caregiver is expected to use, such as CSP containers, devices, and equipment. The~~

~~patient or caregiver practices aseptic and injection technique under the direct observation of a health professional.~~

~~The compounding facility, in conjunction with nursing or medical personnel, is responsible for ensuring initially and on an ongoing basis that the patient or caregiver understands, has mastered, and is capable of and willing to comply with all of these home care responsibilities. This is achieved through a formal, written assessment program. All specified competencies in the patient or caregiver training program are formally assessed. The patient or caregiver is expected to demonstrate to appropriate healthcare personnel mastery of assigned activities before being allowed to administer CSPs unsupervised by a health professional.~~

~~Printed material such as checklists or instructions provided during training may serve as continuing post-training reinforcement of learning or as reminders of specific patient or caregiver responsibilities. Post-training verbal counseling can also be used periodically, as appropriate, to reinforce training and to ensure continuing correct and complete fulfillment of responsibilities.~~

~~PATIENT MONITORING AND ADVERSE EVENTS REPORTING~~

~~Compounding facilities shall clinically monitor patients treated with CSPs according to the regulations and guidelines of their respective state healthcare practitioner licensure boards or of accepted standards of practice. Compounding facilities shall provide patients and other recipients of CSPs with a way to address their questions and report any concerns that they may have with CSPs and their administration devices.~~

~~The SOP manuals of compounding facilities shall describe specific instructions for receiving, acknowledging, and dating receipts, and for recording, or filing, and evaluating reports of adverse events and of the quality of preparation claimed to be associated with CSPs. Reports of adverse events with CSPs shall be reviewed promptly and thoroughly by compounding supervisors to correct and prevent future occurrences. Compounding personnel are encouraged to participate in adverse event reporting and product defects programs of the FDA and USP.~~

~~QUALITY ASSURANCE (QA) PROGRAM~~

~~A provider of CSPs shall have in place a formal QA program intended to provide a mechanism for monitoring, evaluating, correcting, and improving the activities and processes described in this chapter. Emphasis in the QA program is placed on maintaining and improving the quality of systems and the provision of patient care. In addition, the QA program ensures that any plan aimed at correcting identified problems also includes appropriate follow-up to make certain that effective corrective actions were performed.¹¹~~

~~Characteristics of a QA program include the following:~~

- ~~1. Formalization in writing;~~
- ~~2. Consideration of all aspects of the preparations and dispensing of products as described in this chapter, including environmental testing and verification results;~~
- ~~3. Description of specific monitoring and evaluation activities;~~
- ~~4. Specification of how results are to be reported and evaluated;~~
- ~~5. Identification of appropriate follow-up mechanisms when action limits or thresholds are exceeded; and~~

6. ~~Delineation of the individuals responsible for each aspect of the QA program.~~

~~In developing a specific plan, focus is on establishing objective, measurable indicators for monitoring activities and processes that are deemed high risk, high volume, or problem prone. In general, the selection of indicators and the effectiveness of the overall QA program is reassessed on an annual basis.~~

~~ABBREVIATIONS AND ACRONYMS~~

ACD	automated compounding device
ACPH	air changes per hour
ALARA	as low as reasonably achievable
ASHRAE	American Society of Heating, Refrigerating and Air-Conditioning Engineers
BI	biological indicator
BSC	biological safety cabinet
BUD	beyond-use date
CACI	compounding aseptic containment isolator
CAI	compounding aseptic isolator
CDC	Centers for Disease Control and Prevention
CETA	Controlled Environment Testing Association
cfu	colony-forming unit(s)
CSP	compounded sterile preparation
ESTD	closed-system vial transfer device
DCA	direct compounding area
ECV	endotoxin challenge vial
EU	Endotoxin Unit
FDA	Food and Drug Administration
HEPA	high efficiency particulate air
HICPAC	Healthcare Infection Control Practices Advisory Committee
HVAC	heating, ventilation, and air conditioning
IPA	isopropyl alcohol
ISO	International Organization for Standardization
LAFW	laminar airflow workbench
MDVs	multiple-dose vials
MMWR	Morbidity and Mortality Weekly Report
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
PEC	primary engineering control
PET	positron emission tomography
PPE	personnel protective equipment
psi	pounds per square inch
QA	quality assurance
SOP	standard operating procedure
SVI	sterile vial for injection
TSA	trypticase soy agar
USP	United States Pharmacopeia

GLOSSARY

Ante-Area: An ISO Class 8 (see *Table 1*) or better area where personnel hand hygiene and garbing procedures, staging of components, order entry, CSP labeling, and other high-

particulate-generating activities are performed. It is also a transition area that (1) provides assurance that pressure relationships are constantly maintained so that air flows from clean to dirty areas and (2) reduces the need for the heating, ventilating, and air-conditioning (HVAC) control system to respond to large disturbances.¹²

Aseptic Processing: (see *Microbiological Control and Monitoring of Aseptic Processing Environments* (1116)) A mode of processing pharmaceutical and medical products that involves the separate sterilization of the product and of the package (containers, closures, or packaging material for medical devices) and the transfer of the product into the container and its closure under at least ISO Class 5 (see *Table 1*) conditions.

Beyond-Use Date (BUD): (see *General Notices and Requirements and Pharmaceutical Compounding—Nonsterile Preparations* (795)) For the purpose of this chapter, the date or time after which a CSP shall not be stored or transported. The date is determined from the date or time the preparation is compounded.

Biological Safety Cabinet (BSC): A ventilated cabinet for CSPs, personnel, product, and environmental protection having an open front with inward airflow for personnel protection, downward high-efficiency particulate air (HEPA)-filtered laminar airflow for product protection, and HEPA-filtered exhausted air for environmental protection.

Buffer Area: An area where the primary engineering control (PEC) is physically located. Activities that occur in this area include the preparation and staging of components and supplies used when compounding CSPs.

Clean Room: (see *Microbiological Control and Monitoring of Aseptic Processing Environments* (1116) and also the definition of *Buffer Area*) A room in which the concentration of airborne particles is controlled to meet a specified airborne particulate cleanliness class. Microorganisms in the environment are monitored so that a microbial level for air, surface, and personnel gear are not exceeded for a specified cleanliness class.

Compounding Aseptic Containment Isolator (CACI): A compounding aseptic isolator (CAI) designed to provide worker protection from exposure to undesirable levels of airborne drug throughout the compounding and material transfer processes and to provide an aseptic environment for compounding sterile preparations. Air exchange with the surrounding environment should not occur unless the air is first passed through a microbial retentive filter (HEPA minimum) system capable of containing airborne concentrations of the physical size and state of the drug being compounded. Where volatile hazardous drugs are prepared, the exhaust air from the isolator should be appropriately removed by properly designed building ventilation.

Compounding Aseptic Isolator (CAI): A form of isolator specifically designed for compounding pharmaceutical ingredients or preparations. It is designed to maintain an aseptic compounding environment within the isolator throughout the compounding and material transfer processes. Air exchange into the isolator from the surrounding environment should not occur unless the air has first passed through a microbially retentive filter (HEPA minimum).¹³

Critical Area: An ISO Class 5 (see *Table 1*) environment.

Critical Site: A location that includes any component or fluid pathway surfaces (e.g., vial septa, injection ports, beakers) or openings (e.g., opened ampuls, needle hubs) exposed and at risk of direct contact with air (e.g., ambient room or HEPA filtered), moisture (e.g., oral and mucosal secretions), or touch contamination. Risk of microbial particulate contamination of the

critical site increases with the size of the openings and exposure time.

Direct Compounding Area (DCA): A critical area within the ISO Class 5 (see *Table 1*) primary engineering control (PEC) where critical sites are exposed to unidirectional HEPA filtered air, also known as first air.

Disinfectant: An agent that frees from infection, usually a chemical agent but sometimes a physical one, and that destroys disease causing pathogens or other harmful microorganisms but may not kill bacterial and fungal spores. It refers to substances applied to inanimate objects.

First Air: The air exiting the HEPA filter in a unidirectional air stream that is essentially particle free.

Hazardous Drugs: Drugs are classified as hazardous if studies in animals or humans indicate that exposures to them have a potential for causing cancer, development or reproductive toxicity, or harm to organs. (See current NIOSH publication.)

Labeling: [see *General Notices and Requirements* and 21 USC 321 (k) and (m)] A term that designates all labels and other written, printed, or graphic matter on an immediate container of an article or preparation or on, or in, any package or wrapper in which it is enclosed, except any outer shipping container. The term "label" designates that part of the labeling on the immediate container.

Media-Fill Test: (see *Microbiological Control and Monitoring of Aseptic Processing Environments* (1116)) A test used to qualify aseptic technique of compounding personnel or processes and to ensure that the processes used are able to produce sterile product without microbial contamination. During this test, a microbiological growth medium such as Soybean-Casein Digest Medium is substituted for the actual drug product to simulate admixture compounding.³ The issues to consider in the development of a media-fill test are media-fill procedures, media selection, fill volume, incubation, time and temperature, inspection of filled units, documentation, interpretation of results, and possible corrective actions required.

Multiple-Dose Container: (see *General Notices and Requirements* and ⁶⁵⁹ (CN 1 May 2016)) A multiple-unit container for articles or preparations intended for parenteral administration only and usually containing antimicrobial preservatives. The beyond-use date (BUD) for an opened or entered (e.g., needle-punctured) multiple-dose container with antimicrobial preservatives is 28 days (see *Antimicrobial Effectiveness Testing* (51)), unless otherwise specified by the manufacturer.

Negative Pressure Room: A room that is at a lower pressure than the adjacent spaces and, therefore, the net flow of air is *into* the room.¹²

Pharmacy Bulk Package: (see ⁶⁵⁹ (CN 1 May 2016)) A container of a sterile preparation for parenteral use that contains many single doses. The contents are intended for use in a pharmacy admixture program and are restricted to the preparation of admixtures for infusion or, through a sterile transfer device, for the filling of empty sterile syringes. The closure shall be penetrated only one time after constitution with a suitable sterile transfer device or dispensing set, which allows measured dispensing of the contents. The pharmacy bulk package is to be used only in a suitable work area such as a laminar flow hood (or an equivalent clean air compounding area).

Where a container is offered as a pharmacy bulk package, the label shall (a) state prominently

~~“Pharmacy Bulk Package—Not for Direct Infusion,” (b) contain or refer to information on proper techniques to help ensure safe use of the product, and (c) bear a statement limiting the time frame in which the container may be used once it has been entered, provided it is held under the labeled storage conditions.~~

~~**Primary Engineering Control (PEC):** A device or room that provides an ISO Class 5 (see *Table 1*) environment for the exposure of critical sites when compounding CSPs. Such devices include, but may not be limited to, laminar airflow workbenches (LAFWs), biological safety cabinets (BSCs), compounding aseptic isolators (CAIs), and compounding aseptic containment isolators (CACIs).~~

~~**Preparation:** A preparation, or a CSP, that is a sterile drug or nutrient compounded in a licensed pharmacy or other healthcare-related facility pursuant to the order of a licensed prescriber; the article may or may not contain sterile products.~~

~~**Product:** A commercially manufactured sterile drug or nutrient that has been evaluated for safety and efficacy by the FDA. Products are accompanied by full prescribing information, which is commonly known as the FDA-approved manufacturer's labeling or product package insert.~~

~~**Positive Pressure Room:** A room that is at a higher pressure than the adjacent spaces and, therefore, the net airflow is *out of* the room.¹²~~

~~**Single-Dose Container:** (see *General Notices and Requirements* and ~~• (659) • (CN 1 May 2016)~~) A single-dose container is a single-unit container for articles (see *General Notices and Requirements*) or preparations intended for parenteral administration only. It is intended for a single use. A single-dose container is labeled as such. Examples of single-dose containers include prefilled syringes, cartridges, fusion-sealed containers, and closure-sealed containers when so-labeled.~~

~~**Segregated Compounding Area:** A designated space, either a demarcated area or room, that is restricted to preparing low-risk level CSPs with 12-hour or less BUD. Such area shall contain a device that provides unidirectional airflow of ISO Class 5 (see *Table 1*) air quality for preparation of CSPs and shall be void of activities and materials that are extraneous to sterile compounding.~~

~~**Sterilizing Grade Membranes:** Membranes that are documented to retain 100% of a culture of 10^7 microorganisms of a strain of *Brevundimonas (Pseudomonas) diminuta* per square centimeter of membrane surface under a pressure of not less than 30 psi (2.0 bar). Such filter membranes are nominally at 0.22 μm or 0.2 μm nominal pore size, depending on the manufacturer's practice.~~

~~**Sterilization by Filtration:** Passage of a fluid or solution through a sterilizing grade membrane to produce a sterile effluent.~~

~~**Terminal Sterilization:** The application of a lethal process (e.g., steam under pressure or autoclaving) to sealed containers for the purpose of achieving a predetermined sterility assurance level of usually less than 10^{-6} , or a probability of less than one in one million of a nonsterile unit.¹³~~

~~**Unidirectional Flow** (see footnote 3): An airflow moving in a single direction in a robust and uniform manner and at sufficient speed to reproducibly sweep particles away from the critical~~

~~processing or testing area.~~

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APPENDICES

Appendix I. Principal Competencies, Conditions, Practices, and Quality Assurances That Are Required († “shall”) and Recommended († “should”) in USP Chapter (797)

<p>note—This tabular appendix selectively abstracts and condenses the full text of (797) for rapid reference only. Compounding personnel are responsible for reading, understanding and complying with the full text and all official USP terminology, content, and conditions therein.</p>
<p>—</p>
<p>INTRODUCTION</p>
<p>† Chapter purpose is to prevent harm and death to patients treated with CSPs.</p>
<p>† Chapter pertains to preparation, storage, and transportation, but not administration, of CSPs.</p>
<p>† Personnel and facilities to which (797) applies; therefore, for whom and which it may be enforced by regulatory and accreditation authorities.</p>
<p>† Types of preparations designated to be CSPs according to their physical forms, and their sites and routes of administration to patients.</p>
<p>† Compounding personnel must be meticulously conscientious to preclude contact contamination of CSPs both within and outside ISO Class 5 areas.</p>
<p>—</p>
<p>ORGANIZATION</p>
<p>† All compounding personnel shall be responsible for understanding fundamental practices and precautions within USP (797), for developing and implementing appropriate procedures, and for continually evaluating these procedures and the quality of final CSPs to prevent harm.</p>
<p>—</p>
<p>RESPONSIBILITY OF COMPOUNDING PERSONNEL</p>
<p>† Practices and quality assurances required to prepare, store, and transport CSPs that are sterile, and acceptably accurate, pure, and stable.</p>
<p>—</p>
<p>CSP MICROBIAL CONTAMINATION RISK LEVELS</p>
<p>† Proper training and evaluation of personnel, proper cleansing and garbing of personnel, proper cleaning and disinfecting of compounding work environments, and proper maintenance and monitoring of controlled environmental locations (all of which are detailed in their respective sections).</p>
<p>Low-Risk Level CSPs</p>

† Aseptic manipulations within an ISO Class 5 environment using three or fewer sterile products and entries into any container.
† In absence of passing sterility test, store not more than 48 hours at controlled room temperature, 14 days at cold temperature, and 45 days in solid frozen state at -25° to -10° or colder.
† Media fill test at least annually by compounding personnel.
Low-Risk Level CSPs with 12-Hour or Less BUD
† Fully comply with all four specific criteria.
† Sinks should not be located adjacent to the ISO Class 5 primary engineering control.
† Sinks should be separated from the immediate area of the ISO Class 5 primary engineering control device.
Medium-Risk Level CSPs
† Aseptic manipulations within an ISO Class 5 environment using prolonged and complex mixing and transfer, more than three sterile products and entries into any container, and pooling ingredients from multiple sterile products to prepare multiple CSPs.
† In absence of passing sterility test, store not more than 30 hours at controlled room temperature, 9 days at cold temperature, and 45 days in solid frozen state at -25° to -10° or colder.
† Media fill test at least annually by compounding personnel.
High-Risk Level CSPs
† Confirmed presence of nonsterile ingredients and devices, or confirmed or suspected exposure of sterile ingredients for more than one hour to air quality inferior to ISO Class 5 before final sterilization.
† Sterilization method verified to achieve sterility for the quantity and type of containers.
† Meet allowable limits for bacterial endotoxins.
† Maintain acceptable strength and purity of ingredients and integrity of containers after sterilization.
† In absence of passing sterility test, store not more than 24 hours at controlled room temperature, 3 days at cold temperature, and 45 days in solid frozen state at -25° to -10° or colder.
† Media fill test at least semiannually by compounding personnel.
-
PERSONNEL TRAINING AND EVALUATION IN ASEPTIC MANIPULATIONS SKILLS
† Pass didactic, practical skill assessment and media fill testing initially, followed by an annual assessment for a low- and medium-risk level compounding and semi-annual assessment for high-risk level compounding.
† Compounding personnel who fail written tests, or whose media fill test vials result in gross microbial colonization, shall be immediately re-instructed and re-evaluated by expert compounding personnel to ensure correction of all aseptic practice deficiencies.
-
IMMEDIATE-USE CSPs
† Fully comply with all six specified criteria.
-
SINGLE-DOSE AND MULTIPLE-DOSE CONTAINERS
† Beyond use date 28 days, unless specified otherwise by the manufacturer, for closure sealed multiple-dose containers after initial opening or entry.

† Beyond use time of 6 hours, unless specified otherwise by the manufacturer, for closure sealed single dose containers in ISO Class 5 or cleaner air after initial opening or entry.
† Beyond use time of 1 hour for closure sealed single dose containers after being opened or entered in worse than ISO Class 5 air.
† Storage of opened single dose ampuls is not permitted.
-
HAZARDOUS DRUGS AS CSPs
† Appropriate personnel protective equipment.
† Appropriate primary engineering controls (BSCs and CACIs) are used for concurrent personnel protection and exposure of critical sites.
† Hazardous drugs shall be stored separately from other inventory in a manner to prevent contamination and personnel exposure.
† At least 0.01 inch water column negative pressure and 12 air changes per hour in non-cleanrooms in which CACIs are located.
† Hazardous drugs shall be handled with caution at all times using appropriate chemotherapy gloves during receiving, distribution, stocking, inventorying, preparing for administration, and disposal.
† Hazardous drugs shall be prepared in an ISO Class 5 environment with protective engineering controls in place, and following aseptic practices specified for the appropriate contamination risk levels.
† Access to drug preparation areas shall be limited to authorized personnel.
† A pressure indicator shall be installed that can readily monitor room pressurization, which is documented daily.
† Annual documentation of full training of personnel regarding storage, handling, and disposal of hazardous drugs.
† When used, a CSTD shall be used in an ISO Class 5 primary engineering control device.
† At least 0.01 inch water column negative pressure is required for compounding of hazardous drugs.
† Negative pressure buffer area is not required for low volume compounding operations when CSTD is used in BSC or CACI.
† Compounding personnel of reproductive capability shall confirm in writing that they understand the risks of handling hazardous drugs.
† Disposal of all hazardous drug wastes shall comply with all applicable federal and state regulations.
† Total external exhaust of primary engineering controls.
† Assay of surface wipe samples every 6 months.
-
RADIOPHARMACEUTICALS AS CSPs
† Positron Emission Tomography is according to USP chapter (823).
† Appropriate primary engineering controls and radioactivity containment and shielding.

<p>† Radiopharmaceuticals compounded from sterile components, in closed sterile containers, with volume of 100 mL or less for a single dose injection or not more than 30 mL taken from a multiple dose container shall be designated as and conform to the standards for low risk level CSPs.</p>
<p>† Radiopharmaceutical vials, designed for multi use, compounded with technetium-99m, exposed to ISO Class 5 environment and punctured by needles with no direct contact contamination may be used up to the time indicated by manufacturers' recommendations.</p>
<p>† Location of primary engineering controls permitted in ISO Class 8 controlled environment.</p>
<p>† Technetium-99m/Molybdenum-99 generators used according to manufacturer, state, and federal requirements.</p>
<p>† Radiopharmaceuticals prepared as low risk level CSPs with 12 hour or less BUD shall be prepared in a segregated compounding area.</p>
<p>† Materials and garb exposed in patient care and treatment area shall not cross a line of demarcation into the segregated compounding area.</p>
<p>† Technetium-99m/Molybdenum-99 generators must be eluted in ISO Class 8 conditions.</p>
<p>† Segregated compounding area will be designated with a line of demarcation.</p>
<p>† Storage and transport of properly shielded vials of radiopharmaceutical CSPs may occur in a limited access ambient environment without a specific ISO class designation.</p>
<p>-</p>
<p>ALLERGEN EXTRACTS AS CSPs</p>
<p>† Allergen extracts as CSPs are not subject to the personnel, environmental, and storage requirements for all CSP Microbial Contamination Risk Levels when certain criteria are met.</p>
<p>-</p>
<p>VERIFICATION OF COMPOUNDING ACCURACY AND STERILITY</p>
<p>† Review labels and document correct measurements, aseptic manipulations, and sterilization procedures to confirm correct identity, purity, and strength of ingredients in, and sterility of, CSPs.</p>
<p>† Assay finished CSPs to confirm correct identity and, or, strength of ingredients.</p>
<p>† Sterility test finished CSPs.</p>
<p>Sterilization Methods</p>
<p>† Verify that methods achieve sterility while maintaining appropriate strength, purity, quality, and packaging integrity.</p>
<p>† Prove effectiveness by USP chapter (71), equivalent, or superior sterility testing.</p>
<p>Sterilization of High-Risk Level CSPs by Filtration</p>
<p>† Nominal 0.2 µm pore size sterile membranes that are chemically and physically compatible with the CSP.</p>
<p>† Complete rapidly without filter replacement.</p>
<p>† Subject filter to manufacturer's recommended integrity test (e.g., bubble point test) after filtering CSPs.</p>
<p>Sterilization of High-Risk Level CSPs by Steam</p>
<p>† Test to verify the mass of containers to be sterilized will be sterile after the selected exposure duration in the particular autoclave.</p>
<p>† Ensure live steam contacts all ingredients and surfaces to be sterilized.</p>

† Pass solutions through a 1.2-µm or smaller nominal pore size filter into final containers to remove particulates before sterilization.
† Heated filtered air shall be evenly distributed throughout the chamber by a blower device.
† Dry heat shall only be used for those materials that cannot be sterilized by steam, when the moisture would either damage or be impermeable to the materials.
† Sufficient space shall be left between materials to allow for good circulation of the hot air.
† The description of dry heat sterilization conditions and duration for specific CSPs shall be included in written documentation in the compounding facility. The effectiveness of dry heat sterilization shall be verified using appropriate biological indicators and other confirmation.
† The oven should be equipped with a system for controlling temperature and exposure period.
Depyrogenation by Dry Heat
† Dry heat depyrogenation shall be used to render glassware or containers, such as vials free from pyrogens as well as viable microbes.
† The description of the dry heat depyrogenation cycle and duration for specific load items shall be included in written documentation in the compounding facility.
† The effectiveness of the dry heat depyrogenation cycle shall be verified using endotoxin challenge vials (ECVs).
† The bacterial endotoxin test should be performed on the ECVs to verify the cycle is capable of achieving a 3-log reduction in endotoxin.
-
ENVIRONMENTAL QUALITY AND CONTROL
Exposure of Critical Sites
† ISO Class 5 or better air.
† Preclude direct contact (e.g., touch and secretions) contamination.
ISO Class 5 Air Sources, Buffer Areas, and Ante-Areas
† A buffer area is an area that provides at least ISO Class 7 air quality.
† New representations of facility layouts.
† Each compounding facility shall ensure that each source of ISO Class 5 environment for exposure of critical sites and sterilization by filtration is properly located, operated, maintained, monitored, and verified.
† Devices (e.g., computers and printers) and objects (e.g., carts and cabinets) can be placed in buffer areas and shall be verified by testing or monitoring.
Viable and Nonviable Environmental Sampling (ES) Testing
† Environmental sampling shall occur as part a comprehensive quality management program and shall occur minimally when several conditions exist.
† The ES program should provide information to staff and leadership to demonstrate that the engineering controls are maintaining an environment within the compounding area that consistently maintains acceptably low viable and nonviable particle levels.
Environmental Nonviable Particle Testing Program
† Certification and testing of primary (LAFWs, BSCs, CAIs and CACIs) and secondary engineering controls (buffer and ante areas) shall be performed by a qualified individual no less than every six months and whenever the device or room is relocated, altered, or major service to the facility is performed. Certification procedures such as those outlined in the CETA Certification Guide for Sterile Compounding Facilities (CAG-003-2006) shall be used.
Total Particle Counts

<p>† Certification that each ISO classified area (e.g., ISO Class 5, 7 and 8) is within established guidelines shall be performed no less than every 6 months and whenever the LAFW, BSC, CAI, or CACI is relocated or the physical structure of the buffer room or ante-area has been altered.</p>
<p>† Testing shall be performed by qualified operators using current, state-of-the-art electronic equipment with results meeting ISO Class 5, 7, or 8 depending on the requirements of the area.</p>
<p>† All certification records shall be maintained and reviewed by supervising personnel or other designated employee to ensure that the controlled environments comply with the proper air cleanliness, room pressures, and air changes per hour.</p>
<p>Pressure Differential Monitoring</p>
<p>† A pressure gauge or velocity meter shall be installed to monitor the pressure differential or airflow between the buffer area and ante-area, and the ante-area and the general environment outside the compounding area.</p>
<p>† The results shall be reviewed and documented on a log at least every work shift (minimum frequency shall be at least daily) or by a continuous recording device.</p>
<p>† The pressure between the ISO Class 7 and general pharmacy area shall not be less than 5 Pa (0.02 inch water column (w.c.)).</p>
<p>† In facilities where low- and medium-risk level CSPs are prepared, differential airflow shall maintain a minimum velocity of 0.2 meter/second (40 fpm) between buffer area and ante-area.</p>
<p>Environmental Viable Airborne Particle Testing Program—Sampling Plan</p>
<p>† An appropriate environmental sampling plan shall be developed for airborne viable particles based on a risk assessment of compounding activities performed.</p>
<p>† Selected sampling sites shall include locations within each ISO Class 5 environment and in the ISO Class 7 and 8 areas, and the segregated compounding areas at greatest risk of contamination (e.g., work areas near the ISO Class 5 environment, counters near doors, pass-through boxes).</p>
<p>† The plan shall include sample location, method of collection, frequency of sampling, volume of air sampled, and time of day as related to activity in the compounding area and action levels.</p>
<p>† It is recommended that compounding personnel refer to USP Chapter <i>Microbiological Control and Monitoring of Aseptic Processing Environments</i> (1116) and the CDC Guidelines for Environmental Infection Control in Healthcare Facilities 2003 for more information.</p>
<p>Growth Media</p>
<p>† A general microbiological growth medium such as Soybean Casein Digest Medium (also known as trypticase soy broth (TSB) or agar (TSA)) shall be used to support the growth of bacteria.</p>
<p>† Malt extract agar (MEA) or some other media that supports the growth of fungi shall be used in high-risk level compounding environments.</p>
<p>† Media used for surface sampling shall be supplemented with additives to neutralize the effects of disinfecting agents (e.g., TSA with lecithin and polysorbate 80).</p>
<p>Viable Air Sampling</p>
<p>† Evaluation of airborne microorganisms using volumetric collection methods in the controlled air environments shall be performed by properly trained individuals for all compounding risk levels.</p>

<p>† Impaction shall be the preferred method of volumetric air sampling.</p>
<p>† For low-, medium-, and high-risk level compounding, air sampling shall be performed at locations that are prone to contamination during compounding activities and during other activities like staging, labeling, gowning, and cleaning.</p>
<p>† Locations shall include zones of air backwash turbulence within laminar airflow workbench and other areas where air backwash turbulence may enter the compounding area.</p>
<p>† For low-risk level CSPs with 12-hour or less BUD, air sampling shall be performed at locations inside the ISO Class 5 environment and other areas that are in close proximity to the ISO class 5 environment, during the certification of the primary engineering control.</p>
<p>† Consideration should be given to the overall effect the chosen sampling method will have on the unidirectional airflow within a compounding environment.</p>
<p>Air Sampling Devices</p>
<p>† The instructions in the manufacturer's user manual for verification and use of electric air samplers that actively collect volumes of air for evaluation shall be followed.</p>
<p>† A sufficient volume of air (400–1000 liters) shall be tested at each location in order to maximize sensitivity.</p>
<p>† It is recommended that compounding personnel also refer to USP Chapter (1116), which can provide more information on the use of volumetric air samplers and volume of air that should be sampled to detect environmental bioburden excursions.</p>
<p>Air Sampling Frequency and Process</p>
<p>† Air sampling shall be performed at least semiannually (i.e. every 6 months), as part of the re-certification of facilities and equipment for area where primary engineering controls are located.</p>
<p>† A sufficient volume of air shall be sampled and the manufacturer's guidelines for use of the electronic air sampling equipment followed.</p>
<p>† Any facility construction or equipment servicing may require the need to perform air sampling during these events.</p>
<p>Incubation Period</p>
<p>† The microbial growth media plates used to collect environmental sampling are recovered, covers secured (e.g., taped), inverted, and incubated at a temperature and for a time period conducive to multiplication of microorganisms.</p>
<p>† The number of discrete colonies of microorganisms shall be counted and reported as colony-forming units (cfu) and documented on an environmental monitoring form. Counts from air monitoring need to be transformed into cfu/cubic meter of air and evaluated for adverse trends.</p>
<p>† TSA should be incubated at 35° ± 2° for 2–3 days.</p>
<p>† MEA or other suitable fungal media should be incubated at 28° ± 2° for 5–7 days.</p>
<p>Action Levels, Documentation and Data Evaluation</p>
<p>† Sampling data shall be collected and reviewed on a periodic basis as a means of evaluating the overall control of the compounding environment.</p>
<p>† Competent microbiology personnel shall be consulted if an environmental sampling consistently shows elevated levels of microbial growth.</p>
<p>† An investigation into the source of the environmental contamination shall be conducted.</p>

<p>† Any cfu count that exceeds its respective action level should prompt a re-evaluation of the adequacy of personnel work practices, cleaning procedures, operational procedures, and air filtration efficiency within the aseptic compounding location.</p>
<p>† Table titled, Recommended Action Levels for Microbial Contamination should only be used as a guideline</p>
<p>Facility Design and Environmental Controls</p>
<p>† Compounding facilities are physically designed and environmentally controlled to minimize airborne contamination from contacting critical sites.</p>
<p>† Compounding facilities shall provide a comfortable and well-lighted working environment, which typically includes a temperature of 20° or cooler to maintain comfortable conditions for compounding personnel when attired in the required aseptic compounding garb.</p>
<p>† Primary engineering controls provide unidirectional (i.e., laminar) HEPA air at a velocity sufficient to prevent airborne particles from contacting critical sites.</p>
<p>† In-situ air pattern analysis via smoke studies shall be conducted at the critical area to demonstrate unidirectional airflow and sweeping action over and away from the product under dynamic conditions.</p>
<p>† Policies and procedures for maintaining and working within the primary engineering control area shall be written and followed. The policies and procedures will be determined by the scope and risk levels of the aseptic compounding activities used during the preparation of the CSPs.</p>
<p>† The principles of HEPA filtered unidirectional airflow in the work environment shall be understood and practiced in the compounding process in order to achieve the desired environmental conditions.</p>
<p>† Clean rooms for nonhazardous and nonradioactive CSPs are supplied with HEPA that enters from ceilings with return vents low on walls, and that provides not less than 30 air changes per hour.</p>
<p>† Buffer areas maintain 0.02 to 0.05 inch water column positive pressure, and do not contain sinks or drains.</p>
<p>† Air velocity from buffer rooms or zones to ante-areas is at least 40 feet/minute.</p>
<p>† The primary engineering controls shall be placed within a buffer area in such a manner as to avoid conditions that could adversely affect their operation.</p>
<p>† The primary engineering controls shall be placed out of the traffic flow and in a manner to avoid disruption from the HVAC system and room cross-drafts.</p>
<p>† HEPA filtered supply air shall be introduced at the ceiling.</p>
<p>† All HEPA filters shall be efficiency tested using the most penetrating particle size and shall be leak tested at the factory and then leak tested again in situ after installation.</p>
<p>† Activities and tasks carried out within the buffer area shall be limited to only those necessary when working within a controlled environment.</p>
<p>† Only the furniture, equipment, supplies, and other material required for the compounding activities to be performed shall be brought into the room.</p>
<p>† Surfaces and essential furniture in buffer rooms or zones and clean rooms shall be nonporous, smooth, nonshedding, impermeable, cleanable, and resistant to disinfectants.</p>
<p>† The surfaces of ceilings, walls, floors, fixtures, shelving, counters, and cabinets in the buffer area shall be smooth, impervious, free from cracks and crevices, and nonshedding, thereby promoting cleanability, and minimizing spaces in which microorganisms and other contaminants may accumulate.</p>
<p>† The surfaces shall be resistant to damage by disinfectant agents.</p>

† Juncitures of ceilings to walls shall be covered or caulked to avoid cracks and crevices where dirt can accumulate.
† Ceiling tiles shall be caulked around each perimeter to seal them to the support frame.
† The exterior lens surface of ceiling lighting fixtures shall be smooth, mounted flush, and sealed.
† Any other penetrations through the ceiling or walls shall be sealed.
† The buffer area shall not contain sources of water (sinks) or floor drains. Work surfaces shall be constructed of smooth, impervious materials, such as stainless steel or molded plastic, so that they are easily cleaned and disinfected.
† Carts shall be of stainless steel wire, nonporous plastic, or sheet metal construction with good quality, cleanable casters to promote mobility.
† Storage shelving, counters, and cabinets shall be smooth, impervious, free from cracks and crevices, nonshedding, cleanable, and disinfectable.
† Their number, design, and manner of installation the itmes above shall promote effective cleaning and disinfection.
† If ceilings consist of inlaid panels, the panels should be impregnated with a polymer to render them impervious and hydrophobic.
† Dust collecting overhangs, such as ceiling utility pipes, or ledges, such as windowsills, should be avoided.
† Air returns should be mounted low on the wall creating a general top-down dilution of room air with HEPA filtered make-up air.
Placement of Primary Engineering Controls Within ISO Class 7 Buffer Areas
† Primary engineering controls for nonhazardous and nonradioactive CSPs are located in buffer areas, except for CAIs that are proven to maintain ISO Class 5 air when particle counts are sampled 6 to 12 inches upstream of critical site exposure areas during performance of normal inward and outward transfer of materials, and compounding manipulations when such CAIs are located in air quality worse than ISO Class 7.
† Presterilization procedures for high-risk level CSPs, such as weighing and mixing, shall be completed in no worse than an ISO Class 8 environment.
† Primary engineering controls shall be located out of traffic patterns and away from room air currents that could disrupt the intended airflow patterns.
† When isolators are used for sterile compounding, the recovery time to achieve ISO Class 5 air quality shall be documented and internal procedures developed to ensure that adequate recovery time is allowed after material transfer before and during compounding operations.
† When compounding activities require the manipulation of a patient's blood-derived or other biological material (e.g., radiolabeling a patient's or a donor's white blood cells), the manipulations shall be clearly separated from routine material handling procedures and equipment used in CSP preparation activities, and they shall be controlled by specific standard operating procedures in order to avoid any cross-contamination.
† Food, drinks, and items exposed in patient care areas, and unpacking of bulk supplies and personnel cleansing and garbing are prohibited from buffer areas or rooms.
† Demarcation designation between buffer areas or rooms and ante-areas.
† Antiseptic hand cleansing and sterile gloves in buffer areas or rooms.
† Packaged compounding supplies and components, such as needles, syringes, tubing sets, and small and large volume parenterals, should be uncartoned and wiped down with a disinfectant that does not leave a residue (e.g., sterile 70% IPA) when possible in an ante-area, of ISO Class 8 air quality, before being passed into the buffer areas.
Cleaning and Disinfecting the Sterile Compounding Areas

<p>† Trained personnel write detailed procedures including cleansers, disinfectants, and non-shedding wipe and mop materials.</p>
<p>† Cleaning and disinfecting surfaces in the LAFWs, BSCs, CAIs, and CACIs shall be cleaned and disinfected frequently, including at the beginning of each work shift, before each batch preparation is started, every 30 minutes during continuous compounding periods of individual CSPs, when there are spills, and when surface contamination is known or suspected from procedural breaches.</p>
<p>† Trained compounding personnel are responsible for developing, implementing, and practicing the procedures for cleaning and disinfecting the DCAs written in the SOPs.</p>
<p>† Cleaning and disinfecting shall occur before compounding is performed. Items shall be removed from all areas to be cleaned, and surfaces shall be cleaned by removing loose material and residue from spills, e.g., water-soluble solid residues are removed with Sterile Water (for Injection or Irrigation) and low-shedding wipes. This shall be followed by wiping with a residue-free disinfecting agent, such as sterile 70% IPA, which is allowed to dry before compounding begins.</p>
<p>† Work surfaces in ISO Class 7 and 8 areas and segregated compounding areas are cleaned at least daily.</p>
<p>† Dust and debris shall be removed when necessary from storage sites for compounding ingredients and supplies, using a method that does not degrade the ISO Class 7 or 8 air quality.</p>
<p>† Floors in ISO Class 7 and 8 areas are cleaned daily when no compounding occurs.</p>
<p>† IPA (70% isopropyl alcohol) remains on surfaces to be disinfected for at least 30 seconds before such surfaces are used to prepare CSPs.</p>
<p>† Emptied shelving, walls, and ceilings in ante-areas are cleaned and disinfected at least monthly.</p>
<p>† Mopping shall be performed by trained personnel using approved agents and procedures described in the written SOPs.</p>
<p>† Cleaning and disinfecting agents, their schedules of use and methods of application shall be in accordance with written SOPs and followed by custodial and/or compounding personnel.</p>
<p>† All cleaning materials, such as wipers, sponges, and mops, shall be nonshedding, preferably composed of synthetic micro fibers, and dedicated to use in the buffer area, or ante-area, and segregated compounding areas and shall not be removed from these areas except for disposal.</p>
<p>† If cleaning materials are reused (e.g., mops), procedures shall be developed (based on manufacturer recommendations) that ensure that the effectiveness of the cleaning device is maintained and repeated use does not add to the bioburden of the area being cleaned.</p>
<p>† Supplies and equipment removed from shipping cartons shall be wiped with a suitable disinfecting agent (e.g., sterile 70% IPA) delivered from a spray bottle or other suitable delivery method.</p>
<p>† After the disinfectant is sprayed or wiped on a surface to be disinfected, the disinfectant shall be allowed to dry, and during this time the item shall not be used for compounding purposes.</p>
<p>† Sterile 70% IPA wetted gauze pads or other particle-generating material shall not be used to disinfect the sterile entry points of packages and devices.</p>
<p>Personnel Cleansing and Garbing</p>
<p>† Personnel shall also be thoroughly competent and highly motivated to perform flawless aseptic manipulations with ingredients, devices, and components of CSPs.</p>

<p>† Personnel with rashes, sunburn, weeping sores, conjunctivitis, active respiratory infection, and cosmetics are prohibited from preparing CSPs.</p>
<p>† Compounding personnel shall remove personal outer garments; cosmetics; artificial nails; hand, wrist, and body jewelry that can interfere with the fit of gowns and gloves; and visible body piercing above the neck.</p>
<p>† Order of compounding garb and cleansing in ante-area: shoes or shoe covers, head and facial hair covers, face mask, fingernail cleansing, hand and forearm washing and drying; non-shedding gown.</p>
<p>† Order of cleansing and gloving in buffer room or area: hand cleansing with a persistently active alcohol-based product with persistent activity; allow hands to dry; don sterile gloves.</p>
<p>† Routinely disinfect gloves with sterile 70% IPA after contacting nonsterile objects.</p>
<p>† Inspect gloves for holes and replace when breaches are detected.</p>
<p>† Personnel repeat proper procedures after they are exposed to direct contact contamination or worse than ISO Class 8 air.</p>
<p>† These requirements are exempted only for immediate use CSPs and CAIs for which manufacturers provide written documentation based on validated testing that such personnel practices are not required to maintain sterility in CSPs.</p>
<p>-</p>
<p>Personnel Training and Competency Evaluation of Garbing, Aseptic Work Practices and Cleaning/Disinfection Procedures</p>
<p>† Personnel who prepare CSPs shall be trained conscientiously and skillfully by expert personnel, multi-media instructional sources, and professional publications in the theoretical principles and practical skills of garbing procedures, aseptic work practices, achieving and maintaining ISO Class 5 environmental conditions, and cleaning and disinfection procedures.</p>
<p>† This training shall be completed and documented before any compounding personnel begin to prepare CSPs.</p>
<p>† Compounding personnel shall complete didactic training, pass written competence assessments, undergo skill assessment using observational audit tools, and media fill testing.</p>
<p>† Media fill testing of aseptic work skills shall be performed initially before beginning to prepare CSPs and at least annually thereafter for low and medium risk level compounding; and semiannually for high risk level compounding.</p>
<p>† Compounding personnel who fail written tests, observational audits, or whose media fill test vials have one or more units showing visible microbial contamination, shall be reinstructed and re-evaluated by expert compounding personnel to ensure correction of all aseptic work practice deficiencies.</p>
<p>† Compounding personnel shall pass all evaluations prior to resuming compounding of sterile preparations.</p>
<p>† Compounding personnel must demonstrate proficiency of proper hand hygiene, garbing, and consistent cleaning procedures in addition to didactic evaluation and aseptic media fill.</p>
<p>† Cleaning and disinfecting procedures performed by other support personnel shall be thoroughly trained in proper hand hygiene, and garbing, cleaning, and disinfection procedures by a qualified aseptic compounding expert.</p>
<p>† Support personnel shall routinely undergo performance evaluation of proper hand hygiene, garbing, and all applicable cleaning and disinfecting procedures conducted by a qualified aseptic compounding expert.</p>
<p>Competency Evaluation of Garbing and Aseptic Work Practices</p>

<p>‡ Compounding personnel shall be evaluated initially prior to beginning compounding CSPs and whenever an aseptic media fill is performed using a Sample Form for Assessing Hand Hygiene and Garbing Related Practices of Compounding Personnel and the personnel glove fingertip sampling procedures.</p>
<p>Aseptic Work Practice Assessment and Evaluation via Personnel Glove Fingertip Sampling</p>
<p>‡ Monitoring of compounding personnel glove fingertips shall be performed for all CSP risk level compounding.</p>
<p>‡ Glove fingertip sampling shall be used to evaluate the competency of personnel in performing hand hygiene and garbing procedures in addition to educating compounding personnel on proper work practices.</p>
<p>‡ All personnel shall demonstrate competency in proper hand hygiene and garbing procedures in addition to aseptic work practices.</p>
<p>‡ Sterile contact agar plates shall be used to sample the gloved fingertips of compounding personnel after garbing to assess garbing competency and after completing the media fill preparation.</p>
<p>‡ Gloves shall not be disinfected with sterile 70% IPA immediately prior to sampling.</p>
<p>Garbing and Gloving Competency Evaluation</p>
<p>‡ Compounding personnel shall be visually observed during the process of performing hand hygiene and garbing procedures.</p>
<p>‡ The visual observation shall be documented on a Sample Form for Assessing Hand Hygiene and Garbing Related Practices of Compounding Personnel and maintained to provide a permanent record of and long term assessment of personnel competency.</p>
<p>Gloved Fingertip Sampling</p>
<p>‡ Immediately after the compounder completes the hand hygiene and garbing procedure, the evaluator shall collect a gloved fingertip and thumb sample from both hands of the compounder onto appropriate agar plates by lightly pressing each finger tip into the agar.</p>
<p>‡ The plates shall be incubated for the appropriate incubation period and at the appropriate temperature.</p>
<p>‡ All employees shall successfully complete an initial competency evaluation and gloved fingertip/thumb sampling procedure (0 cfu) no less than three times before initially being allowed to compound CSPs for human use.</p>
<p>‡ After completing the initial gowning and gloving competency evaluation, re-evaluation of all compounding personnel shall occur at least annually for low- and medium-risk level CSPs and semiannually for high-risk level CSPs before being allowed to continue compounding CSPs.</p>
<p>‡ Gloves shall not be disinfected with sterile 70% IPA prior to testing.</p>
<p>‡ The sampled gloves shall be immediately discarded and proper hand hygiene performed after sampling. The nutrient agar plates shall be incubated as stated below.</p>
<p>‡ The cfu action level for gloved hands shall be based on the total number of cfu on both gloves and not per hand.</p>
<p>‡ Results should be reported separately as number of cfu per employee per hand (left hand, right hand).</p>
<p>Incubation Period</p>
<p>‡ At the end of the designated sampling period, the agar plates are recovered, covers secured, inverted and incubated at a temperature and for a time period conducive to multiplication of microorganisms. Trypticase soy agar (TSA) with lecithin and polysorbate 80 shall be incubated at 35°± 2° for 2-3 days.</p>

Aseptic Manipulation Competency Evaluation
† All compounding personnel shall have their aseptic technique and related practice competency evaluated initially during the media fill test procedure and subsequent annual or semiannual media fill test procedures on the Sample Form for Assessing Aseptic Technique and Related Practices of Compounding Personnel.
Media-Fill Test Procedure
† The skill of personnel to aseptically prepare CSPs shall be evaluated using sterile fluid bacterial culture media fill verification.
† Media-filled vials shall be incubated within a range of 35° ± 2° for 14 days.
Surface Cleaning and Disinfection Sampling and Assessment
† Surface sampling shall be performed in all ISO-classified areas on a periodic basis and can be accomplished using contact plates and/or swabs and shall be done at the conclusion of compounding.
† Locations to be sampled shall be defined in a sample plan or on a form.
Cleaning and Disinfecting Competency Evaluation
† Compounding personnel and other personnel responsible for cleaning shall be visually observed during the process of performing cleaning and disinfecting procedures during initial personnel training on cleaning procedures, changes in cleaning staff and at the completion of any Media-Fill Test Procedure.
† Visual observation shall be documented on a Sample Form for Assessing Cleaning and Disinfection Procedures and maintained to provide a permanent record of, and long-term assessment of, personnel competency.
Surface Collection Methods
† Immediately after sampling a surface with the contact plate, the sampled area shall be thoroughly wiped with a non-shedding wipe soaked in sterile 70% IPA.
† Results should be reported as cfu per unit of surface area.
Action Levels, Documentation, and Data Evaluation
† Environmental sampling data shall be collected and reviewed on a routine basis as a means of evaluating the overall control of the compounding environment.
† If an activity consistently shows elevated levels of microbial growth, competent microbiology personnel shall be consulted.
† An investigation into the source of the contamination shall be conducted.
† When gloved fingertip sample results exceeds action levels after proper incubation, a review of hand hygiene and garbing procedures as well as glove and surface disinfection procedures and work practices shall be performed and documented.
† Any cfu count that exceeds its respective action level should prompt a re-evaluation of the adequacy of personnel work practices, cleaning procedures, operational procedures, and air filtration efficiency within the aseptic compounding location.
-
SUGGESTED STANDARD OPERATING PROCEDURES
† All facilities are required to have these, and they must include at least the items enumerated in this section.
-
FINISHED PREPARATION RELEASE CHECKS AND TESTS
Inspection of Solution Dosage Forms and Review of Compounding Procedures

<p>‡ Review procedures and documents to ensure sterility, purity, correct identities and amounts of ingredients, and stability.</p>
<p>‡ Visually inspect for abnormal particulate matter and color, and intact containers and seals.</p>
<p>Sterility Testing</p>
<p>‡ High-risk level CSPs prepared in batches of more than 25 identical containers, or exposed longer than 12 hours at 2° to 8°, and 6 hours at warmer than 8° before being sterilized.</p>
<p>Bacterial Endotoxin (Pyrogen) Testing</p>
<p>‡ High-risk level CSPs, excluding those for inhalation and ophthalmic administration, prepared in batches of more than 25 identical containers, or exposed longer than 12 hours at 2° to 8°, and 6 hours at warmer than 8°, before being sterilized.</p>
<p>Identity and Strength Verification of Ingredients</p>
<p>‡ Written procedures to verify correct identity, quality, amounts, and purities of ingredients used in CSPs.</p>
<p>‡ Written procedures to ensure labels of CSPs contain correct names and amounts or concentrations of ingredients, total volumes, beyond-use dates, storage conditions, and route(s) of administration.</p>
<p>-</p>
<p>STORAGE AND BEYOND-USE DATING</p>
<p>Determining Beyond-Use Dates</p>
<p>‡ Use the general criteria in USP (795) in the absence of direct stability-indicating assays or authoritative literature that supports longer durations.</p>
<p>-</p>
<p>MAINTAINING STERILITY, PURITY, AND STABILITY OF DISPENSED AND DISTRIBUTED CSPs</p>
<p>‡ Written procedures for proper packaging, storage, and transportation conditions to maintain sterility, quality, purity, and strength of CSPs.</p>
<p>Redispensed CSPs</p>
<p>‡ When sterility, and acceptable purity, strength, and quality can be ensured.</p>
<p>‡ Assignment of sterility storage times and stability beyond-use dates that occur later than those of originally dispensed CSPs must be based on results of sterility testing and quantitative assay of ingredients.</p>
<p>Packaging and Transporting CSPs</p>
<p>‡ Packaging maintains physical integrity, sterility, stability, and purity of CSPs.</p>
<p>‡ Modes of transport that maintain appropriate temperatures and prevent damage to CSPs.</p>
<p>-</p>
<p>PATIENT OR CAREGIVER TRAINING</p>
<p>‡ Multiple component formal training program to ensure patients and caregivers understand the proper storage, handling, use, and disposal of CSPs.</p>
<p>-</p>
<p>PATIENT MONITORING AND ADVERSE EVENTS REPORTING</p>

† Written standard procedures describe means for patients to ask questions and report concerns and adverse events with CSPs, and for compounding supervisors to correct and prevent future problems.
† Adverse events and defects with CSPs reported to FDA's MedWatch and USP's MEDMARX programs.
-
GLOSSARY
† Twenty-eight terms are defined and integral to complying with USP (797).

Appendix II. Common Disinfectants Used in Health Care for Inanimate Surfaces and Noncritical Devices, and Their Microbial Activity and Properties¹

Chemical Category of Disinfectant							
		Isopropyl alcohol	Accelerated hydrogen peroxide	Quaternary Ammonium (e.g., dodecyl dimethyl ammonium chloride)	Phenolics	Chlorine (e.g., sodium hypochlorite)	Iodophors (e.g., povidone-iodine)
Concentration Used	-	60-95%	0.5% ³	0.4-1.6% aq	0.4-1.6% aq	100-5000 ppm	30-50 ppm
Microbial Inactivation ²	Bacteria	+	+	+	+	+	+
	Lipophilic viruses	+	+	+	+	+	+
	Hydrophilic viruses	±	+	±	±	+	±
	M. tuberculosis	+	+	±	+	+	±
	Mycotic agents (fungi)	+	+	+	+	+	±
Important Chemical & Physical Properties	Bacterial Spores	-	-	-	-	+	-
	Shelf life > 1 week	+	+	+	+	+	+
	Corrosive or deleterious effects	±	-	-	-	±	±
	Non-evaporable residue	-	-	+	+	-	+
	Inactivated by organic matter	+	±	+	±	+	+
	Skin irritant	±	-	+	+	+	±
	Eye irritant	+	-	+	+	+	+
Respiratory irritant	-	-	-	-	+	-	
Systemic toxicity	+	-	-	+	+	+	+

Key to abbreviation and symbols: aq = diluted with water; ppm = parts per million; + = yes; - = no; ± = variable results.

¹ Modified from World Health Organization, Laboratory Bio-Safety Manual 1983 and Rutala WA, "Antisepsis, disinfection and sterilization in the hospital and related institutions," *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, DC, 1995, pages 227-245.

² Inactivation of the most common microorganisms (i.e., bacteria) occurs with a contact time of ≤1 minute; inactivation of spores requires longer contact times (e.g., 5-10 minutes for 5,000 ppm chlorine solution against *C. difficile* spores). Reference: Perez J, Springthorpe VS, Sattar SA, "Activity of selected oxidizing microbicides against the spores of *Clostridium difficile*: Relevance to environmental control," *American Journal of Infection Control*, August 2005, pages 320-325.

³ Accelerated hydrogen peroxide is a new generation of hydrogen peroxide-based germicides in which the potency and performance of the active ingredient have been enhanced and accelerated through the use of appropriate acids and detergents.

Appendix III. Sample Form for Assessing Hand Hygiene and Garbing Related Practices of Compounding Personnel

-	
Printed name and position/title of person assessed:	-
Name of facility or location:	-
-	
Hand Hygiene and Garbing Practices: The qualified evaluator will check each space for which the person being assessed has acceptably completed the described activity, prints N/A if the activity is not applicable to the assessment session or N/O if the activity was not observed.*	

-	-	
-	Presents in a clean appropriate attire and manner.	
-	Wears no cosmetics or jewelry (watches, rings, earrings, etc. piercing jewelry included) upon entry into ante areas.	
-	Brings no food or drinks into or stored in the ante areas or buffer areas.	
-	Is aware of the line of demarcation separating clean and dirty sides and observes required activities.	
-	Dons shoe covers or designated clean area shoes one at a time, placing the covered or designated shoe on clean side of the line of demarcation, as appropriate.	
-	Dons beard cover if necessary.	
-	Dons head cover assuring that all hair is covered.	
-	Dons face mask to cover bridge of nose down to include chin.	
-	Performs hand hygiene procedure by wetting hands and forearms and washing using soap and warm water for at least 30 seconds.	
-	Dries hands and forearms using lint free towel or hand dryer.	
-	Selects the appropriate sized gown examining for any holes, tears, or other defects.	
-	Dons gown and ensures full closure.	
-	Disinfects hands again using a waterless alcohol based surgical hand scrub with persistent activity and allows hands to dry thoroughly before donning sterile gloves.	
-	Dons appropriate sized sterile gloves ensuring that there is a tight fit with no excess glove material at the fingertips.	
-	Examines gloves ensuring that there are no defects, holes, or tears.	
-	While engaging in sterile compounding activities, routinely disinfects gloves with sterile 70% IPA prior to work in the direct compounding area (DCA) and after touching items or surfaces that may contaminate gloves.	
-	Removes PPE on the clean side of the ante area.	
-	Removes gloves and performs hand hygiene.	
-	Removes gown and discards it, or hangs it on hook if it is to be reused within the same work day.	
-	Removes and discards mask, head cover, and beard cover (if used).	
-	Removes shoe covers or shoes one at a time, ensuring that uncovered foot is placed on the dirty side of the line of demarcation and performs hand hygiene again. (Removes and discards shoe covers every time the compounding area is exited).	
-		
*The person assessed is immediately informed of all unacceptable activities (i.e., spaces lacking check marks, N/A, or N/O) and shown and informed of specific corrections.		
—	-	-
Signature of Person Assessed	Printed Name	Date
—	-	-

Signature of Qualified Evaluator	Printed Name	Date
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Appendix IV. Sample Form for Assessing Aseptic Technique and Related Practices of Compounding Personnel

-	
Printed name and position/title of person assessed:	-
Name of facility or location:	-
-	
<p>Aseptic Technique, Safety, and Quality Assurance Practices: The qualified evaluator checks each space for which the person being assessed has acceptably completed the described activity, prints N/A if the activity is not applicable to the assessment session or N/O if the activity was not observed.*</p>	
-	-
-	Completes the Hand Hygiene and Garbing Competency Assessment Form.
-	Performs proper hand hygiene, garbing, and gloving procedures according to SOPs.
-	Disinfects ISO Class 5 device surfaces with an appropriate agent.
-	Disinfects components/vials with an appropriate agent prior to placing into ISO Class 5 work area.
-	Introduces only essential materials in a proper arrangement in the ISO Class 5 work area.
-	Does not interrupt, impede, or divert flow of first air to critical sites.
-	Ensures syringes, needles, and tubing remain in their individual packaging and are only opened in ISO Class 5 work area.
-	Performs manipulations only in the appropriate DCA of the ISO Class 5 device.
-	Does not expose critical sites to contact contamination or worse than ISO Class 5 air.
-	Disinfects stoppers, injection ports, and ampul necks by wiping with sterile 70% IPA and allows sufficient time to dry.
-	Affixes needles to syringes without contact contamination.
-	Punctures vial stoppers and spikes infusion ports without contact contamination.
-	Labels preparation(s) correctly.
-	Disinfects sterile gloves routinely by wiping with sterile 70% IPA during prolonged compounding manipulations.
-	Cleans, sets up, and calibrates automated compounding device (e.g., "TPN compounder") according to manufacturer's instructions.
-	Disposes of sharps and waste according to institutional policy or recognized guidelines.
-	

*The person assessed is immediately informed of all unacceptable activities (i.e., spaces lacking check marks, N/A, or N/O) and shown and informed of specific corrections.		
—	-	-
Signature of Person Assessed	Printed Name	Date
—	-	-
Signature of Qualified Evaluator	Printed Name	Date

Appendix V. Sample Form for Assessing Cleaning and Disinfection Procedures

-	-
Printed name and position/title of person assessed:	-
Name of facility or location:	-
-	-
Cleaning and Disinfection Practices: The qualified evaluator will check each space for which the person being assessed has acceptably completed the described activity, prints N/A if the activity is not applicable to the assessment session or N/O if the activity was not observed.*	
-	-
Daily Tasks:	
-	Prepares correct concentration of disinfectant solution according to manufacturer's instructions.
-	Uses appropriately labeled container for the type of surface to be cleaned (floor, wall, production bins, etc.).
-	Documents disinfectant solution preparation.
-	Follows garbing procedures when performing any cleaning activities.
-	At the beginning of each shift, cleans all ISO Class 5 devices prior to compounding in the following order: walls, IV bar, automated compounders, and work surface.
-	Uses a lint free wipe soaked with sterile 70% IPA or other approved disinfectant solution and allows to dry completely.
-	Removes all compounder components and cleans all ISO Class 5 areas as stated above at the end of each shift.
-	Cleans all counters and easily cleanable work surfaces.
-	Mops floors, using the mop labeled "floors," starting at the wall opposite the room entry door; mops floor surface in even strokes toward the operator. Moves carts as needed to clean entire floor surface. Use of a microfiber cleaning system is an acceptable alternative to mops.
-	In the ante-area, cleans sink and all contact surfaces; cleans floor with a disinfectant solution or uses microfiber cleaning system.
-	-
Monthly Tasks:	
-	Performs monthly cleaning on a designated day. Prepares a disinfectant solution as stated in daily tasks that is appropriate for the surfaces to be cleaned.

-	Cleans buffer area and ante-area ceiling, walls, and storage shelving with a disinfectant solution and a mop or uses a microfiber cleaning system.	
-	Once ISO Class 5 area is clean, cleans compounding room ceiling, followed by walls and ending with the floor. Uses appropriate labeled mops or microfiber cleaning system.	
-	Cleans all buffer area totes and storage shelves by removing contents and using a germicidal detergent soaked lint free wipe, cleans the inside surfaces of the tote and then the entire exterior surfaces of the tote. Allows totes to dry. Prior to replacing contents into tote, wipes tote with sterile 70% IPA to remove disinfectant residue. Uses new wipe as needed.	
-	Cleans all buffer area carts by removing contents and using germicidal detergent soaked lint free wipe, cleans all carts starting with the top shelf and top of post, working down to wheels. Cleans the under side of shelves in a similar manner. Uses a new wipe for each cart. Allows to dry. Wipes carts with sterile 70% IPA wetted lint free wipe to remove any disinfectant residue. Uses new wipe as needed.	
-	Cleans buffer area chairs, the interior and exterior of trash bins, and storage bins using disinfectant solution soaked lint free wipe.	
-	Documents all cleaning activities as to who performed such activities with date and time noted.	
-		
*The person assessed is immediately informed of all unacceptable activities (i.e., spaces lacking check marks, N/A, or N/O) and shown and informed of specific corrections:		
—	-	-
Signature of Person Assessed	Printed Name	Date
—	-	-
Signature of Qualified Evaluator	Printed Name	Date



1. INTRODUCTION AND SCOPE

This chapter describes the minimum practices and quality standards to be followed when preparing compounded sterile human and animal drugs (compounded sterile preparations, or CSPs). These practices and standards must be used to prevent harm, including death, to human and animal patients that could result from 1) microbial contamination (nonsterility), 2) excessive bacterial endotoxins, 3) variability from the intended strength of correct ingredients, 4) chemical and physical contaminants, and/or 5) use of ingredients of inappropriate quality.

1.1 Scope

CSP AFFECTED

The requirements and standards described in this chapter must be used to ensure the sterility of any CSP. Although the list below is not exhaustive, the following must be sterile:

- Injections
- Aqueous bronchial inhalations

- Baths and soaks for live organs and tissues
- Irrigations for internal body cavities (i.e., any space that does not freely communicate with the environment outside of the body)
- Ophthalmics
- Implants

For the compounding of hazardous drugs, see *Hazardous Drugs—Handling in Healthcare Settings* (800).

PERSONNEL AND SETTINGS AFFECTED

This chapter applies to all persons who prepare CSPs (e.g., pharmacists, pharmacy technicians, physicians, veterinarians, and nurses) at all places where CSPs are prepared (e.g., hospitals and other healthcare institutions, patient treatment sites, infusion facilities, pharmacies, and physicians' or veterinarians' practice sites).

The compounding organization's leadership and all employees involved in preparing, storing, and transporting CSPs are responsible for 1) ensuring that the applicable practices and quality standards in this chapter are continually and consistently applied to their operations, and 2) proactively identifying and remedying potential problems within their operations.

SPECIFIC PRACTICES

Administration of medications: This chapter is not intended to address administration of sterile medications. Administration of sterile medications should be performed in accordance with the Centers for Disease Control and Prevention's Safe Injection Practices¹ and the manufacturer's or compounder's labeling of the sterile medication.

Proprietary bag and vial systems: Docking and activation of proprietary bag and vial systems (e.g., ADD-Vantage®, Mini Bag Plus®, addEASE®) strictly in accordance with the manufacturer's instructions for immediate administration to an individual patient is not considered compounding. However, aseptic technique must be followed when attaching the proprietary bag and vial system.

Docking of the proprietary bag and vial systems for future activation and administration is considered compounding and must be performed in accordance with this chapter, with the exception of 12. *Establishing Beyond-Use Dates and In-Use Times*. Beyond-use dates (BUDs) for proprietary bag and vial systems must be assigned in accordance with the manufacturer's instructions provided in product labeling.

Reconstitution or dilution: Reconstituting or diluting a conventionally manufactured sterile product with no intervening steps strictly in accordance with the manufacturer's labeling for administration to an individual patient is not considered compounding. However, aseptic technique must be followed during preparation, and procedures must be in place to minimize the potential for contact with nonsterile surfaces and introduction of particulate matter or biological fluids.

Any other reconstitution or dilution of a conventionally manufactured sterile product is considered compounding and must be performed in accordance with this chapter.

Repackaging: Repackaging of a conventionally manufactured sterile product from its original primary container into another primary container must be performed in accordance with the requirements in this chapter for CSPs, including assignment of BUDs and in-use times as

described in 12. *Establishing Beyond-Use Dates and In-Use Times*.

1.2 Factors Affecting the Risks Associated with CSPs

CSPs can be compounded using only sterile starting ingredients or using some or all nonsterile starting ingredients. If all of the components used to compound a drug are sterile to begin with, the sterility of the components must be maintained during compounding to produce a sterile compounded preparation. If one or more of the starting components being used to compound is not sterile, the sterility of the compounded preparation must be achieved through a sterilization process, such as terminal sterilization in the final sealed container or sterile filtration, and then maintained through subsequent manipulations of the preparation. When compounding with nonsterile starting ingredients, the quality of the components and the effectiveness of the sterilization step are particularly critical to achieving a sterile preparation. In all cases, failure to achieve and/or maintain sterility of CSPs can lead to serious harm, including death. Personnel engaged in compounding and handling CSPs must strictly adhere to the standards in this chapter throughout the compounding process and until the preparation reaches the intended patient(s).

The risks to the sterility associated with a particular CSP depend on a number of factors, including the following:

- Batch size
- Complexity of the compounding process (e.g., number of manipulations involved; whether starting with nonsterile or sterile components)
- Inherent nature of the drug being compounded (e.g., whether the drug is susceptible to microbial growth; whether the preparation will be preservative free)
- Complexity of the compounding operation (e.g., multiple people in the cleanroom at the same time; multiple CSPs being prepared at the same time; activity in the surrounding areas)
- Length of time between the start of compounding (including making a stock solution) and administration of the drug to the patient

Ultimately, the risk to the population of patients is lower if the compounding is done for an individual patient as compared to when the compounding is done in a batch for multiple patients. When applying the standards in this chapter, the risks of a particular compounding operation must be considered, and steps commensurate with these risks must be taken to ensure a quality CSP.

1.3 Risk Categories

Consistent with this risk-based approach, this chapter distinguishes between two categories of CSPs, Category 1 and Category 2, primarily by the conditions under which they are made and the time within which they will be used. Category 1 CSPs are those assigned a maximum BUD of 12 hours or less at controlled room temperature or 24 hours or less if refrigerated if made in accordance with all of the applicable standards for Category 1 CSPs in this chapter. Category 2 CSPs are those that may be assigned a BUD of greater than 12 hours at room temperature or greater than 24 hours if refrigerated (see 12. *Establishing Beyond-Use Dates and In-Use Times*) if made in accordance with all of the applicable standards for Category 2 CSPs in this chapter. See *Table 1* for a summary comparison of the minimum requirements in this chapter for Category 1 and 2 CSPs.

This chapter describes minimum requirements that apply to compounding of all CSPs, and also

to repackaging of sterile products. If a compounder does not meet all of the Category 2 requirements, the CSP or repackaged sterile product will be considered a Category 1, and the shorter BUD applicable to Category 1 CSPs must be assigned. The minimum requirements not specifically described as applicable to Category 1 or Category 2, such as minimum training and competency testing and personal hygiene for personnel, are applicable to compounding of all CSPs and repackaging of sterile products.

Table 1. Summary Comparison of Minimum Requirements for Category 1 and Category 2 CSPs^a

	Category 1 CSPs	Category 2 CSPs
Personnel Qualifications		
Visual observation of hand hygiene and garbing	Quarterly	Quarterly
Gloved fingertip sampling	Quarterly	Quarterly
Media fill testing	Quarterly	Quarterly
Personal Protective Equipment		
See Table 2.		
Buildings and Facilities		
Primary engineering control (PEC)	Not required to be placed in a classified area	Required to be placed in a classified area
Recertification	Every 6 months	Every 6 months
Environmental Monitoring		
Nonviable airborne monitoring	Every 6 months	Every 6 months
Viable airborne monitoring	Monthly	Monthly
Surface sampling	Monthly	Monthly
Release Testing		
Physical inspection	Required	Required
Sterility testing	Not required	Based on assigned BUD
Endotoxin testing	Not required	Required if prepared from nonsterile ingredient(s) ^b
BUD		
BUD assignment	≤12 hours at controlled room temperature or ≤24 hours if refrigerated	>12 hours at controlled room temperature or >24 hours if refrigerated
<p>^a This table summarizes the requirements that apply specifically to Category 1 and Category 2 CSPs. There are numerous requirements in the chapter that are not summarized in this table because they apply to all CSPs, regardless of whether they are Category 1 or Category 2.</p> <p>^b See exemptions in 10.3 Bacterial Endotoxins Testing.</p>		

1.4 Urgent-Use CSPs

A CSP may be prepared in worse than International Organization for Standardization (ISO) Class

5 air quality (see 4.1 *Protection from Airborne Contaminants*) in rare circumstances when a CSP is needed urgently (e.g., cardiopulmonary resuscitation) for a single patient, and preparation of the CSP under conditions described for Category 1 or Category 2 would subject the patient to additional risk due to delays in therapy. In these circumstances, the compounding procedure must be a continuous process not to exceed 1 hour, and administration of the CSP must begin immediately upon completion of preparation of the CSP. Aseptic technique must be followed during preparation, and procedures must be in place to minimize the potential for contact with nonsterile surfaces, introduction of particulate matter or biological fluids, and mix-ups with other CSPs.

1.5 Roadmap through Chapter

The chapter is organized as follows:

1. Introduction and Scope
2. Personnel Qualifications—Training, Evaluation, and Requalification
3. Personal Hygiene and Personal Protective Equipment
4. Buildings and Facilities
5. Environmental Monitoring
6. Cleaning and Disinfecting Compounding Areas
7. Equipment and Components
8. Sterilization and Depyrogenation
9. SOPs and Master Formulation and Compounding Records
10. Release Testing
11. Labeling
12. Establishing Beyond-Use Dates and In-Use Times
13. Quality Assurance and Quality Control
14. CSP Storage, Handling, Packaging, and Transport
15. Complaint Handling and Adverse Event Reporting
16. Documentation
17. Radiopharmaceuticals as CSPs

Glossary

Appendices

Appendix 1. Acronyms

Appendix 2. Common Disinfectants Used in Health Care for Inanimate Surfaces and Noncritical Devices, and Their Microbial Activity and Properties

2. PERSONNEL QUALIFICATIONS—TRAINING, EVALUATION, AND REQUALIFICATION

Because the failure of compounding personnel to follow procedures and adhere to quality standards poses the greatest risk of CSP contamination, all personnel involved in the preparation and handling of CSPs must be trained and qualified and must undergo annual refresher training and requalification in appropriate sterile compounding standards and practices. Training, qualification, and requalification of personnel must be documented.

Each compounding facility must develop a written training program that describes the required training, the frequency of training, and the process for evaluating the performance of individuals involved in sterile compounding. This program should equip personnel with the appropriate knowledge and train them in the required skills necessary to perform their assigned

tasks. This section describes the minimum qualifications for personnel preparing and handling CSPs, including the training, evaluation, and requalification of such personnel.

Separate from the formal competency testing and requalification described in this section, supervisors of compounding personnel should observe compounding activities on a daily basis and take immediate corrective action if deficient practices are observed.

2.1 Demonstrating Proficiency in Core Competencies

Before beginning to prepare CSPs, personnel must complete training and be able to demonstrate proficiency in the theoretical principles and hands-on skills of sterile manipulations and in achieving and maintaining appropriate environmental conditions. Successful completion must be demonstrated through written testing and hands-on demonstration of skills. Proficiency must be demonstrated in at least the following core competencies:

- Hand hygiene and garbing
- Cleaning and disinfection
- Measuring and mixing
- Aseptic manipulation
- Proper cleanroom behavior
- Methods of sterilization and depyrogenation, if applicable
- Use of equipment
- Documentation of the compounding process (e.g., master formulation and compounding records)
- Understanding the direction of the HEPA-filtered unidirectional airflow within the ISO Class 5 area
- Proper use of PECs
- The potential impact of personnel activities such as moving materials into and out of the compounding area

The following sections describe in detail the competency testing that must be conducted initially for all sterile compounding personnel in garbing and hand hygiene and aseptic manipulation, and the requirements for retraining and requalification.

2.2 Competency Testing in Garbing and Hand Hygiene

Gloved fingertip/thumb sampling is important because direct touch contamination is the most likely source of microorganisms. Gloved fingertip sampling evaluates a compounding person's competency in correctly performing hand hygiene and garbing (see *Box 2-1*). All persons performing compounding must successfully complete an initial competency evaluation, including visual observation and gloved fingertip/thumb sampling [zero colony-forming units (CFUs)] no fewer than three times before being allowed to compound CSPs, to demonstrate that they can perform the procedure consistently. After the initial competency evaluation, compounding personnel must successfully complete gloved fingertip/thumb sampling quarterly (no more than a total of three CFUs). Each fingertip/thumb evaluation must occur after separate, full hand hygiene and garbing procedures.

Compounding personnel must be visually observed by competent personnel while performing hand hygiene and garbing procedures (see *3. Personal Hygiene and Personal Protective Equipment*). The visual audit must be documented and the documentation maintained to provide a permanent record and long-term assessment of personnel competency.

Sampling must be performed on sterile gloves inside of an ISO Class 5 PEC. If conducting gloved

fingertip/thumb sampling in a Restricted Access Barrier System (RABS) [e.g., Compounding Aseptic Isolator (CAI) or Compounding Aseptic Containment Isolator (CACI)] or an isolator, samples must be taken from the sterile gloves placed over the gauntlet gloves. In addition, gloved fingertip/thumb sampling must be performed after completing the media-fill preparation without applying sterile alcohol or any other agent that could interfere with the ability of the gloved fingertip test to assess the adequacy of aseptic work practices.

Box 2-1 Gloved Fingertip Sampling and Testing Procedures

- Use two plates filled with nutrient agar containing neutralizing agents (e.g., lecithin and polysorbate 80) in a size range of 24- to 30-cm² in size.
- Do NOT disinfect gloves with sterile 70% isopropyl alcohol (IPA) or any other disinfectant immediately before touching the agar plate because this could cause a false-negative result.
- Collect a gloved fingertip and thumb sample from both hands by lightly pressing each fingertip into the agar. Use a separate plate for each hand.
- Re-cover the agar plates without further contact with agar. Label the plates with a personnel identifier, right or left hand, date, and time.
- Immediately discard the gloves after sampling.
- Invert the plates and incubate them at a temperature and for a time period conducive to multiplication of microorganisms (e.g., 20°–35° for 5 days).
- Record the number of CFU per hand (left hand, right hand).
- Determine whether the CFU action level for gloved hands (i.e., zero CFU initially or three CFU thereafter) is exceeded by counting the total number of CFUs on both gloves, not per hand.

2.3 Competency Testing in Aseptic Manipulation

After successful completion of the initial hand hygiene and garbing competency evaluation, all sterile compounding personnel must have their sterile technique and related practices evaluated during a media-fill test (see *Box 2-2*). Evaluation results must be documented and the documentation maintained to provide a permanent record and long-term assessment of personnel competency.

Box 2-2 Media-Fill Testing Procedures

- When performing these testing procedures, use the most difficult and challenging compounding procedures and processing conditions encountered by the person during a work shift (e.g., the most manipulations, most complex flow of materials, longest time to compound), replacing all the components used in the CSPs with microbial growth medium.
 - Include all normal processing steps and incorporate worst-case conditions, including sterilizing filtration if used.
 - Do not interrupt the test once it has begun, unless the normal work day involves interruptions.
 - If all of the starting components are sterile to begin with, transfer sterile fluid microbial culture medium, such as sterile soybean-casein digest, into the same types of container-closure systems commonly used at the facility to evaluate a person's skill at aseptically processing CSPs into finished dosage forms.
 - If some of the starting components are nonsterile to begin with, use a nonsterile commercially available medium, such as soybean-casein digest powder, to make a 3% solution. Prepare the nonsterile culture medium according to the manufacturer's instructions and manipulate it in a manner that reflects nonsterile-to-sterile compounding activities.
 - Incubate media-filled vials at 20°–35° for a minimum of 14 days. If two temperatures are used for incubation of media-filled samples, incubate the filled containers for at least 7 days at the lower temperature (20°–25°) followed by 7 days at 30°–35°. Failure is indicated by visible turbidity or other visual manifestations of growth in the medium in one or more container-closure unit(s) on or before 14 days. Investigate media-fill failures to determine possible causes (e.g., sterilizing filter failure). Document and discuss investigational findings with personnel before any re-testing.
 - If using a purchased pre-prepared microbial growth medium, either verify that the growth medium is growth promoting, or obtain a certificate of analysis (COA) from the supplier of the growth medium to ensure that it will support the growth of microorganisms.
 - If using a microbial growth medium prepared in-house, the growth promotion capability of the medium must be demonstrated and documented (see *Sterility Tests* (71)).
- Always store microbial growth media in accordance with manufacturer instructions and use them before their expiration date.

2.4 Reevaluation, Retraining, and Requalification

REQUALIFICATION AFTER FAILURE

Persons who fail written tests; visual observation of hand hygiene, garbing, and aseptic technique; gloved fingertip/thumb sampling; or media-fill tests must undergo immediate requalification through additional training by competent compounding personnel. Personnel who fail visual observation of hand hygiene, garbing, and aseptic technique; gloved fingertip/thumb sampling; or media-fill tests must pass three successive reevaluations in the deficient area before they can resume compounding of sterile preparations.

REFRESHER TRAINING PROGRAM

Compounding personnel must successfully complete annual refresher training in the core competencies listed in *2.1 Demonstrating Proficiency in Core Competencies*. Successful completion must be demonstrated through written testing and hands-on demonstration of skills.

TIMING OF REEVALUATION AND REQUALIFICATION

- Visual observation—Compounding personnel must be visually observed while performing hand hygiene and garbing procedures initially and then at least quarterly.
- Gloved fingertip sampling—Compounding personnel must perform fingertip/thumb sampling three times initially and then quarterly to confirm their competency and work practices. Fingertip sampling conducted as part of a routine media-fill test can be counted in fulfilling these reevaluation requirements.
- Media-fill testing—After initial qualification, conduct media-fill tests of all personnel engaged in compounding CSPs at least quarterly to evaluate aseptic technique and requalify them.
- Cleaning and disinfecting—Retrain and requalify personnel in cleaning and disinfecting compounding areas after a change in cleaning and disinfecting procedures.
- After a pause in compounding—Personnel who have not compounded CSPs in more than 3 months must be requalified in all core competencies before resuming compounding duties.

If compounding is done less frequently than the frequencies specified above (e.g., quarterly), personnel reevaluation and requalification must occur before each compounding session begins.

3. PERSONAL HYGIENE AND PERSONAL PROTECTIVE EQUIPMENT

Because personnel preparing CSPs are the most likely cause of CSP contamination, compounding personnel must maintain proper personal hygiene and use personal protective equipment (PPE).

Personnel suffering from rashes, sunburn, oozing tattoos or sores, conjunctivitis, active respiratory infection, or other active communicable disease must be excluded from working in compounding areas until their conditions are resolved.

3.1 Personnel Preparation

Compounding personnel must take appropriate steps to prevent microbial contamination of CSPs. Squamous cells are normally shed from the human body at a rate of 10^6 or more per hour, and those skin particles are laden with microorganisms.^{2,3} Before entering a designated compounding area, compounding staff must remove any items that are not easily cleanable and that are not necessary for compounding. For example, personnel must:

- Remove personal outer garments (e.g., bandanas, coats, hats, jackets, scarves, sweaters, and vests)
- Remove all cosmetics because they shed flakes and particles
- Remove all hand, wrist, and other exposed jewelry or piercings (e.g., rings, watches, bracelets, earrings, and lip or eyebrow rings) that can interfere with the effectiveness of PPE (e.g., fit of gloves, cuffs of sleeves, and eye protection). Cover any jewelry that cannot be removed (e.g., surgically implanted jewelry)
- Remove ear buds, headphones, and cell phones, or other similar devices
- Keep natural nails clean and neatly trimmed to minimize particle shedding and avoid glove punctures. Nail polish, artificial nails, and extenders must be removed.

3.2 Hand Hygiene

Hand hygiene is required before initiating any compounding activities and when re-entering the

ante-area after a break in compounding activity. After donning shoe covers, head and facial hair covers, and face masks, hand hygiene must be conducted (see *Box 3-1*). Hands must be washed with unscented soap and water. Alcohol hand sanitizers alone are not sufficient. Do not combine antimicrobial soaps and handrubs with alcohol-based products because of potential adverse dermatologic reactions. Brushes are not recommended for hand hygiene because of the potential for skin irritation and increased bacterial shedding. Dry hands and forearms with either low-lint disposable towels or wipes. After hands are washed and dried, perform hand antisepsis using a suitable alcohol-based handrub with sustained antimicrobial activity immediately before donning sterile gloves. Follow the manufacturer's instructions for application times, and apply the product to dry hands only. [Note—Soap must not be added to a partially empty soap dispenser. This practice of "topping off" dispensers can lead to bacterial contamination of soap.]

Box 3-1 Hand Hygiene Procedures

- Remove debris from underneath fingernails, if present, using a nail cleaner under warm running water.
- Wash hands and forearms up to the elbows with unscented soap and water for at least 30 seconds.
- Dry hands and forearms to the elbows completely with low-lint disposable towels or wipes.
- Immediately prior to donning sterile gloves, apply a suitable alcohol-based handrub with sustained antimicrobial activity, following the manufacturer's instructions for application times, and use a sufficient amount of product to keep the hands wet for the duration of the application time.
- Allow hands to dry thoroughly before donning sterile gloves.

3.3. Garb and Glove Requirements

The garb and glove requirements for CSPs depend on the category of CSP and type of PEC used. *Table 2* summarizes the minimum garb and glove requirements for CSPs.

Table 2. Minimum Garb and Glove Requirements

CSP Category	PEC type	Minimum Requirement
Category 1	Any	<ul style="list-style-type: none"> • Non-cotton, low-lint, disposable gown or coveralls • Low-lint, disposable covers for shoes • Low-lint, disposable covers for head and facial hair that cover the ears and forehead • Sterile gloves and sterile sleeves^a
Category 2	Laminar airflow system (LAFS) and biological safety cabinet (BSC)	<ul style="list-style-type: none"> • Non-cotton, low-lint, disposable gowns or coveralls • Low-lint, disposable covers for shoes • Low-lint, disposable covers for head and facial hair that cover the ears and forehead • Mask • Sterile gloves and sterile sleeves^a • Eye shield is optional
Category 2	RABS (CAI or CACI) or isolator	<ul style="list-style-type: none"> • Non-cotton, low-lint, disposable gowns or coveralls • Low-lint, disposable covers for shoes and hair • Sterile gloves
<p>^a If a sterile gown is used, the use of sterile sleeves is optional.</p>		

Personnel intending to enter a buffer area or segregated compounding area must put on protective clothing. Protective clothing must be put on in an order that eliminates the greatest risk of contamination. As noted previously, put on shoe covers, head and facial hair covers, face masks, and gown, before completing hand cleansing procedures and then put on sterile gloves and sterile sleeves (if used). If sterile gowns are used, put on sterile gloves and gowns after hand cleansing procedures. Garbing and degarbing should not occur in the ante-area at the same time.

GOWNS

Visibly soiled gowns must be changed immediately. Gowns and other garbing items must be segregated and stored before use in an enclosure to prevent contamination (e.g., away from sinks to avoid splashing). Coveralls and sterile gowns must not be reused.

GLOVES

Gloves must be sterile and powder free. Use only gloves that have been tested by the manufacturer for compatibility with alcohol disinfection. Before putting on gloves, perform hand hygiene as described in *Box 3-1*. Hands must be completely dry before putting on sterile gloves. Unless donning a sterile gown, sterile gloves must be the last item put on before handling anything in the buffer or segregated compounding area and before compounding begins in the PEC. If donning a sterile gown, put on the gloves first, then the sterile gown.

Routine application of sterile 70% IPA to gloves must occur throughout the compounding process and whenever nonsterile surfaces (e.g., vials, counter tops, chairs, carts) are touched. Contaminated gloved hands can be disinfected by rubbing sterile 70% IPA solution onto all contact surface areas of the gloves and letting the gloved hands dry thoroughly.

Gloves on hands and gauntlet sleeves on RABS and isolators must be inspected routinely by the personnel using them for holes, punctures, or tears and must be replaced immediately if such defects are detected. Sterile gloves must be placed over the gauntlet gloves of the RABS and isolators.

EXITING AND REENTERING COMPOUNDING AREAS

When compounding personnel exit the buffer or segregated compounding area during a work shift, a nonsterile gown can be removed and retained in the ante or segregated compounding area if not visibly soiled, to be re-donned during that same work shift only. Coveralls and sterile gowns may not be reused and must be replaced with new ones. Shoe covers, hair and facial hair covers, face masks, head covering, gloves, and sleeves may not be reused and must be replaced with new ones. Goggles must be either sterilized or disinfected with sterile 70% IPA before each use. Hand hygiene must be performed before resuming sterile compounding.

COMPOUNDING HAZARDOUS DRUGS

For PPE requirements when handling hazardous drugs, refer to (800).

4. BUILDINGS AND FACILITIES

Buildings and facilities in which compounding will be taking place must be designed, built, outfitted, and maintained properly to prevent airborne contamination of CSPs. Areas related to compounding operations in such facilities (i.e., ante-area, buffer area, segregated compounding area, and PEC) must be separated from areas not directly related to compounding and must be appropriately controlled to achieve and maintain required air quality classification levels (see *Table 3*), depending on the nature of the operation being performed in the specific area.

A facility's design must ensure that the movement of personnel, equipment, and components into and out of the compounding area does not disrupt air quality in the area or create a route of contamination. The number of operations being performed, the number of personnel in the compounding area (and in adjacent areas), and the complexity of the compounding procedures are critical factors that determine whether a facility will be able to maintain control of environmental conditions. All of these factors must be taken into account when designing and outfitting a facility in which sterile compounding will be performed.

This section describes applicable air quality standards and the appropriate design of buildings and facilities intended for the preparation of CSPs. It describes in detail the materials to be used and the steps to be taken in designing facilities to ensure suitable conditions. This section also discusses the environmental controls that must be in place to ensure achievement and maintenance of sterility for CSPs.

4.1 Protection from Airborne Contaminants

Buildings and facilities used in compounding must be designed to prevent airborne contamination of the area in which sterile compounding occurs. Without proper design and controls, airborne contaminants are likely to reach the area where compounding occurs, increasing the risk that CSPs will be exposed to microbial contamination.

APPLICABLE AIR QUALITY STANDARDS

The internationally accepted standards for air quality in controlled environments are described

in *Table 3* and referenced throughout this chapter.

Table 3. ISO Classification of Particulate Matter in Room Air

ISO Class	Particle Count ^a /m ³
3	35.2
4	352
5	3,520
6	35,200
7	352,000
8	3,520,000
^a Limits for number of particles $\geq 0.5 \mu\text{m}$ measured under typical operating conditions.	

DESIGN REQUIREMENTS TO MAINTAIN AIR QUALITY

For compounding Category 1 or 2 CSPs, buildings and facilities intended for compounding CSPs must be designed so that air quality increases with movement through separate operational areas to the PEC. Separate areas of operation must be appropriately controlled, depending on the necessary level of air quality. Classified areas in which the air quality is controlled include ante-areas, buffer areas, and PECs.

- Ante-areas must meet at least ISO Class 8 standards. Typically, personnel hand hygiene and garbing procedures, staging of components, order entry, CSP handling, and other activities that potentially generate high levels of particulates are performed in this area. Ante-areas are also transition areas to ensure that proper air pressure relationships are maintained between designated areas.
- A buffer area must provide at least ISO Class 7 air quality. Activities in this area must be especially carefully controlled to avoid affecting the air quality in the area where CSP preparation occurs.
- Areas intended for CSP preparation must meet ISO Class 5 standards. ISO Class 5 standards are achieved through use of a PEC, such as a LAFS, BSC, CAI, CACI, or isolator.

A PEC used for compounding may be placed in an unclassified, segregated compounding area (see below) if only Category 1 CSPs are compounded in the PEC.

4.2 Facility Design and Environmental Controls

In addition to minimizing airborne contamination and protecting CSPs, compounding facilities must be designed and controlled to provide a well-lit and comfortable working environment, with appropriate temperature and humidity for compounding personnel wearing the required garb. The room must be maintained at a temperature of 20° or cooler and a humidity below 60% at all times. Temperature and humidity must be controlled through an efficient heating, ventilation, and air conditioning (HVAC) system rather than through use of humidifiers and dehumidifiers, which can contain standing water that can contribute to microbial contamination.

It is the responsibility of compounding facility management to ensure that each operational area related to CSP preparation meets the ISO-classified air quality standard appropriate for the activities to be conducted there and, specifically, that the ISO Class 5 areas are optimally located, operated, maintained, monitored, and verified to have appropriate air quality.

DESIGN OF A COMPOUNDING FACILITY

A compounding facility generally consists of separate, designated operational clean areas, including an ante-area, a buffer area, and a PEC, or a segregated compounding area containing a PEC where CSPs are prepared. See *Placement and Use of Primary Engineering Controls* for the requirements for a segregated compounding area. The ante-area must be separated from the surrounding, unclassified sections of the building to reduce the risk of contaminants being blown, dragged, tracked, or otherwise introduced into the high-efficiency particulate air (HEPA)-filtered clean environment. This separation must be continuously maintained and monitored (see 5. *Environmental Monitoring*). When compounding Category 2 CSPs, the ISO Class 8 ante-area and the ISO Class 7 buffer area must be separate rooms, with walls and doors between them and controls to prevent the flow of lower-quality air into the more controlled areas.

The PEC must be located in the buffer area or the segregated compounding area so as to avoid conditions that could adversely affect their separate operations. For example, strong air currents from opened doors, personnel traffic, or air streams from the HVAC system(s) can disrupt the unidirectional airflow of an open-faced PEC such as a laminar airflow workbench (LAFW). Compounding personnel can also create disruptions in airflow by their own movements adjacent to the PEC, their manipulations within the PEC, and by placing objects onto work surfaces within the PEC. Access of personnel to controlled areas must be limited. For example, only authorized personnel and materials required for compounding and cleaning should be permitted in the buffer area.

Due to the interdependence of the various rooms or areas that make up a compounding facility, it is essential to carefully define and control the dynamic interactions permitted between areas and rooms. When designing doors, consider door closures, door surfaces, and the swing of the door, all of which can affect airflow.

Airlocks and interlocking doors can be used to facilitate better control of air balance between a higher classified area and an area of lesser air quality (e.g., between the buffer area and ante-area), or between a classified area and an unclassified area (e.g., between the ante-area and an uncontrolled area such as a hallway). If a pass-through is used, it must only be opened one door at a time; both doors must never be opened at the same time.

It is critical to adequately control materials (e.g., supplies, equipment, and utensils) as they move from lesser to higher classified areas to prevent the influx of contaminants. For this reason, when designing a facility, consider the movement of materials.

When designing the facility, consider whether all materials used can be easily cleaned. Avoid using door seals and sweeps that are difficult to clean. Hands-free access doors are preferred. Do not use tacky mats in ISO-classified areas.

THE CSP PROCESSING ENVIRONMENT

All CSPs must be prepared in a PEC, which provides an ISO Class 5 environment (with the exception of Urgent-Use CSPs, see 1.4 *Urgent-Use CSPs*). The compounding environment must continuously meet ISO Class 5 or better conditions for 0.5- μ m particles and must exclude microbial contamination during compounding of CSPs (typical operating conditions).

HEPA filters and unidirectional (laminar) airflow are used to maintain the appropriate airborne particulate classification of the area. Unidirectional airflow must be maintained in the PEC at all times. HEPA-filtered air must be supplied to the PEC at a velocity sufficient to sweep particles

away from critical sites and maintain unidirectional airflow during operations. Proper design and control prevents turbulence and creation of eddies or stagnant air in the PEC.

Air must be introduced through HEPA filters located at the ceiling of the buffer area containing the PEC, and returns should be mounted low on the wall, creating a general top-down dilution of area air through HEPA-filtered air.

PLACEMENT AND USE OF PRIMARY ENGINEERING CONTROLS

Proper placement of the PEC is critical to ensuring an ISO Class 5 environment for compounding CSPs.

LAFS: Provides an ISO Class 5 or better environment for sterile compounding. A LAFS provides smooth, unidirectional HEPA-filtered airflow that is designed to prevent contamination of a sterile compounding environment. The LAFS can consist of either a LAFW or a HEPA filter alone creating an ISO Class 5 zone within an ISO Class 7 room, as long as unidirectional airflow is maintained.

The LAFS must be located out of traffic patterns and away from room air currents that could disrupt the intended airflow patterns. If used to prepare only Category 1 CSPs, the ISO Class 5 environment can be obtained by placing a LAFW in a segregated compounding area. If used to prepare Category 2 CSPs, the LAFS must be located within a restricted access buffer area with an ISO Class 7 or better air quality.

BSC: A ventilated cabinet with an open front and inward and downward HEPA-filtered airflow and HEPA-filtered exhaust. A BSC must be located out of traffic patterns and away from room air currents that could disrupt the intended airflow patterns. A BSC used to prepare only Category 1 CSPs can be placed in an unclassified area. If used to prepare Category 2 CSPs, the BSC must be located within a restricted access buffer area with an ISO Class 7 or better air quality. If a BSC is used to prepare hazardous drugs, see (800).

RABS: Can include a CAI or a CACI, and can be used to provide an ISO Class 5 environment for preparing CSPs (see *Microbiological Control and Monitoring of Aseptic Processing Environments* (1116)). A RABS is different from an isolator (see description of isolators below). In a RABS, glove ports are used to provide physical separation between the surrounding area and the aseptic manipulations. If used to prepare Category 2 CSPs, the area surrounding the RABS must meet ISO Class 7 or better air quality.

All transport ports on the RABS must be closed during compounding. When a RABS is used, the recovery time after opening to achieve ISO Class 5 air quality must be documented, and internal procedures must be developed to ensure that adequate recovery time is allowed after opening and closing the RABS, both before and during compounding operations.

Isolator: Provides isolation from the surrounding area and maintains ISO Class 5 air quality during typical operating conditions. The following standards must be met to qualify as an isolator:

- High-integrity transfer ports are used to move supplies, ingredients, components, and devices into and out of the isolator.
- The isolator is decontaminated using a generator that distributes a sporicidal chemical agent throughout the isolator chamber.
- The isolator maintains constant overpressure of at least 0.05-inch water column.
- The manufacturer has provided documentation that the isolator will continuously meet

ISO Class 5 conditions, including during material transfer.

If ISO Class 5 classification is achieved using an isolator that meets the requirements above, the isolator can be located in an ISO Class 8 area and used to prepare Category 2 CSPs. In addition, when using an isolator, some functions, such as hand washing, can be done in the ISO Class 8 area. Water sources such as sinks and drains must be located at least 1 meter from the isolator. If the isolator does not meet the requirements above, it is considered a RABS that must be located within at least an ISO Class 7 area to prepare Category 2 CSPs, or within a segregated compounding area to prepare Category 1 CSPs.

Segregated Compounding Areas: In some situations, a PEC may be located within an unclassified area, without a buffer or ante-area. This type of design is called a segregated compounding area. Category 2 CSPs must never be compounded in segregated compounding areas; only Category 1 CSPs can be compounded in facilities with such designs. It is critical to locate a segregated compounding area away from unsealed windows, doors that connect to the outdoors, and significant traffic flow. A segregated compounding area must not be located adjacent to construction sites, warehouses, food preparation areas, or other environmental control challenges. The impact of activities that will be conducted around or adjacent to the segregated compounding area must be considered carefully when designing such an area, and the perimeter of the segregated compounding area must be defined.

ACTIVITIES IN RELATION TO THE PEC

The facility where CSPs are prepared must be designed so that activities such as hand hygiene and gowning will not adversely affect the ability of the PEC to function as designed. In facilities with ante-areas and buffer areas, the sink used for hand hygiene must not be placed in the buffer area. The sink should be placed in the ante-area to allow for hand washing before entering the buffer area. In a segregated compounding area, the sink must be located at least 1 meter from the PEC.

AIR-EXCHANGE REQUIREMENTS

For facilities designed with ante-areas and buffer areas, adequate HEPA-filtered airflow to the buffer and ante-areas is required to maintain the appropriate cleanliness classification during compounding activities. Airflow adequacy is measured in terms of the number of air changes per hour (ACPH). Factors that should be considered when determining appropriate air-exchange requirements for an area include the maximum number of personnel permitted to work in the area, the number of particulates that may be generated from activities and processes in the area, and the effects of temperature.

An ISO Class 7 buffer or ante-area supplied with HEPA-filtered air must measure an ACPH of not less than 30, and the ACPH may need to be higher to maintain the classification, depending on the factors previously described. The ACPH of 30 can include recirculated HEPA-filtered air, but at least half (a minimum of 15 ACPH) must be HEPA-filtered fresh air.

If an isolator that meets the specifications described above is used to achieve ISO Class 5 air quality, air exchange requirements in the room where the isolator is located can be reduced to 15 ACPH.

ESTABLISHING AND MAINTAINING PRESSURE DIFFERENTIALS

To prevent the flow of poorer quality air from one area to another area of higher air quality

classification, except when a segregated compounding area is used, a minimum differential positive pressure of 0.02-inch water column is required to separate each ISO-classified area. The pressure differential between the ISO Class 7 area and the general pharmacy area must not be less than 0.02-inch water column.

A pressure gauge or velocity meter must be used to monitor the pressure differential or airflow between the ante-area and buffer area and between the ante-area and the general environment outside the classified areas. The results must be reviewed and documented on a log at least daily or by a continuous recording device.

4.3 Constructing Areas to Achieve Easily Cleanable Conditions

The surfaces of ceilings, walls, floors, fixtures, shelving, counters, and cabinets in a classified area or in a segregated compounding area must be smooth, impervious, free from cracks and crevices, and non-shedding, thereby promoting cleanability and minimizing spaces in which microorganisms and other contaminants can accumulate. Surfaces must be resistant to damage by disinfectants. Junctures between the ceiling and the walls must be coved or sealed to eliminate cracks and crevices where dirt can accumulate. If ceilings consist of inlaid panels, the panels must be impregnated with a polymer to render them impervious and hydrophobic, and they must be sealed.

Walls must be constructed of durable material (e.g., heavy-gauge polymer). Panels must be locked together and sealed. If gypsum board is used, it must be epoxy-coated. Floors must be overlaid with wide sheet vinyl flooring with heat-welded seams and coving to the sidewall. Classified areas and segregated compounding areas must not contain dust-collecting overhangs, such as utility pipes, or ledges, such as windowsills. The exterior lens surface of ceiling light fixtures must be smooth, mounted flush, and sealed. Any other penetrations through the ceiling or walls must be sealed. The buffer area or area inside the perimeter of a segregated compounding area cannot contain water sources (e.g., sinks) or floor drains.

Work surfaces must be constructed of smooth, impervious materials, such as stainless steel or molded plastic, so that they can be easily cleaned and disinfected.

4.4 Placement and Movement of Materials

Only furniture, storage shelving, counters, cabinets, supplies, and other materials necessary for performing compounding activities are permitted in buffer or segregated compounding areas. Any objects located in buffer or segregated compounding areas must be smooth, impervious, free from cracks and crevices, non-shedding, and easily cleaned and disinfected. Their number, design, and manner of installation must promote effective cleaning and disinfecting. Certain items are not permitted in buffer areas. These include, but are not limited to, coarse cardboard, external shipping containers, and nonessential paper (e.g., paper towels and tissues).

Carts used to transport components or equipment into classified areas must be constructed from stainless steel, nonporous plastic, or sheet metal, with good quality, cleanable casters to promote mobility and ensure ease of disinfection.

Certain devices (e.g., computers) and objects (e.g., carts and cabinets) essential to compounding can be located in the segregated compounding area, but must be located at an appropriate distance from the PEC so that they have no detrimental effects on the air quality inside the PEC. The appropriate distance must be determined by considering the surrounding environment and the activities conducted in it.

Before being brought into a buffer area or segregated compounding area, objects must be cleaned and disinfected. Equipment and other items used in a buffer area or a segregated compounding area should not be removed except for calibration, servicing, or other activities associated with proper maintenance. If removed, these items must be cleaned and disinfected before they are returned to the buffer area or segregated compounding area.

4.5 Certification and Recertification of Facilities

Before a facility is used to compound either Category 1 or Category 2 CSPs, it must be certified by an independent, qualified individual as meeting its design and air quality specifications (see *Table 3*). It is important to place special emphasis on certifying the ISO Class 5 areas. During certification of ISO Class 5 areas, air sampling must be performed inside the PEC and the surrounding ISO-classified areas. Routine staff activity during compounding-related processes must be simulated during certification.

Certification of the PEC must include:

- **Airflow Testing** to determine acceptability of the air velocity and volume, the air exchange rate, and room pressure cascade to ensure that air consistently flows from clean to dirty areas, and that the appropriate quality of air is maintained under typical operating conditions.
- **HEPA Filter Integrity Testing** using the most penetrating particle size. HEPA filters must be leak tested at the factory and then leak tested again after installation and as part of recertification.
- **Total Particle Counts Testing** under typical operating conditions by qualified operators using current, state-of-the-art electronic equipment.
- **Smoke Studies** for each PEC under full operational processing conditions to demonstrate unidirectional airflow and sweeping action over and away from the product(s).

Certification of other ISO-classified areas must include:

- **Airflow Testing** to determine acceptability of the air velocity and volume, the air exchange rate, and room pressure cascade to ensure that air consistently flows from clean to dirty areas and that the appropriate quality of air is maintained under typical operating conditions.

Classified areas must be recertified if there are changes to the area such as redesign, construction, or replacement or relocation of the PEC, or alteration in the configuration of the room that could affect airflow or air quality. Recertification must be done at least every 6 months.

All certification and recertification records must be reviewed by supervising personnel or other designated employees to ensure that the controlled environments comply with the proper standards and records must be maintained in accordance with the requirements in *16. Documentation*.

4.6 Design and Construction of Facilities for Compounding with Hazardous Drugs

For design of facilities in which compounding with hazardous drugs will occur, see (800).

5. ENVIRONMENTAL MONITORING

An effective environmental monitoring program provides meaningful information on the quality of

the compounding environment and any environmental trends in surrounding areas. In addition, an effective environmental monitoring program will identify potential routes of contamination, allowing for implementation of corrections to prevent CSP contamination. Sterile compounding facilities must develop and implement written environmental monitoring procedures (see 9. *SOPs and Master Formulation and Compounding Records*). All environmental sampling and results must be documented, and records must be maintained in accordance with the requirements in 16. *Documentation*.

5.1 General Monitoring Requirements

Sterile compounding facilities must be qualified initially using environmental air and surface sampling as described below to establish a baseline level of environmental quality. After initial qualification, the environment in which sterile compounding activities are performed must be monitored regularly to ensure that the environment remains suitable for sterile compounding.

Environmental monitoring involves the collection and review of environmental samples from various air and surface locations to detect airborne and surface contaminants. Data from sampling are then used to assess airborne nonviable particulate and microbial contamination risks, potential routes of contamination, and the adequacy of disinfection procedures. Data collected from environmental sampling must be reviewed regularly to detect elevated levels of microbial bioburden, elevated levels of nonviable particulates, or other adverse changes within the environment.

Data from air and surface sampling must be reviewed in conjunction with personnel data to assess the state of environmental control and to identify CSP contamination risks. Prompt corrective action in response to any adverse data is essential to maintain the necessary environmental quality for CSP preparation. Data must also be reviewed following corrective actions to confirm that the actions taken have been effective in achieving the required air and surface quality levels (see *Table 3* and *Table 5*).

Routine environmental sampling during compounding operations must be conducted to confirm that the environmental quality in ISO-classified areas is maintained. Sampling also must be performed in any of the following circumstances.

- As part of the certification of new facilities and equipment
- As part of recertification, following any servicing of facilities or equipment (see 4. *Buildings and Facilities*)
- In response to identified problems (e.g., sterility failures; a complaint of patient infection when the CSP is considered to be a potential source of the infection)
- In response to identified trends (e.g., repeated positive fingertip sampling results or failed media fill simulations; repeated observations of air or surface contamination)

The sampling program must include: 1) nonviable airborne particulate sampling; 2) viable airborne particulate sampling; and 3) surface sampling, including but not limited to equipment, work surfaces, and room surfaces.

To obtain an environmental sample that is representative of the full operating conditions at the facility, environmental air sampling (both viable and nonviable) must be conducted during periods of typical activity (i.e., when compounding is occurring). However, the sampling program must be designed and conducted in a manner that minimizes the chance that the sampling itself will contribute to contamination of the CSP, the operator, or the environment.

The sampling program must be developed based on an understanding of risk factors, including but not limited to criticality of the environment sampled, number and types of activities conducted in the room being monitored, maximum number of personnel that may be working in the room at one time, and how the CSPs will be exposed to the immediate environment during compounding. The sampling program must contain a listing of the sample locations, procedures for collecting samples, frequency of sampling, size of sample (e.g., surface area, volume of air), time of day sampled in relation to activities in the compounding area, and levels that will trigger corrective action. Sampling timing and locations should be carefully selected based on their relationship to the operation performed in the area. Sampling locations, frequencies, and timing must be clearly described in a facility's established Standard Operating Procedure (SOP). It is important to sample locations posing the most contamination risk to the CSP (i.e., the PEC), and sampling locations should be selected that are likely to be representative of the conditions throughout the area.

Graphic presentation of the results collected over a period of time can be useful in identifying trends, or for indicating that a significant change has occurred, even when the results fall within the specified limits.

It is important that personnel be trained in the proper operation of the air sampling equipment used to ensure reproducible sampling. All air sampling devices must be serviced and calibrated at appropriate intervals (i.e., as recommended by the manufacturer).

5.2 Monitoring Air Quality for Nonviable Airborne Particles

Because maintaining appropriate air quality is essential to the overall contamination prevention strategy for sterile compounding, it is imperative that all engineering control equipment function as designed and that the levels of airborne particles remain within acceptable limits during compounding operations (see *Table 3*). A monitoring program for nonviable airborne particles must be developed and implemented to measure the performance of the engineering controls that are being used to provide the specified levels of air cleanliness (e.g., in the PEC and ISO Class 7 and 8 areas).

AIR SAMPLING TIMING AND LOCATIONS

Air sampling sites must be selected in all classified areas. Measurements of air cleanliness must be taken in each PEC, at locations where there is greatest risk to the exposed CSPs, containers, and closures. Measurements of air cleanliness in other classified areas, including the buffer area and ante-area, should be taken at representative locations that reflect the quality of air in the area. When conducting sampling of the PEC, care should be taken to avoid disturbing the unidirectional airflow.

Total particle counts of all ISO-classified areas must be conducted during typical operations every 6 months.

DATA EVALUATION AND ACTION LEVELS

If levels measured during the nonviable air sampling program exceed the criteria in *Table 3* for the appropriate ISO classification levels of the area sampled when measured under typical operating conditions, an investigation of the cause must be conducted and corrective action must be taken to prevent future deviations. When nonviable air sampling results for an ISO Class 5 PEC exceed the criteria in *Table 3*, all compounding activities must cease in that PEC and a corrective action plan must be implemented immediately. When nonviable air sampling

results for ISO Class 7 or 8 areas exceed the criteria in *Table 3*, a corrective action plan must be implemented immediately. In such a case, if compounding is continued, the BUDs for any CSPs compounded must not exceed the BUDs for Category 1 CSPs until the area is successfully recertified. Some examples of corrective action include a procedural improvement, such as enhanced disinfection; a process or facility improvement; or HEPA filter replacement or repair. The extent of the investigation should be consistent with the type of excursion, and should include an evaluation of trends.

5.3 Monitoring Air Quality for Viable Airborne Particles

An environmental sampling program for viable airborne particles must be developed and implemented to assess microbiological air quality in all classified areas. The goals of an environmental sampling program are to determine whether contamination is present at unacceptable levels and to assess whether proper personnel practices are being followed and proper environmental conditions maintained.

AIR SAMPLING TIMING AND LOCATIONS

Air sampling sites must be selected in all classified areas. When conducting sampling of the PEC, care should be taken to avoid disturbing unidirectional airflow. See *Box 5-1* for active air sampling procedures. Active air sampling of all ISO-classified areas must be conducted during typical operating conditions at least monthly. Active air sampling is required in each ISO-classified area (e.g., PEC and ISO Class 7 and 8 areas). A general microbiological growth medium that supports the growth of bacteria and fungi, such as trypticase soy agar (TSA) or soybean-casein digest medium, must be used. Samples must be incubated at 20°–25° for 5–7 days and then at 30°–35° for 2–3 additional days. A microbiological incubator that is monitored to maintain the required temperature must be used to incubate the samples. The microbiological incubator must be placed in a location outside of a cleanroom or segregated compounding area. All sampling activities must be performed by properly trained individuals.

Box 5-1 Active Air Sampling Procedures for Viable Airborne Monitoring

- Decontaminate sampling equipment according to the manufacturer's instructions and handle aseptically.
- When media are brought into an ISO-classified area, wipe the wrapping with sterile 70% IPA using a low-lint sterile wipe before removing the media from their packaging.
- Examine media used to collect samples for damage or contamination, and handle in an aseptic manner. Discard contaminated or damaged media and conduct an investigation to determine the cause of the damage or contamination. If a damaged sampling device or packaging is identified (e.g., cracks or foreign bodies on the media surface, or discoloration), examine the entire lot of devices from that vendor to determine whether other devices are damaged or contaminated.
- Using an active air sampling device, test at least 1 cubic meter or 1,000 liters of air from each area sampled.
- At the end of the designated sampling, retrieve the medium and cover it to protect it from external contamination. Protect media from physical damage and keep at appropriate temperatures during transport to the incubator.
- Invert the media plates and incubate the medium at 20°–25° for 5–7 days and then at 30°–35° for 2–3 additional days.
- Examine the media plates for growth daily during normal business hours and record the total number of discrete colonies of microorganisms as CFU per cubic meter of air on an environmental sampling form based on sample type, sample location, and sample date.

DATA EVALUATION AND ACTION LEVELS

Evaluate counts against the action levels in *Table 4*, and examine counts in relation to previous data to identify adverse results or trends. If levels measured during the viable air sampling program reach or exceed the levels in *Table 4*, corrective actions must be taken, including repeat air sampling. If a CFU count is identified below the action levels in *Table 4*, primary screening and characterization must be performed (see *Microbial Characterization, Identification, and Strain Typing* (1113)). Highly pathogenic microorganisms (e.g., gram-negative rods, coagulase positive staphylococcus, molds and yeasts) are potentially fatal to patients receiving CSPs and must be immediately remedied through cleaning and disinfection, regardless of CFU count. If levels measured during viable air sampling exceed the levels in *Table 4*, the genus must be identified, and when possible, identify the species of any microorganism recovered, with the assistance of a credentialed microbiology laboratory.

Table 4. Action Levels for Viable Airborne Particle Air Sampling^a

ISO Class	Air Sampling Action Levels (CFU/m ³) ^b
5	≥1
7	≥10
8	≥100
<p>^a Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice—US Dept of Health and Human Services, Food and Drug Administration, (FDA) September 2004.</p> <p>^b All action levels must be based on sampling in the vicinity of exposed materials/articles during compounding operations.</p>	

5.4 Sampling Surfaces for Contamination

Surface sampling is an important component of the maintenance of a suitably controlled environment for compounding CSPs, especially because transfer of microbial contamination from improperly disinfected work surfaces via inadvertent touch contact by compounding personnel is a potential source of contamination of CSPs. Surface sampling is useful for evaluating facility and work surface cleaning and disinfecting procedures, and employee competency in work practices such as cleaning and disinfection of component/vial surfaces.

Surface sampling for microbial contamination must be performed in all ISO-classified areas. All sampling sites and procedures must be described in the facility’s SOP.

SAMPLING TIMING AND LOCATIONS

When conducted, surface sampling must be performed at the conclusion of compounding activities, but before the area has been cleaned and disinfected. Media used for surface sampling must be supplemented with additives to neutralize the effects of any residual disinfecting agents (e.g., TSA with lecithin and polysorbate 80).

Multiple locations must be sampled at least monthly within each ISO-classified area, including the following (see (1116)):

- The interior of the PEC and equipment contained in it
- Staging or work areas near the PEC
- Frequently touched surfaces

- Pass-through chambers

SAMPLING PROCEDURES

Contact sampling devices (e.g., plates, paddles, or slides) containing microbial growth media must be used for sampling flat surfaces. Sterile swabs wetted with sterile water can be used when sampling irregular surfaces and difficult-to-reach locations in classified areas, such as crevices, corners, and spaces between surfaces.

Surface sampling devices must contain general microbial growth media (e.g., soybean casein digest media) supplemented with neutralizing additives (e.g., lecithin and polysorbate 80). Use a surface sampling device (e.g., plates, paddles, or slides) in the size range of 24- to 36-cm². Contact sampling devices must be certified by the manufacturer to meet growth promotion tests in *Microbial Enumeration Tests* (61). Contact plates must have a raised convex surface. Plates must be stored according to the manufacturer's recommendation. See *Box 5-2* for the procedures for conducting surface sampling with contact sampling devices. Follow the manufacturer's instructions for using sampling swabs.

Box 5-2 Using Devices for Flat Surface Sampling

- Examine media used to collect samples for damage or contamination and handle in an aseptic manner. Discard contaminated or damaged media and conduct an investigation to determine the cause of the damage or contamination. If a damaged sampling device or packaging is identified (e.g., cracks or foreign bodies on the media surface, or discoloration), examine the entire lot of devices from that vendor to determine whether other devices are damaged or contaminated.
- If using commercially prepared devices, wipe the wrapping with sterile 70% IPA using a low-lint sterile cloth before removing the devices from their packaging.
- Remove the cover from the contact sampling device and firmly press the media surface onto the surface to be sampled. The contact sampling device will leave a residue of growth medium on the sample site. After sampling, use a low-lint sterile wipe to thoroughly clean the sampled area with sterile water and disinfect with sterile 70% IPA.
- After exposure, cover each contact sampling device to protect it from further contamination.
- Invert the plates and incubate the contact sampling devices at 20°–25° for 5–7 days and then at 30°–35° for 2–3 additional days.
- Examine the sampling devices for growth daily during normal business hours, and record the observed count at each time point. At the final time point, record the total number of discrete colonies of microorganisms (CFU/sample) on the environmental sampling record based on sample type, sample location and sample date.

DATA EVALUATION AND ACTION LEVELS

If levels measured during surface sampling exceed the criteria in *Table 5*, an investigation of the cause must be conducted and corrective action must be taken to prevent future deviations. When surface sampling results for an ISO Class 5 PEC exceed the criteria in *Table 5*, all compounding activities must cease in that PEC. When surface sampling results for ISO Class 7 or 8 areas exceed the criteria in *Table 5*, a corrective action plan must be implemented immediately. In such a case, if compounding is continued, the BUDs for any CSPs compounded must not exceed the BUDs for Category 1 CSPs until the surfaces are retested and the results fall below action levels in *Table 5*. Some examples of corrective action include a procedural, facility, or equipment improvement. The extent of the investigation should be consistent with

the type of excursion and should include an evaluation of trends.

Table 5. Action Levels for Surface Sampling

ISO Class	Work Surfaces Sampled Using Contact Plates (CFU/plate) ^a	Work Surfaces Sampled Using Swabs (CFU/25 cm ² or per sample) ^a	Non-work Surfaces Sampled Using Contact Plates (CFU/plate) ^b	Non-work Surfaces Sampled Using Swabs (CFU/25 cm ² or per sample) ^b
5	>3	>3	N/A ^c	N/A ^c
7	>5	>5	>10	>10
8	>25	>25	>50	>50
<p>^a Work surfaces are those surfaces that are in direct contact with materials used in compounding. These action levels are based on the expectation that materials will be disinfected before introduction to an ISO Class 5 area.</p> <p>^b Non-work surfaces are those surfaces that do not come into direct contact with materials used in compounding.</p> <p>^c All surfaces within the ISO Class 5 area are considered work surfaces.</p>				

6. CLEANING AND DISINFECTING COMPOUNDING AREAS

Surfaces in compounding areas are a major source of microbial contamination of CSPs. Therefore, scrupulous attention must be paid to cleaning and disinfection. Cleaning and disinfecting the surfaces in sterile compounding areas must occur on a regular basis at the intervals noted in *Table 6*. Cleaning and disinfection must be repeated when spills occur; when surfaces, floors, and walls are visibly soiled; and when microbial contamination is known to have been, or is suspected of having been, introduced into the compounding areas.

If compounding is done less frequently than the cleaning frequencies specified below (e.g., once a week or once a month), cleaning must occur before each compounding session begins, instead of according to the frequencies described in *Table 6*.

Table 6. Minimum Frequency for Cleaning and Disinfecting Surfaces in Classified and Segregated Compounding Areas

Site	Minimum Frequency
PEC (except for an isolator)	At the beginning and end of each shift; before each batch; no longer than 30 minutes following the previous surface disinfection when ongoing compounding activities are occurring; after spills; and when surface contamination is known or suspected
Isolator (as defined in 4. Buildings and Facilities)	Clean the isolator each time it is opened; decontaminate the isolator once it is closed after each time it has been opened, or after each cleaning cycle, if cleaning occurs without opening
Work surfaces outside the PEC (e.g., buffer area and/or segregated compounding area)	Daily
Floors	Daily
Walls	Monthly
Ceilings	Monthly
Storage shelving	Monthly

6.1 Disinfectants

Cleaning and disinfection agents must be selected and used with careful consideration of compatibilities, effectiveness, and inappropriate or toxic residues (see *Appendix 2 and Disinfectants and Antiseptics* (1072)). The selection and use of disinfectants must be guided by microbicidal activity, inactivation by organic matter, residue, and shelf life. Sporicidal agents must be used at least weekly to clean all ISO-classified and segregated compounding areas (see *Disinfectants and Antiseptics* (1072), *Classification of Disinfectants, Table 2, General Classification of Antiseptics, Disinfectants, and Sporicidal Agents*).

The frequency, methods, and locations of disinfection agent use must be established in written SOPs, in accordance with the manufacturer's instructions, and followed by environmental services (i.e., custodial) or compounding personnel.

6.2 Cleaning Tools

All cleaning tools (e.g., wipes, sponges, and mop heads) must be sterile and low-lint, preferably composed of synthetic microfibers and dedicated for use in buffer or ante-areas or segregated compounding areas. All cleaning tools must be cleaned and re-sterilized after each use. They must be discarded after an appropriate amount of time, to be determined based on the condition of the materials. Disposal must involve collecting them in suitable plastic bags and removing them from classified and segregated compounding areas, with minimal agitation so as not to disperse contaminants into the air.

6.3 Cleaning and Disinfecting Floors, Ceilings, Walls, and Shelving

Floors in all ISO-classified and segregated compounding areas should be cleaned by mopping with a cleaning and disinfection agent once daily at a time when no aseptic operations are in progress. Mopping should be in the direction of clean to dirty areas. Mopping must be performed by trained personnel using approved agents and procedures, which must be described in written SOPs. In all ISO-classified and segregated compounding areas, the walls, ceilings, and shelving must be cleaned and disinfected monthly.

6.4 Cleaning and Disinfecting Work Surfaces

For both Category 1 and Category 2 CSPs, cleaning and disinfecting work surfaces in the PEC are the most critical steps before preparing CSPs. These surfaces must be cleaned and disinfected more frequently than other surfaces such as walls and ceilings. With the exception of isolators (as defined in 4. *Buildings and Facilities*), all surfaces in the PEC must be cleaned at the beginning and end of each work shift; no longer than 30 minutes following the previous surface disinfection when ongoing compounding activities are occurring; when there are spills; and when surface contamination is known or suspected from procedural breaches. Additionally, surfaces in the PEC in direct contact with materials used in compounding must be cleaned before starting each batch of CSPs. When using an isolator, cleaning must be done when the isolator is opened. Decontaminate the isolator once it is closed after each time it has been opened, or after each cleaning cycle, if cleaning occurs without opening. See *Box 6-1* for a summary of procedures for cleaning and disinfecting visibly soiled areas in the PEC.

Box 6-1 Procedures for Cleaning and Disinfecting Visibly Soiled Areas in the PEC

- Remove all items on the surface and remove loose material and residue from spills using a suitable cleaning agent.
- Use sterile water for injection or irrigation and sterile low-lint wipes to remove water-soluble solid residues.
- Wipe the affected area with a disinfectant (e.g., sterile 70% IPA).
- Allow the surface to dry before beginning compounding.

Work surfaces in the buffer, ante, and segregated compounding areas must be cleaned and disinfected at least daily, and storage sites for compounding ingredients and supplies must remain free from dust and debris. This must be achieved using a method that does not diminish the ISO Class 7 or 8 air quality.

6.5 Cleaning and Disinfecting Compounding Supplies

No shipping or other external cartons are allowed into the buffer or ante-areas or segregated compounding areas. Before compounding supplies are introduced into buffer areas, they must be wiped with a suitable disinfectant (e.g., sterile 70% IPA) that is delivered from a spray bottle or other suitable delivery method. After the disinfectant is sprayed or wiped on the surface to be disinfected, the disinfectant must be allowed to dry, during which time the item cannot be used.

6.6 Disinfecting Critical Sites

Critical sites (e.g., vial stoppers, ampul necks, and intravenous bag septums) must be disinfected by wiping them with sterile 70% IPA swabs that are commercially available in individual foil-sealed packages (or a comparable method). The IPA must be allowed to dry before piercing stoppers with sterile needles or breaking the necks of ampuls. The sterile 70% IPA swabs used for disinfecting critical sites and devices must not contact any other object before contacting the critical site.

7. EQUIPMENT AND COMPONENTS

7.1 Equipment

The equipment used for compounding CSPs must be of appropriate design and adequate size. The equipment also must be of suitable composition such that the surfaces that contact

components are not reactive, additive, or sorptive, and therefore, will not affect or alter the purity of the CSP. Equipment in direct contact with the CSP and container-closure system must be sterilized and depyrogenated using methods appropriate for the equipment and container-closure system (see 8. *Sterilization and Depyrogenation*, and *Sterilization and Sterility Assurance of Compendial Articles* (1211), *Methods of Sterilization*).

Equipment must be suitably located to facilitate sterile compounding operations. It must be consistently capable of operating properly and within acceptable tolerance limits. Compounding personnel must establish, maintain, and follow written procedures for the calibration, maintenance, and use of the equipment, as well as monitoring it for proper function. Personnel must also maintain results from equipment calibration, annual maintenance reports, and other routine maintenance records in accordance with the requirements in 16. *Documentation*.

Automated compounding devices (ACDs) are designed to streamline the labor-intensive processes involved in the compounding of multiple-ingredient preparations (e.g., parenteral nutrition) by automatically delivering the individual ingredients in a predetermined sequence under computerized control. ACDs can improve the accuracy and precision of the compounding process, compared with manual compounding methods.

When using ACDs, compounding personnel must conduct an accuracy assessment of the ACD each day it is used to compound CSPs. The volume and weight accuracy of the ACD must be determined, based on manufacturer recommendations, to ensure that the correct quantities of ingredients are delivered to the final container. The precision of the ACD can be monitored based on an assessment of day-to-day variations in its accuracy measures. Compounding personnel must keep a daily record of the accuracy measures and must review the results at least weekly to identify trends over time.

7.2 Components

Compounding personnel must establish, maintain, and follow written procedures for the selection and inventory control of all CSP components, including all ingredients (i.e., bulk active pharmaceutical ingredients (APIs) and inactive ingredients), containers, and closures. These written procedures must be followed for all components, from receipt to consumption.

COMPONENT SELECTION

Compounders must use qualified vendors. A vendor is qualified when there is evidence to support its ability to supply a material that consistently meets all quality specifications. Qualification must include an evaluation of the vendor's reputation and reliability.

Ingredients that are the subject of a *USP* or *NF* monograph must be used when available. APIs used in compounding must be manufactured by an FDA-registered facility. Each API must be accompanied by a valid COA that includes the specifications and test results and shows that the API meets the monograph, if one exists, and any additional specifications required to appropriately compound the CSP. Other bulk ingredients should be accompanied by a valid COA that shows that the ingredient meets the monograph, if one exists, and any additional specifications for the ingredient.

When ingredients other than APIs cannot be obtained from an FDA-registered facility, compounders must use professional judgment in selecting an acceptable and reliable source. When ingredients are obtained from an unregistered facility, the compounder must establish the identity, strength, purity, and quality of the ingredients obtained from that supplier by

reasonable means. These means may include checking each lot of the component when received, or periodically verifying quality by testing a sample of components obtained from that supplier to determine whether the COAs for ingredients sourced from that supplier accurately reflect the characteristics of the ingredients.

When components of compendial quality are not obtainable, components of high quality such as those that are chemically pure, analytical reagent grade, or American Chemical Society (ACS)-certified may be used. However, these components should be used cautiously because the standards for analytical reagents or ACS-grade materials do not consider whether the presence of any impurity raises human or animal safety concerns.

Careful consideration and evaluation of nonsterile ingredient sources is especially warranted when the CSP will be administered into the vascular system, central nervous system, or eyes.

Each lot of commercially available sterile, depyrogenated containers and container-closure systems must be accompanied by a COA or other documentation showing conformance with established specifications.

COMPONENT RECEIPT

Upon receipt of each lot of a component, a visual inspection must be performed to ensure that the ingredient appears to be what it is represented to be; the lot must also be examined for evidence of deterioration and other aspects of unacceptable quality. Facility personnel must verify the labeling and condition of the component [e.g., whether the outer packaging is damaged and whether temperature-sensing indicators show that the component has been exposed to excessive temperature(s)].

Analytical results in the vendor-supplied COA for each lot of incoming ingredient must be inspected against the compounding facility's current specification sheet to ensure that the acceptance criteria are met. If there is a compendial monograph for any ingredient received, facility personnel must verify that the COA for the ingredient demonstrates that the ingredient has met the acceptance criteria of all specified monograph tests for that lot and includes the test results.

Any ingredients found to be of unacceptable quality must be promptly rejected, clearly labeled as rejected, and segregated to prevent their use before appropriate disposal. Any other lots of that ingredient from that vendor must be examined to determine whether other lots have the same defect.

The date of receipt by the compounding facility must be clearly and indelibly marked on each ingredient package, except for finished dosage forms obtained from FDA-registered manufacturers. For each ingredient, information including receipt date, quantity received, supplier's name, lot number, expiration date, and results of any in-house or third-party testing performed must be recorded. Compounding personnel must keep a written record of each shipment of components received, in accordance with the recordkeeping requirements described in *16. Documentation*.

COMPONENT EVALUATION BEFORE USE

Before use, all components must be re-inspected. Ingredient packages must be inspected to detect container breaks, looseness of the cap or closure, and deviation from the expected appearance, aroma, and texture of the contents that might have occurred during storage.

Sterile container-closures and sterile devices must be visually inspected to ensure that they are free from defects that could compromise sterility, and are otherwise suitable for their intended use.

Compounding personnel must ascertain before use that ingredients for CSPs are of the correct identity and appropriate quality and have been stored under appropriate conditions. The following information should be used to make this determination: prescription or medication order, compounding record, master formulation record (if used), vendor labels and COAs, product labeling, and knowledge of the compounding facility storage conditions and practices.

If the correct identity, purity, strength, and sterility of ingredients and other components intended for preparation of CSPs cannot be confirmed (e.g., containers of ingredients with incomplete labeling, unlabeled syringes, opened ampuls, punctured vial stoppers, flexible intravenous bags), they must be promptly rejected, clearly labeled as rejected, and segregated to prevent their use before appropriate disposal.

COMPONENT HANDLING AND STORAGE

All components must be handled and stored in a manner that prevents contamination, mix-ups, and deterioration. Ingredients must be stored in tightly closed containers under temperature, humidity, and lighting conditions consistent with those indicated in official monographs or specified by the suppliers/manufacture. Moisture-sensitive ingredients must be stored in tight, well-closed containers.

Packages of ingredients that lack a vendor's expiration date must not be used after 1 year from receipt by the compounding facility, unless appropriate inspection and testing indicates that the ingredient has retained its purity and quality for use in CSPs.

For information on handling and storage of hazardous drugs, see (800).

8. STERILIZATION AND DEPYROGENATION

Each CSP must be sterile and pyrogen-free before release. When selecting the sterilization method for each CSP, personnel must take into consideration the nature of the components, its physical and chemical properties, and the intended container-closure system. The sterilization method used must sterilize the CSP while maintaining its physical and chemical stability (i.e., appropriate strength, purity, quality), and the packaging integrity of the CSP. Utensils and materials in direct contact with the components, the CSP, and the container-closure system must be sterilized and depyrogenated using appropriate methods (see *Sterilization of Compendial Articles* (1229)). If sterilization and depyrogenation of container-closure systems is performed on site, the efficacy of each process must be established and documented, and the process must be shown to be reproducible. CSPs that are terminally sterilized are expected to use a process that achieves a sterility assurance level (SAL) of 10^{-6} . An SAL of 10^{-6} is equivalent to a probability that 1 unit in a million is nonsterile. Generally, an SAL value cannot be applied to CSPs that are aseptically filled into a sterile container following sterilization.

The following must be considered when selecting an appropriate sterilization method:

- Terminal sterilization (e.g., dry heat, steam, or irradiation) is the preferred method, unless the specific CSP or container-closure system cannot tolerate terminal sterilization.
- Filtration is not an option if compounding a suspension when the suspended particles

are removed by the filter being used, which could affect the strength of the CSP.

- Dry heat is not an option if a CSP component is labile when exposed to the temperatures used.
- Steam sterilization is not an option if moisture, pressure, or the temperatures used would degrade the CSP.

A description of the sterilization and depyrogenation process, including the temperature, pressure (if applicable), duration, and permissible load conditions for each cycle, must be included in the facility's written SOPs (see 9. *SOPS and Master Formulation and Compounding Records.*)

In addition, the SOPs must include a schedule and method for establishing and periodically verifying the effectiveness of the sterilization and depyrogenation methods selected, as well as the method for maintaining and cleaning the sterilizers and depyrogenation equipment.

The following sections provide general guidance on specific sterilization methods.

8.1 Sterilization by Filtration

See *Sterilizing Filtration of Liquids* (1229.4). Commercially available sterile filters must be certified by the manufacturer as suitable for pharmaceutical use when used to sterilize CSPs. Sterilizing filters must be sterile and pyrogen-free and have a nominal pore size of 0.2 or 0.22 μm . They must be certified by the manufacturer to retain at least 10^7 microorganisms of a strain of *Brevundimonas diminuta* per square centimeter of upstream filter surface area under conditions similar to those in which the CSPs will be filtered (i.e., pressure, flow rate, and volume filtered).

The person responsible for selecting the sterilization method must ascertain from appropriate information sources that the sterilizing-grade membrane filter selected is chemically and physically compatible with the specific formulation of the CSP. For example, CSPs containing water-miscible alcohols may cause undetectable damage to filter integrity and shrinkage of microorganisms to sizes smaller than the filter's nominal pore size.

The responsible person must ensure, directly or from appropriate documentation from the supplier, that the filters 1) are chemically and physically stable at the pressure and temperature conditions that will be used; 2) have enough capacity to filter the required volumes; and 3) will yield a sterile filtrate while maintaining pre-filtration pharmaceutical quality, including strength of ingredients of the specific CSP. The filter dimensions and the preparation to be sterilized by filtration should permit the sterilization process to be completed without the need for replacement of the filter during the process. When CSPs are known to contain excessive particulate matter, to maximize the efficiency of the final sterilizing filtration, a pre-filtration step should be performed using a filter of larger nominal pore size, or a separate filter of larger nominal pore size should be placed upstream of (i.e., prior to) the sterilizing filter to remove gross particulate contaminants before the CSP is passed through the sterilizing grade filter. Excessive particulate matter requiring a prefiltration step also could be a signal of an inappropriate formulation, and therefore the formulation and the process should be assessed to ensure that they are appropriate; if necessary, they should be modified. Filter units used to sterilize CSPs must be subjected to the manufacturers' recommended post-use integrity test, such as a bubble point test.

8.2 Sterilization by Steam Heat

See *Steam Sterilization by Direct Contact* (1229.1). The process of thermal sterilization using saturated steam under pressure (i.e., autoclaving) is the preferred method for terminal sterilization of aqueous preparations in their final, sealed container–closure system. Steam heat sterilization is not an option if moisture, pressure, or the temperatures used would degrade the CSP. Steam heat sterilization is also used to sterilize many components (e.g., elastomeric closures) and some types of equipment. To achieve sterility, all materials must be directly exposed to steam under adequate pressure for the length of time necessary, as determined by use of appropriate biological indicators, to render the items sterile (i.e., kill any microorganisms, including bacterial spores that might be present). This is usually between 20 and 60 minutes at 121° saturated steam under a pressure of 15 psi. The duration of the exposure period must include sufficient time for the CSP or other items to reach the sterilizing temperature. The CSP and other items must remain at the sterilizing temperature for the duration of the sterilization period. The sterilization cycle should be designed to achieve a SAL of 10^{-6} .

CSPs must be placed in suitable trays to allow steam to reach the CSPs without entrapment of air. Flat, stainless steel trays with low sides or ventilated bottoms will permit steam contact. When preparing plastic, glass, and metal devices or other items for steam sterilization, the items must be wrapped in low-lint protective fabric or paper or sealed in envelopes that will permit steam penetration and prevent post sterilization microbial contamination. Immediately before filling ampuls and vials that will be steam sterilized, solutions must be passed through a filter having a nominal pore size of not larger than 1.2 μm for removal of particulate matter.

Sealed containers must be able to generate steam internally. Stoppered and crimped empty vials must contain a small amount of moisture to generate steam. Deep containers, such as beakers and graduated cylinders, should be placed on their sides to prevent air entrapment, or should have a small amount of water placed in them when steam sterilized. Porous materials and those items with occluded pathways (e.g., tubing) should only be sterilized by steam if the autoclave chamber has suitable cycles for dry goods, such as a pre-vacuum process to remove air before steam is sent into the chamber. Elastomeric closures and many other dry goods will need a drying cycle after steam exposure to remove condensed or absorbed moisture.

The effectiveness of steam sterilization must be established and verified with each sterilization run or load by using appropriate biological indicators, such as spores of *Geobacillus stearothermophilus*, ATCC 12980, ATCC 7953 or equivalent (see *Biological Indicators for Sterilization* (1035)), and other confirmation methods such as physicochemical indicators and integrators (see *Sterilization—Chemical and Physicochemical Indicators and Integrators* (1209)).

The steam supplied must be free of contaminants and generated using clean water. The seals on the doors of autoclave chambers should be examined visually every day they are used for cracks or other damage, and the seal surfaces should be kept clean. A data recorder or chart must be used to monitor each cycle and to examine for cycle irregularities (e.g., deviations in temperature or pressure).

Because the temperatures used to achieve sterilization by steam heat are lower than those used to achieve depyrogenation, materials in direct contact with the CSP (e.g., the container–closure system) must first undergo a depyrogenation process (e.g., dry heat or rinsing with pyrogen-free water) before being sterilized using steam heat, unless the materials used are

certified to be pyrogen-free (see *Depyrogenation* <1228>).

8.3 Sterilization by Dry Heat

See *Dry Heat Sterilization* <1229.8>. Dry heat can be used only for those items that cannot be sterilized by steam or other means, when either the moisture would damage the material or the wrapping material is impermeable. Sterilization by dry heat requires higher temperatures and longer exposure times than sterilization by steam. The duration of the exposure period must include sufficient time for the CSP or other items to reach the sterilizing temperature. The CSP and other items must remain at the sterilizing temperature for the duration of the sterilization period.

Dry heat sterilization is usually done in an oven designed for sterilization at a temperature of 160° or higher, although sterilization processes at lower temperatures have been developed and validated. If lower temperatures are used, they must be shown to achieve effective sterilization (see *Dry Heat Sterilization* <1229.8>, *Validation of Dry Heat Sterilization*, *Biological Indicators*).

Heated air must be evenly distributed throughout the chamber, which is typically done by an air blower. The oven must be equipped with temperature controls and a timer. During sterilization, sufficient space must be left between materials to allow for good circulation of the hot air. A data recorder or chart must be used to monitor each cycle and the data must be reviewed to identify cycle irregularities (e.g., deviations in temperature or exposure time).

The effectiveness of the dry heat sterilization method must be established and verified with each sterilization run or load using appropriate biological indicators such as spores of *Bacillus atrophaeus*, ATCC 9372, (see <1035>) and other confirmation methods (e.g., temperature-sensing devices).

Because the temperatures used to achieve sterilization by dry heat are lower than those used to achieve depyrogenation, materials in direct contact with the CSP (e.g., the container-closure system) must first undergo a depyrogenation process (e.g., dry heat or rinsing with pyrogen-free water) before being sterilized using dry heat, unless the materials used are certified to be pyrogen-free (see <1228>).

8.4 Depyrogenation by Dry Heat

See <1228>. Dry heat depyrogenation must be used to render glassware and other thermostable containers pyrogen-free. Depyrogenation processes typically operate at a range of temperatures from approximately 170° up to about 400°, depending on the exposure time. For example, a typical cycle would hold the items at 250° for 30 minutes. The duration of the exposure period must include sufficient time for the items to reach the depyrogenation temperature. The items must remain at the depyrogenation temperature for the duration of the depyrogenation period.

The effectiveness of the dry heat depyrogenation cycle must be established and verified annually using endotoxin challenge vials (ECVs) to demonstrate that the cycle is capable of achieving a ≥ 3 -log reduction in endotoxins (see *Bacterial Endotoxins Test* <85>).

9. SOPS AND MASTER FORMULATION AND COMPOUNDING RECORDS

Every compounding facility must establish and follow written SOPs for sterile compounding. The

SOPs must ensure that the entire compounding operation is well designed, functions as designed, and will yield CSPs that are safe for administration to patients. The compounding process for CSPs must be described in SOPs.

A Master Formulation Record is required when CSPs are prepared in a batch for multiple patients or when CSPs are prepared from nonsterile ingredients. A Master Formulation Record documents the ingredients, specific procedures, equipment to be used, and testing required for each CSP. A Compounding Record is required for every CSP prepared and requires documentation by all individuals involved in the actual preparation of the CSP.

9.1 Creating and Following SOPs

Facilities preparing CSPs must develop SOPs on all aspects of the compounding operation. All personnel who conduct or oversee compounding activities must be trained in the SOPs and are responsible for ensuring that they are followed. All compounding personnel must:

- Be able to immediately recognize potential problems, deviations, or errors associated with preparing a CSP (e.g., related to equipment, facilities, materials, personnel, compounding process, or testing) that could potentially result in contamination or other adverse impact on CSP quality
- Report any problems, deviations, or errors to the compounding supervisor or designee, who must take corrective actions

Compounding supervisors must ensure that SOPs are appropriate and are fully implemented, which includes ensuring that staff demonstrate consistency and competency in performing every procedure that relates to their job function. Compounding supervisors must also ensure that appropriate follow-up occurs if problems, deviations, or errors are identified.

9.2 Creating Master Formulation Records

A Master Formulation Record must be created for CSPs prepared in a batch for multiple patients or for CSPs prepared from nonsterile ingredients. Any changes or alterations to the Master Formulation Record must be performed only by authorized personnel and must be documented. *Box 9-1* lists the information that must be included in a Master Formulation Record.

Box 9-1 Master Formulation Record

A Master Formulation Record must include at least the following information:

- Name, strength, and dosage form of the CSP
- Physical description of the final preparation
- Identities and amounts of all ingredients and appropriate container-closure systems
- Complete instructions for preparing the CSP, including equipment, supplies, and a description of the compounding steps
- BUD and storage requirements
- Quality control procedures (e.g., pH, filter integrity, and visual inspection)
- Sterilization method, if applicable (e.g., filter, steam, or dry heat)
- Any other information needed to describe the operation and ensure its repeatability (e.g., adjusting pH and tonicity and temperature)

9.3 Creating Compounding Records

A Compounding Record must be created by the compounder preparing the CSP to document the compounding process. The Compounding Record or inventory control system must permit traceability of all ingredients. The Master Formulation Record (when used) can be used as the basis for preparing the Compounding Record. For example, a copy of the Master Formulation

Record can be made that contains spaces for recording the information needed to complete the Compounding Record. It is critical that the Compounding Record document in detail any deviations from the process outlined in the Master Formulation Record and any problems or errors experienced during the compounding of the CSP. *Box 9-2* lists the information that must be included in a Compounding Record.

Each Compounding Record must be reviewed and approved before the CSP is released (signature or initials and date).

Box 9-2 Compounding Records

Compounding Records must include at least the following information:

- Name, strength, and dosage form of the CSP
- Master Formulation Record reference for the preparation, when used
- Date and time of preparation of the CSP
- Assigned internal identification number (e.g., prescription or lot number)
- Signature or initials of individuals involved in each step (e.g., technician or pharmacist)
- Name, vendor or manufacturer, lot number, and expiration date of each ingredient and container–closure system
- Weight or measurement of each ingredient
- Documentation of the calculations made to determine and verify quantities and/or concentrations of components, if appropriate
- Documentation of quality control procedures in accordance with the SOP (e.g., filter integrity, pH, and visual inspection)
- Any deviations from the Master Formulation Record, if used, and any problems or errors experienced during the compounding of the CSP
- Total quantity compounded
- Assigned BUD
- Duplicate container label if prepared in a batch

10. RELEASE TESTING

At the completion of compounding, before release and dispensing, the CSP must be inspected as described below to determine whether the physical appearance of the CSP is as it should be and to confirm that the CSP and its labeling match the prescription or medication order.

The physical inspection described in *10.1 Physical Inspection of CSP* must be performed on all CSPs before they are released. In addition, sterility and bacterial endotoxin testing must be performed in some cases (see *12. Establishing Beyond-Use Dates and In-Use Times*), as described in *10.2 Sterility Testing* and *10.3 Bacterial Endotoxins Testing*. All checks and inspections, and any other tests or checks necessary to ensure the quality of the CSP (e.g., assays), must be included in the facility's SOP (see *9. SOPS and Master Formulation and Compounding Records*). Additional quality assurance and quality control activities are discussed in *13. Quality Assurance and Quality Control*.

10.1 Physical Inspection of CSP

After compounding, and as a condition of release, each individual CSP unit must be inspected to identify any apparent physical defect. Each individual injectable CSP unit must be inspected against a lighted white background and a black background for evidence of visible particulates or other foreign matter, or discoloration. Some CSPs also must be visually checked for certain characteristics (e.g., emulsions must be checked for phase separation). Pre-release inspection

also must include a visual inspection of container–closure integrity (e.g., checking for leakage, cracks in the container, or improper seals). CSPs with observed defects must be immediately discarded, or marked and segregated from acceptable units in a manner that prevents them from being released or dispensed.

When a CSP will not be released or dispensed promptly after preparation, a pre-release inspection must be conducted immediately before it is released or dispensed to make sure that the CSP does not exhibit any defects, such as precipitation, cloudiness, or leakage, which may develop during storage. A CSP with such defects must be immediately discarded, or marked and segregated from acceptable units in a manner that prevents it from being released or dispensed.

10.2 Sterility Testing

Category 1 CSP BUDs apply regardless of whether sterility testing is conducted (see *Table 7*). If a Category 2 CSP is assigned a BUD that requires sterility testing (see *Table 8*), the testing must be performed in a manner consistent with $\langle 71 \rangle$, with the exception, in some cases, of the batch sizes specified in *Sterility Tests* $\langle 71 \rangle$, *Table 3. Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch*. If the number of units of CSPs to be prepared in a single batch is less than the number of units needed for testing, additional units may be required to be compounded to be able to conduct sterility testing. For batch sizes of 1–39 units, each sterility test must be performed using a number of units equal to 10% of the batch size, rounded up to the next whole number. For batch sizes of 40 or more units, the sample sizes specified in *Sterility Tests* $\langle 71 \rangle$, *Table 3* must be used.

When the CSP formulation permits, the Membrane Filtration method is the method of choice for sterility testing. The preferred alternative is the Direct Inoculation of the Culture Medium method; both methods are described in $\langle 71 \rangle$.

If sterility testing will be conducted, ideally the results should be obtained before dispensing to patient(s). If it is anticipated that there will be situations in which there may be an urgent need to dispense a CSP before the results of the sterility testing are known, a written procedure (SOP) must be developed and followed; this SOP must describe how these situations will be handled. In addition, this SOP must require frequent observation of the incubating test specimen and must require immediate recall of the dispensed CSP (if possible) or immediate notification of the patient’s prescriber, if any evidence of microbial growth is found during the test.

Positive sterility test results must prompt a rapid and systematic investigation into the causes of the sterility failure, including identification of the contaminating organism (at least to the genus level) and any aspects of the facility, process, or personnel that may have contributed to the sterility failure. The source of the contamination, if identified, must be corrected, and the facility should determine whether the conditions causing the sterility failure affect other CSPs. The investigation and resulting corrective actions must be documented.

10.3 Bacterial Endotoxins Testing

All Category 2 CSPs made from one or more nonsterile ingredients, except those for inhalation and topical ophthalmic administration, must be tested to ensure that they do not contain excessive bacterial endotoxins (see $\langle 85 \rangle$ and *Pyrogen Test* $\langle 151 \rangle$). A CSP does not need to be tested for bacterial endotoxins if the COA for the nonsterile ingredient lists the endotoxins

burden, or if the compounding facility has predetermined the endotoxins burden of the nonsterile ingredient and found it acceptable, and the material is stored under cool and dry conditions.

In the absence of a bacterial endotoxins limit in an official monograph or other CSP formula source, the CSP must not exceed the endotoxins limit calculated as described in (85) for the appropriate route of administration.

11. LABELING

CSPs must be labeled with adequate, legible identifying information to prevent errors during storage, dispensing, and use. The term labeling designates all labels and other written, printed, or graphic matter on an article's immediate container or on, or in, any package or wrapper in which it is enclosed, except any outer shipping container. The term label designates that part of the labeling on the immediate container. See *Labeling (7), Labels and Labeling for Products and Other Categories, Compounded Preparations*.

The label must, at a minimum, display prominently and understandably the following information:

- Assigned internal identification number (e.g., prescription or lot number)
- Brand and/or generic name(s), or active ingredient(s) and amounts or concentrations
- Dosage form
- Total amount, if it is not obvious from the container
- Storage conditions
- BUD and when appropriate, an in-use time (see *12. Establishing Beyond-Use Dates and In-Use Times*)
- Whether it is a single-dose or multiple-dose container
- Indication that the preparation is compounded

The labeling must, at a minimum, display prominently and understandably the following information:

- Patient name, or for animal drugs, owner's name and species of patient
- Route of administration, if known
- Any special handling instructions
- Any warning statements that are applicable
- Name, address, and contact information of the compounder if the CSP is to be sent outside of the facility in which it was compounded

Labeling operations must be controlled to prevent labeling errors and CSP mix-ups. A final check must be conducted to verify that the correct and complete label has been affixed to the finished CSP. All labels must also comply with applicable state laws and regulations.

12. ESTABLISHING BEYOND-USE DATES AND IN-USE TIMES

Each CSP label must state the date beyond which the preparation cannot be used and must be discarded. A CSP may also be labeled with an in-use time within which it must be used after it has been opened or punctured. A number of critical parameters must be considered before establishing these dates. It is also important to understand the various terms that are used in discussion of these dates.

12.1 Terminology

A number of terms are used to describe the time period during which a drug is considered to retain its desired characteristics so that it can be safely administered to a patient to achieve the desired therapeutic effect.

The expiration date identifies the time during which a conventionally manufactured drug product may be expected to maintain its labeled identity, strength, quality, and purity, provided it is kept under the labeled storage conditions. The expiration date limits the time during which a conventionally manufactured product may be dispensed or used. Expiration dates are determined based on product-specific studies that evaluate the specific formulation of a drug product in the specific container in which it is to be stored and under the conditions to which it could be exposed. Temperature, humidity, and light are some of the factors that can affect whether and how much a product degrades over time. An expiration date is determined by taking representative samples from batches and placing them in storage under controlled conditions and then testing them at scheduled intervals to determine whether they meet specifications throughout their labeled shelf lives. When an expiration date is stated only in terms of the month and the year, it is a representation that the intended expiration date is the last day of the stated month.

A BUD is included on the label of each CSP to indicate the date or date and hour after which the CSP must not be used, because its required quality characteristics (e.g., sterility, strength, purity) cannot be ensured. The term expiration date is not appropriate for CSPs, because the types of full stability studies conducted by manufacturers to establish expiration dates for conventionally manufactured products are not typically performed for CSPs. BUDs for CSPs are calculated in terms of hours or days.

An in-use time refers to the time before which a conventionally manufactured product or a CSP must be used after it has been opened or needle punctured (e.g., after a container closure of a vial has been penetrated).

12.2 Critical Parameters to Be Considered in Establishing a BUD

Time is a critical factor in establishing a BUD. The more time that passes between the compounding of a CSP and its administration to a patient, the greater the risk of harm to the patient if the stability or sterility of the CSP has been compromised. With respect to sterility, this is especially the case if microbial contamination is present from the outset. BUDs for CSPs should be established conservatively to ensure that the drug maintains its required characteristics until administration to reduce the risk to patients of receiving a contaminated or degraded preparation. Both sterility and stability considerations must be taken into account when establishing a BUD.

STERILITY CONSIDERATIONS

Microbial contamination of a CSP poses a significant risk to patients, and if the CSP is contaminated during preparation, the risk increases the longer the time the CSP is stored before administration.

When establishing a BUD for a CSP, it is critical that compounding personnel carefully consider all of the possible ways that the sterility of the CSP could be compromised over time. The following factors related to sterility must be carefully considered:

- Whether the CSP will be tested for sterility, and the results will be known, before the CSP is released or dispensed

- Whether the CSP will be terminally sterilized in its final container (provided the drug and its container–closure can withstand the terminal sterilization process) (see 8. *Sterilization and Depyrogenation*)
- Whether the CSP contains a preservative, or is inherently susceptible to microbial survival or growth, if contaminated
- Whether the container–closure system and sealing method will ensure the integrity of the CSP until administration to the patient

STABILITY CONSIDERATIONS

Over time, active ingredient(s) in a CSP may degrade, reducing the strength of the preparation and/or producing harmful impurities. Additionally, the container–closure system may degrade, which can lead to several potential deleterious effects, such as: 1) reducing the integrity of the CSP; 2) leaching of harmful chemicals into the preparation from the container–closure system; and/or 3) absorption of the active ingredient onto the container, thereby reducing potency. When establishing a BUD for a CSP, it is critical that compounding personnel carefully consider all of the possible ways that the physical or chemical characteristics of the CSP could change over time. The following issues must be carefully considered:

- The chemical and physical stability properties of the drug and/or its formulation (i.e., if the drug and its formulation are known to degrade over time and/or under certain storage conditions)
- The compatibility of the container–closure system with the finished preparation (e.g., consider leachables, interactions, and storage conditions of the components)

In addition:

- If the CSP includes components from conventionally manufactured product(s), the BUD of the CSP must not exceed the shortest remaining expiration date of any of the starting components.
- If the CSP includes components from other compounded preparations, the BUD of the final CSP must not exceed the shortest remaining BUD of any of the starting CSP components.

12.3 Establishing a BUD for a CSP

BUDs for CSPs must be established in accordance with *Table 7* for Category 1 CSPs and *Table 8* for Category 2 CSPs. The BUDs specified in the tables indicate the hours or days after the CSP is prepared and beyond which the CSP cannot be used. The BUD is determined from the time the CSP is compounded. One day is equivalent to 24 hours.

The BUDs in *Table 7* and *Table 8* for CSPs are based on the risk of microbial contamination, not the physical or chemical stability of the CSP, and involve the following assumptions:

- The CSP and its components can remain chemically and physically stable for the BUD period
- None of the factors identified in *12.2 Critical Parameters to Be Considered in Establishing a BUD*, would require a shorter BUD

If there is any indication, based on the factors described in *12.2. Critical Parameters to be Considered in Establishing a BUD*, that the particular CSP formulation will not remain chemically or physically stable for the specified period, a shorter BUD must be assigned based on the time period during which the CSP is expected to remain chemically and physically stable.

Table 8 establishes the longest permitted BUDs for Category 2 CSPs, based the following variables:

METHOD OF ACHIEVING STERILITY

Because terminal sterilization using a verified method provides reasonable assurance that a CSP will be sterile, *Table 8* allows longer BUDs for CSPs that are terminally sterilized and not sterility tested than for aseptically prepared CSPs that are not sterility tested. Not all CSPs can be terminally sterilized, and if the CSP is aseptically prepared (e.g., using only sterile products or sterilized by filtration), the shorter BUDs in *Table 8* for aseptically prepared CSPs that are not sterility tested must not be exceeded.

STARTING COMPONENTS

An aseptically prepared CSP compounded from one or more nonsterile starting component has a higher risk of microbial contamination than an aseptically prepared CSP compounded only from sterile starting components. *Table 8* allows for longer BUDs for CSPs aseptically prepared from only sterile starting components.

WHETHER THE CSP WILL BE STERILITY TESTED AND THE RESULTS KNOWN BEFORE THE DRUG IS RELEASED OR DISPENSED

Sterility testing (see *10.2 Sterility Testing*) before releasing or dispensing a CSP can provide additional assurance of the absence of contamination. When the results of sterility testing are known before dispensing, a longer BUD is permitted in *Table 8*. If sterility testing is not performed, a shorter BUD is required in *Table 8*.

If a sterility test is performed and there is an urgent need to dispense the CSP before sterility test results become available, a CSP can be dispensed to a patient before the end of the sterility testing period if:

- The prescriber specifically requests dispensing before completion of the sterility test, and the request is documented
- The patient and the prescriber are notified of any microbial growth during the sterility testing. The species of microbial contaminant is reported to the prescriber to ensure appropriate medical therapy following exposure to a contaminated CSP

PRESENCE OF A PRESERVATIVE

Although a preservative must not be considered a substitute for good aseptic practices, preservatives can be added to multiple-dose CSPs because they may inhibit the growth of microorganisms for short periods of time. If a CSP contains a preservative whose effectiveness for the length of the BUD has been verified based on antimicrobial effectiveness testing (see *Antimicrobial Effectiveness Testing* (51)), the BUD in *Table 8* for a CSP containing a preservative can be assigned. Shorter BUDs are required for preservative-free CSPs, as compared to CSPs that contain a verified preservative.

The particular CSP formulation must pass antimicrobial effectiveness testing in accordance with (51) at the completion of the sterility test (if conducted) or at the time of preparation (if sterility testing is not performed). The test must be completed and the results obtained on the specific formulation before any of the CSP is released or dispensed. The test needs to be conducted only once on each formulation in the particular container-closure system in which it will be stored or released/dispensed.

STORAGE CONDITIONS

The specified conditions under which a CSP will be stored are important in determining an appropriate BUD. Storage in a refrigerator or in a freezer (see *Packaging and Storage Requirements* (659)) has been shown to slow the growth of microorganisms. Therefore, *Tables 7 and 8* allow for longer BUDs for CSPs stored in colder conditions than when stored at controlled room temperature.

Storage under frozen conditions places the container–closure under physical stress, and the degree of stress may depend on the formulation and other factors. Therefore, if a Category 2 CSP is to be stored in a freezer, the integrity of the CSP in the particular container–closure system in which it will be stored must have been demonstrated for 45 days at frozen storage. A container–closure integrity test needs to be conducted only once on each formulation and fill volume in the particular container–closure system in which it will be stored or released/dispensed. Once the CSP is thawed, the CSP must not be re-frozen.

It must be recognized that CSPs may be stored under different storage conditions before they are used (e.g., they may first be frozen, and then thawed in the refrigerator, and finally kept at controlled room temperature before administration). The storage time of a CSP must not exceed the original BUD placed on the CSP for its labeled storage conditions, and BUDs are not additive. For example, a CSP cannot be stored for 4 days at controlled room temperature, then 7 days refrigerated, and then 45 days in a freezer, for a total of 56 days. Once a CSP is stored under a condition that would require a shorter BUD (i.e., controlled room temperature), the CSP must be used within the shorter timeframe for that storage condition (in this example, 4 days).

Table 7. BUDs for Category 1 CSPs^a

	Storage Conditions	
	Controlled Room Temperature (20°–25°)	Refrigerator (2°–8°)
BUD	≤12 hours	≤24 hours
^a The BUDs specified in the table indicate the hours after the Category 1 CSP is prepared beyond which the CSP cannot be used. The BUD is determined from the time the CSP is compounded.		

Table 8. BUDs for Category 2 CSPs^a

		Preparation Characteristics		Storage Conditions			
	Method of Achieving Sterility	Sterility Testing Performed	Preservative Added	Controlled Room Temperature (20°–25°)	Refrigerator (2°–8°)	Freezer (–25° to –10°) ^b	
BUD	Aseptically prepared CSPs	No	No	Prepared from one or more nonsterile starting component 4 days	Prepared from one or more nonsterile starting component 7 days	Prepared from one or more nonsterile starting component 45 days	
				Prepared from only sterile starting components 6 days	Prepared from only sterile starting components 9 days	Prepared from only sterile starting components 45 days	
		Yes	No	Yes ^c	28 days	42 days	45 days
				No	28 days	42 days	45 days
			Yes ^d	Yes ^d	42 days	42 days	45 days
				No	14 days	28 days	45 days
	Terminally Sterilized CSPs	No	Yes ^c	28 days	42 days	45 days	
			No	28 days	42 days	45 days	
		Yes	Yes ^d	42 days	42 days	45 days	
			No	14 days	28 days	45 days	

^a The BUDs specified in the table indicate the days after the Category 2 CSP is prepared beyond which the CSP cannot be used. The BUD is determined from the time the CSP is compounded. One day is equivalent to 24 hours.

^b The integrity of the container–closure system with the particular CSP in it must have been demonstrated for 45 days at frozen storage. The container–closure integrity test needs to be conducted only once on each formulation in the particular container–closure system in which it will be stored or released/dispensed.

^c The particular CSP formulation must pass antimicrobial effectiveness testing in accordance with (51) at the time of preparation. The test must be completed and the results obtained on the specific formulation before any of the CSP is dispensed. The test needs to be conducted only once on each formulation in the particular container–closure system in which it will be stored or released/dispensed.

^d The particular CSP formulation must pass antimicrobial effectiveness testing in accordance with (51) at the completion of sterility test (i.e., 14 days after preparation). The test must be completed and the results obtained on the specific formulation before any of the CSP is dispensed. The test needs to be conducted only once on each formulation in the particular container–closure system in which it will be stored or released/dispensed.

12.4 Establishing In-Use Times

The in-use time is the time before which a conventionally manufactured product or a CSP must be used after it has been opened or needle-punctured. The in-use time assigned cannot exceed the expiration date of the conventionally manufactured product or the BUD of a CSP. The in-use time may be dependent on the type of product or CSP and the environment where the manipulations occur (e.g., ISO Class 5, or worse than ISO Class 5). *Table 9* specifies the in-use times for conventionally manufactured products and CSPs that are opened, stored, and used for sterile compounding in ISO Class 5 or better air quality. *Table 10* specifies the in-use times for conventionally manufactured products and CSPs that are opened and/or stored in worse than ISO Class 5 air quality.

Table 9. In-Use Times for Conventionally Manufactured Products and CSPs Opened, Stored, and Used for Sterile Compounding in ISO Class 5 or Better Air Quality

Components	In-Use Time
Conventionally Manufactured Sterile Product	
Ampuls	Use <i>immediately</i> after opening and passing through a sterile particulate filter
Pharmacy Bulk Package	As specified by the manufacturer
Single-dose container (e.g., bag, bottle, syringe, or vial)	6 hours
Multiple-dose container	28 days, unless otherwise specified by the manufacturer
CSP	
Compounded single-dose container	6 hours
Compounded stock solutions	6 hours
Compounded multiple-dose container ^a	28 days, unless otherwise specified by the original compounder
<p>^a The particular CSP formulation must pass antimicrobial effectiveness testing in accordance with (51) at the completion of the sterility test (if conducted) or at the time of preparation (if sterility testing is not performed). The test must be completed and the results obtained on the specific formulation before any of the CSP is released or dispensed. The test needs to be conducted only once on each formulation in the particular container-closure system in which it will be stored or released/dispensed.</p>	

Table 10. In-Use Times for Conventionally Manufactured Products and CSPs Opened and/or Stored in Worse than ISO Class 5 Air^a

Components	In-Use Time
Conventionally Manufactured Sterile Product	
Ampuls	Use <i>immediately</i> after opening and passing through a sterile particulate filter
Pharmacy Bulk Package	Not applicable. Contents of pharmacy bulk packages must be used only in an ISO Class 5 or better environment.
Single-dose container (e.g., bag, bottle, syringe, vial)	Use for a single patient within the time specified by the manufacturer, or by the end of the case or procedure, whichever comes first. Discard remainder.
Multiple-dose container	28 days, unless otherwise specified by the manufacturer
CSP	
Compounded single-dose container	Use for a single patient immediately. Discard remainder.
Compounded multiple-dose container ^b	28 days, unless otherwise specified by the original compounder
<p>^a Compounding or repackaging must not occur in worse than ISO Class 5 air.</p> <p>^b The particular CSP formulation must pass antimicrobial effectiveness testing in accordance with (51) at the completion of the sterility test (if conducted) or at the time of preparation (if sterility testing is not performed). The test must be completed and the results obtained on the specific formulation before any of the CSP is released or dispensed. The test needs to be conducted only once on each formulation in the particular container-closure system in which it will be stored or released/dispensed.</p>	

13. QUALITY ASSURANCE AND QUALITY CONTROL

A quality assurance (QA) and quality control (QC) program is necessary to ensure that consistently high-quality CSPs are prepared. QA is a set of written processes that, at a minimum, verifies, monitors, and reviews the adequacy of the compounding process. QC is the observation of techniques and activities that demonstrate that requirements are met.

Each facility must have a formal, written QA and QC program that establishes a system of adherence to procedures, prevention and detection of errors and other quality problems, and appropriate corrective actions when needed. A facility’s QA program must be formally established and documented in SOPs that ensure that all aspects of the preparation of CSPs are conducted in accordance with this chapter and applicable federal, state, and local laws and regulations.

The QA program must, at a minimum, address the following functions:

- Personnel qualifications and training:
 - Periodically review personnel files for each employee to determine whether personnel continue to meet basic qualifications, are obtaining required training, and are getting qualified and requalified in accordance with the specified frequencies (e.g., gloved fingertip/thumb sampling)
 - Assess staff performance, including aseptic techniques, cleanroom behavior, and

other critical activities (e.g., media-fill testing)

- Component selection and handling:
 - Carefully select, and ensure ongoing qualification of suppliers and service providers (e.g., chemical vendors and external testing laboratories)
 - Select ingredients that are the subject of a *USP* or *NF* monograph and that are manufactured at an FDA-registered facility, when available
 - Inspect incoming components against their COAs to ensure that they meet their specifications
 - Quarantine, properly dispose of, and investigate components that do not meet their specifications
- Design and maintenance of the building, facility, and equipment:
 - Review the adequacy of the design of the building, facility, and equipment; when changes are made, assess their effects to make sure they do not adversely affect the operation (e.g., certification)
 - Ensure that facilities and equipment used in compounding are installed, operated, and maintained properly per appropriate and pre-established specifications
 - Detect adverse trends in environmental monitoring data to take preventive action and corrective action
- Compounding process:
 - Approve Master Formulation Records (when used), or any changes to them, before they are implemented
 - Review Compounding Records for accuracy, completeness, and conformance to established specifications
 - Review final labeling against prescription or medication orders
 - Ensure that all errors, process problems, or deviations from procedures are documented
 - Investigate any error, deviation, out-of-specification result, or complaint, and implement, oversee, and document appropriate corrective action to prevent recurrence
- Final CSP release:
 - Assess the final CSP before release (e.g., physical inspection, sterility testing, and analytical testing)
 - Review internal or external testing programs (if used) for conformance with applicable standards (e.g., sterility testing and endotoxin testing)
- Documentation:
 - Establish SOPs and assess conformance to SOPs
 - Establish and assess conformance with document control and records management procedures
 - Establish, maintain, and follow written procedures for handling all written and oral complaints regarding a CSP

The roles and duties of personnel responsible for each aspect of the QA program must be described in the SOPs. Designated personnel responsible for the QA program must have adequate training, experience, responsibility, and authority to perform these duties.

The overall QA program must be assessed annually.

14. CSP STORAGE, HANDLING, PACKAGING, AND TRANSPORT

Appropriate processes or techniques for storing, handling, packaging, and transporting CSPs must be in place and must also be outlined in SOPs. Personnel who will be storing, handling, packaging, and transporting CSPs within the facility must be properly trained in accordance with the relevant SOPs.

14.1 Storing CSPs within the Compounding Facility

To help ensure that CSP quality is retained while the CSP is stored at the compounding facility, compounding personnel must monitor conditions in the drug storage areas. A controlled temperature area must be checked at least once daily to determine whether the temperature remains within the appropriate range, and the results must be documented on a temperature log. If the compounding facility uses a continuous temperature recording device, compounding personnel must verify at least once daily that the recording device is functioning properly. In addition, the compounding facility must adhere to appropriate procedures for all controlled temperature areas to ensure that such spaces are not subject to prolonged temperature fluctuations (e.g., by leaving a refrigerator door open too long).

When it is known that a CSP has been exposed to temperatures that exceed storage temperature limits, (i.e., temperatures warmer than the warmest labeled limit or temperatures exceeding 40° for more than 4 hours), the CSP should be discarded.

14.2 Handling of CSPs

CSPs must be handled properly while in the compounding facility to maintain CSP quality and packaging integrity. For example, techniques should be in place to prevent the depression of syringe plungers or dislodging of syringe tips. Additionally, disconnection of system components (e.g., where CSPs are dispensed with administration sets attached to them) must be prevented throughout the BUD or until administration of the CSP.

14.3 Packaging of CSPs

Compounding personnel must select and use packaging materials that will maintain the physical integrity, sterility, and stability of the CSPs. Packaging materials must protect CSPs from damage, leakage, contamination, degradation, and adsorption, while simultaneously protecting transport personnel from harm. The facility must have written SOPs that describe appropriate shipping containers and insulating and stuffing materials based on the product specifications, information from vendors, and knowledge of the mode of transport. For example, when CSPs are transported within the facility through pneumatic tube systems, foam padding or inserts may be useful for preventing breakage and spills. Compounding personnel must continuously monitor the effectiveness and reliability of the packaging materials.

Alternative modes of transport and/or special packaging may be needed to protect the quality of CSPs. The use of tamper-evident closures and seals on CSP ports can provide an additional measure of security that can help ensure product integrity, regardless of the transport method used. If the CSP is sensitive to light, light-resistant packaging materials must be used. In some cases, the CSP should be packaged in a special container (e.g., a cooler) to protect it from temperature fluctuations.

14.4 Transporting CSPs

Compounding personnel must select modes of transport that are expected to deliver properly

packed CSPs in an undamaged, sterile, and stable condition. Inappropriate handling and transport can adversely affect the quality of CSPs in general, particularly certain CSPs with unique stability concerns. For example, the physical shaking that might occur during pneumatic tube transport, or undue exposure to heat or light, must be considered and addressed on a preparation-specific basis. Compounding personnel must include specific handling instructions on the exteriors of containers that are used to transport CSPs.

14.5 Handling of Hazardous Drugs

For information on the storage, packaging, handling, and transport of hazardous drugs, see (800).

15. COMPLAINT HANDLING AND ADVERSE EVENT REPORTING

Compounding facilities must provide patients and other CSP recipients with a way to submit questions and report any concerns or complaints they may have regarding a CSP. SOPs must be developed, implemented, and followed for the receipt, acknowledgment, and handling of all complaints about the quality and labeling of, or possible adverse reactions to, a specific CSP.

15.1 Complaint Handling

A qualified individual must review all complaints to determine whether the complaint indicates a potential quality problem with the CSP. If so, a thorough investigation into the cause of the problem must be initiated and completed promptly. The investigation must consider whether the quality problem extends to other batches of the same CSP, or to other CSPs that could have been affected. Corrective action, if necessary, must be implemented immediately for all potentially affected CSPs. When warranted, consider whether to initiate a recall of potentially affected CSPs and whether to cease sterile compounding operations until all underlying problems have been identified and corrected.

A written record of each complaint must be kept, regardless of the source (e.g., e-mail, telephone, mail). The record must contain the name of the complainant, the date the complaint was received, the nature of the complaint, and the response to the complaint. In addition, to the extent that the information is known, the following should be recorded: the name and strength of the CSP, the prescription or medication order number, and the lot number, if one is assigned. The record must also include the findings of any investigation and any follow-up. Complaint records must be easily retrievable for review and evaluation for possible trends and must be retained in accordance with the recordkeeping requirements in *16. Documentation*. A CSP that is returned in connection with a complaint must be quarantined until it is destroyed after completion of the investigation and in accordance with applicable federal, state, and local laws and regulations.

15.2 Adverse Event Reporting

Reports of potential adverse events involving a CSP must be reviewed promptly and thoroughly by compounding personnel. The reports must be handled in accordance with the procedures for handling complaints as described in section *15.1 Complaint Handling*, as well as the record retention requirements described in *16. Documentation*. Relevant healthcare professionals and patients must be informed as appropriate. If required, adverse events must be reported in accordance with applicable state and local laws and regulations. In addition, serious or unexpected adverse events associated with a CSP should be reported to the FDA through the

MedWatch program for human drugs and Form FDA 1932a for animal drugs.

16. DOCUMENTATION

All facilities where CSPs are prepared must have and maintain written documentation to demonstrate compliance with this chapter, including all SOPs, Master Formulation Records (when used), Compounding Records, laboratory and equipment records, prescriptions or medication orders, and all information related to complaints.

All records must be legible and stored in a manner that prevents their deterioration and/or loss. Records can be kept electronically. Records must be maintained either at the facility or at another location that is readily accessible within a reasonable period of time.

All records specific to the compounding of a particular CSP (e.g., Master Formulation Record, Compounding Record, and testing results) must be kept for at least 3 years after the BUD of the CSP, or as required by state laws and regulations, whichever is longer. Facility design and initial qualification records must be kept as long as the facility is in operation. All other records must be kept for at least 3 years, or as required by state laws and regulations, whichever is longer. Examples include records related to personnel training and qualification, equipment maintenance and calibration, receipt of components, environmental monitoring, complaints, and quality assurance.

Recordkeeping must also comply with all applicable federal laws and regulations.

17. RADIOPHARMACEUTICALS AS CSPS

Radiopharmaceuticals are associated with risks of radiation exposure to healthcare practitioners and unintentional radiation exposure to patients. Compounding of radiopharmaceuticals must comply with applicable federal, state, and local laws and regulations such as those from the Nuclear Regulatory Commission (NRC), FDA, and State Boards of Pharmacy.

Unless done in strict conformance with the manufacturer's package insert, any further use or handling and manipulation of conventionally manufactured radiopharmaceutical product is considered compounding and must follow the standards in this chapter and applicable federal, state, and local laws and regulations. Radiation exposures must be kept as low as reasonably achievable (the ALARA principle). Therefore, appropriate shielding must be used to help minimize radiation exposure. Additional equipment needed for radiation control when compounding may include, but is not limited to:

- Radiation detectors
- Static and handheld monitors
- Handheld monitors
- Lead (or other appropriate) shielding
- Shielded waste cans
- Non-shedding absorbent mats
- L-Blocks
- Tongs
- Syringe, vial, and elution shields
- Syringe re-cappers
- Final unit dose containment shielding
- Dose calibrators

- Dippers and dipper lifts

Radioisotope generator systems (e.g., Tc-99m/Mo-99, Rb-82/Sr-82, Ga-68/Ge-68) must be stored and eluted (operated) under conditions recommended by the manufacturer and applicable state and federal regulations. The generators must be eluted in an ISO Class 8 or cleaner air environment that allows special generator shielding, airflow requirements, and the use of lifting devices (e.g., cranes and/or wenchers) due to the weight of the generator and shielding. Radioisotope generators producing radioisotopes with a half-life of 15 minutes or less (e.g., Rb-82/Sr-82) can be eluted in accordance with the manufacturer's instructions at the point of care (e.g., at the bedside or in the patient care area). Visual inspection of radiopharmaceutical CSPs containing high concentrations of radioactivity (e.g., for color and absence of particulate material) must be performed in accordance with ALARA principles to limit acute and chronic radiation exposure of the inspecting personnel.

All compounding personnel must be properly gowned and garbed as described in *3. Personal Hygiene and Personal Protective Equipment*. However, personnel compounding radiopharmaceuticals are permitted to use personal radiation dosimeters. Personal radiation dosimeters can be film, thermoluminescent, or electronic. Whole-body badge radiation dosimeters must be worn underneath the gown, whereas ring/wrist badges that measure the dose at the extremities must be worn under gloves. If personnel are going from a cleanroom to a patient care area, all PPE must be changed before leaving one area and entering the other.

When compounding activities require the manipulation of a patient's blood-derived or other biological material (e.g., radiolabeling of white blood cells), the manipulation must be performed in a separate, dedicated ISO Class 7 area that contains a PEC. All blood manipulations in the radiolabeling process, except for the centrifuge steps, must be performed inside the dedicated PEC. Dedicated equipment must be used for all blood manipulations. Strict SOPs must be developed and implemented to minimize the risk of patient-to-patient cross-contamination.

Some radiopharmaceutical preparations (e.g., volatile or gaseous preparations such as iodine or xenon) may require pressurization configurations that are different from those described in *4. Buildings and Facilities*. In these cases, the facility must comply with applicable federal, state, and local laws and regulations.

Nonradioactive compounds may be compounded in the same compounding area in which radioactive compounds have been prepared, provided the following takes place:

- The ISO Class 5 area is decontaminated and monitored for radioactivity above background levels.
- The dose calibrator is left inside the PEC.
- The PEC is operated in accordance with all the standards in this chapter when nonradioactive CSPs are being prepared.

GLOSSARY

Airlock: A space with interlocked doors, constructed to maintain air pressure control when items move between two adjoining areas (generally with different air cleanliness standards). The intent of an airlock is to prevent ingress of particulate matter and microbial contamination from a lesser-controlled area.

Ante-area: An ISO Class 8 or cleaner area where personnel hand hygiene and garbing procedures and other activities that generate high particulate levels are performed. The ante-

area is the transition area between the unclassified area of the facility and the buffer area. [Note—The ante-area is sometimes referred to as an ante-room when solid doors and walls are present.]

Aseptic processing or preparation: A process by which separate, sterile components (e.g., drugs, containers, or closures) are brought together under conditions that maintain their sterility. The components can either be purchased as sterile or, when starting with nonsterile components, can be separately sterilized prior to combining (e.g., by membrane filtration, autoclave).

Batch: More than one unit of CSP prepared in a single process and intended to have uniform characteristics and quality, within specified limits.

Beyond-use date (BUD): The date or time after which a CSP cannot be used and must be discarded. The date or time is determined from the date or time when the preparation was compounded.

Biological safety cabinet (BSC): A ventilated cabinet with unidirectional HEPA-filtered airflow and HEPA-filtered exhaust to protect the worker from hazardous drugs. A BSC used to prepare a CSP must be capable of providing an ISO Class 5 environment for preparation of the CSP.

Buffer area: An ISO Class 7 (or ISO Class 8 if using an isolator) or cleaner area where the PEC that generates and maintains an ISO Class 5 environment is physically located.

Category 1 CSP: A CSP assigned a BUD of 12 hours or less at controlled room temperature or 24 hours or less refrigerated.

Category 2 CSP: A CSP assigned a BUD of greater than 12 hours at controlled room temperature or greater than 24 hours refrigerated that is compounded in accordance with all applicable standards for Category 2 CSPs in this chapter.

Certificate of analysis (COA): A report from the supplier of a component, container, or closure that accompanies the supplier's material and contains the specifications and results of all analyses and a description of the material.

Classified space: A space that maintains an air cleanliness classification based on the International Organization for Standardization (see also definition for ISO Class).

Cleanroom: An ISO-classified room in which the concentration of airborne particles is controlled to meet a specified airborne-particulate cleanliness class to prevent particle and microbial contamination of CSPs.

Compounded sterile preparation (CSP): A preparation intended to be sterile that is created by combining, diluting, pooling, or otherwise altering a drug product or bulk drug substance. A product produced by reconstituting a conventionally manufactured product for an individual patient strictly in accordance with the directions contained in the approved labeling provided by the product manufacturer is not considered a CSP for the purposes of this chapter.

Compounding aseptic containment isolator (CACI): A type of RABS that uses HEPA filtration to provide an ISO Class 5 clean air environment designed for the compounding of sterile hazardous drugs.

Compounding aseptic isolator (CAI): A type of RABS that uses HEPA filtration to provide an ISO Class 5 clean air environment designed for compounding of sterile non-hazardous drugs.

Compounded stock solution: A compounded solution to be used in the preparation of multiple units of a finished CSP.

Container–closure system: The sum of packaging components that together contain and protect the dosage form. This includes primary packaging components and secondary packaging components, if the latter are intended to provide additional protection.

Conventionally manufactured product: A pharmaceutical dosage form, usually the subject of an FDA-approved application, and manufactured under current good manufacturing practice conditions. Conventionally manufactured products are not compounded preparations.

Critical site: A location that includes any component or fluid pathway surfaces (e.g., vial septa, injection ports, and beakers) or openings (e.g., opened ampuls and needle hubs) that are exposed and at risk of direct contact with air (e.g., ambient room or HEPA filtered), moisture (e.g., oral and mucosal secretions), or touch contamination.

Direct compounding area: A critical area within the ISO Class 5 PEC where critical sites are exposed to unidirectional HEPA-filtered air, also known as first air.

Disinfectant: A chemical agent used on inanimate surfaces and objects to destroy fungi, viruses, and bacteria, but not necessarily their spores.

Expiration date: Date placed on a conventionally manufactured product to limit the time during which it can be used.

Filter integrity test: A test (e.g., bubble point test) of the integrity of a sterilizing grade filter performed after the filtration process to detect whether the integrity of the filter has been compromised.

First air: The air exiting the HEPA filter in a unidirectional air stream.

Hazardous drug: Any drug identified by at least one of the following six criteria: carcinogenicity, teratogenicity or developmental toxicity, reproductive toxicity in humans, organ toxicity at low dose in humans or animals, genotoxicity, or new drugs that mimic existing hazardous drugs in structure or toxicity.

In-use time: The time before which a conventionally manufactured product or a CSP must be used after it has been opened or needle punctured (e.g., after a container closure of a vial has been penetrated).

ISO Class: An air quality classification from the International Organization for Standardization.

Isolator: An enclosure that provides HEPA-filtered ISO Class 5 unidirectional air operated at a continuously higher pressure than its surrounding environment and is decontaminated using an automated system. It uses only decontaminated interfaces or rapid transfer ports for materials transfer.

Label: A display of written, printed, or graphic matter on the immediate container of any article.

Labeling: All labels and other written, printed, or graphic matter that are 1) on any article or any of its containers or wrappers, or 2) accompanying such an article.

Laminar airflow system (LAFS): A device or zone within a buffer area that provides an ISO Class 5 or better environment for sterile compounding. The system provides a unidirectional

HEPA-filtered airflow.

Laminar airflow workbench (LAFW): A device that is a type of LAFS that provides an ISO Class 5 or better environment for sterile compounding. The device provides a unidirectional HEPA-filtered airflow.

Media fill test: A simulation used to qualify processes and personnel engaged in sterile compounding to ensure that the processes and personnel are able to produce sterile CSPs without microbial contamination.

Microbial contamination: The presence of microorganisms in, or on, an item.

Multiple-dose container: A container of sterile medication for parenteral administration (e.g., injection or infusion) that is designed to contain more than one dose of the medication. A multiple-dose container is usually required to meet the antimicrobial effectiveness testing criteria. See *Container Content for Injections (697)*, *Determination of Volume of Injection in Containers*, *Multi-Dose Containers*.

Pass-through: An enclosure with seals on interlocking doors that are positioned between two spaces for the purpose of minimizing particulate transfer while moving materials from one space to another.

Pharmacy bulk package: A conventionally manufactured sterile product for parenteral use that contains many single doses intended for use in a pharmacy admixture program. A pharmacy bulk package may either be used to prepare admixtures for infusion or, through a sterile transfer device, for the filling of empty sterile syringes.

Positive-pressure room: A room that is maintained at higher pressure than the adjacent spaces, and therefore the net airflow is out of the room.

Primary engineering control (PEC): A device or zone that provides an ISO Class 5 environment for sterile compounding.

Preservative: A substance added to inhibit microbial growth or to prevent decomposition or undesirable chemical changes.

Pyrogen: A substance that induces a febrile reaction in a patient.

Pyrogen-free: A substance lacking sufficient endotoxins or other fever-inducing contamination to induce a febrile or pyrogenic response.

Quality assurance (QA): A system of procedures, activities, and oversight that ensures that operational and quality standards are consistently met.

Quality control (QC): The sampling, testing, and documentation of results that, taken together, ensure that specifications have been met before release of the preparation.

Reconstitution: The process of adding a diluent to a powdered medication to prepare a sterile solution or suspension.

Release testing: Testing performed to ensure that a preparation meets appropriate quality characteristics.

Repackaging: The act of removing a conventionally manufactured sterile product from its original primary container and placing it into another primary container, usually of smaller size.

Responsible person: The individual accountable for an activity.

Restricted access barrier system (RABS): An enclosure that provides HEPA-filtered ISO Class 5 unidirectional air that allows for the ingress and/or egress of materials through defined openings that have been designed and validated to preclude the transfer of contamination, and that generally are not to be opened during operations. Examples of RABS include CAIs and CACIs.

Segregated compounding area: A designated, unclassified space, area, or room that contains a PEC and is suitable for preparation of Category 1 CSPs only.

Single-dose containers: A container of sterile medication for parenteral administration (e.g., injection or infusion) that is designed for use with a single patient as a single injection/infusion. A single-dose container usually does not contain a preservative.

Specification: The tests, analytical methods, and acceptance criteria to which a drug substance, drug product, CSP, component, container-closure system, equipment, or other material used in drug preparation must conform to be considered acceptable for its intended use.

Stability: The extent to which a CSP retains physical and chemical properties and characteristics within specified limits throughout its BUD.

Sterility: The freedom from viable microorganisms.

Sterility testing: A documented and established laboratory procedure for detecting viable microbial contamination in a sample or preparation.

Sterilizing-grade membranes: Filter membranes that are documented to retain 100% of a culture of 10^7 microorganisms of a strain of *Brevundimonas diminuta* per cm^2 of membrane surface under a pressure of not less than 30 psi. Such filter membranes are nominally 0.22- μm or 0.2- μm pore size.

Sterilization by filtration: Passage of a gas or liquid through a sterilizing-grade membrane to consistently yield filtrates that are sterile.

Terminal sterilization: The application of a lethal process (e.g., dry heat, steam, irradiation) to sealed containers for the purpose of achieving a predetermined SAL of greater than 10^{-6} or a probability of less than one in one million of a nonsterile unit.

Unclassified space: A space not required to meet any air cleanliness classification based on the International Organization for Standardization (ISO).

Unidirectional airflow: Air within a PEC moving in a single direction in a uniform manner and at sufficient speed to reproducibly sweep particles away from the direct compounding area or testing area.

Verification: Confirmation that a method, process, or system will perform as expected under the conditions of actual use.

APPENDICES

Appendix 1. Acronyms

ACD	Automated compounding device
ACPH	Air changes per hour
ALARA	As low as reasonably achievable
API	Active pharmaceutical ingredient
BSC	Biological safety cabinet
BUD	Beyond-use date
CACI	Compounding aseptic containment isolator
CAI	Compounding aseptic isolator
CFU	Colony-forming units
COA	Certificate of analysis
CSP	Compounded sterile preparation
ECV	Endotoxin challenge vial
FDA	Food and Drug Administration
HEPA	High-efficiency particulate air
HVAC	Heating, ventilation, and air conditioning
IPA	Isopropyl alcohol
ISO	International Organization for Standardization
LAFS	Laminar airflow system
LAFW	Laminar airflow workbench
NRC	Nuclear Regulatory Commission
PEC	Primary engineering control
PPE	Personal protective equipment
QA	Quality assurance
QC	Quality control
RABS	Restricted access barrier system
SAL	Sterility assurance level
SOP	Standard operating procedure
TSA	Trypticase soy agar

Appendix 2. Common Disinfectants Used in Health Care for Inanimate Surfaces and Noncritical Devices, and Their Microbial Activity and Properties^a

Chemical Category of Disinfectant							
		Isopropyl Alcohol	Accelerated Hydrogen Peroxide	Quaternary Ammonium (e.g., dodecyl dimethyl ammonium chloride)	Phenolics	Chlorine (e.g., sodium hypochlorite)	Iodophors (e.g., povidone-iodine)
Concentration Used		60%–95%	0.5% ^b	0.4%–1.6% aq	0.4%–1.6% aq	100–5000 ppm	30–50 ppm
Microbial Inactivation ^c	Bacteria	+	+	+	+	+	+
	Lipophilic viruses	+	+	+	+	+	+
	Hydrophilic viruses	±	+	±	±	+	±
	M. tuberculosis	+	+	±	+	+	±
	Mycotic agents (fungi)	+	+	+	+	+	±
	Bacterial spores	–	–	–	–	+	–
Important Chemical and Physical Properties	Shelf life >1 week	+	+	+	+	+	+
	Corrosive or deleterious effects	±	–	–	–	±	±
	Non-evaporable residue	–	–	+	+	–	+
	Inactivated by organic matter	+	±	+	±	+	+
	Skin irritant	±	–	+	+	+	±
	Eye irritant	+	–	+	+	+	+
	Respiratory irritant	–	–	–	–	+	–
	Systemic toxicity	+	–	+	+	+	+
Key to abbreviation and symbols: aq = diluted with water; ppm = parts per million; + = yes; – = no; ± = variable results.							
<p>^a Modified from World Health Organization, Laboratory Bio Safety Manual 1983 and Rutala WA. Antisepsis, disinfection and sterilization in the hospital and related institutions. <i>Man Clin Microbiol.</i> Washington D.C.: American Society for Microbiology;1995, 227–245.</p> <p>^b Accelerated hydrogen peroxide is a new generation of hydrogen peroxide-based germicides in which the potency and performance of the active ingredient have been enhanced and accelerated through the use of appropriate acids and detergents.</p> <p>^c Inactivation of the most common microorganisms (i.e., bacteria) occurs with a contact time of ≤1 minute; inactivation of spores requires longer contact times (e.g., 5–10 minutes for 5,000 ppm chlorine solution against <i>C. difficile</i> spores). Perez J, Springthorpe VS, Sattar SA. Activity of selected oxidizing microbicides against the spores of <i>Clostridium difficile</i>: relevance to environmental control. <i>Am J Infect Con.</i> 2005;33(6):320–325.</p>							

▲USP40

[±] U.S. Food and Drug Administration, Guidance for Industry, *Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice*, September 2004.

² Guidelines for Environmental Infection Control in Health Care Facilities, Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC), MMWR, vol. 52, no. RR-10, June 6, 2003, figure 3, pg. 12.

³ NSF/ANSI 49.

⁴ ~~ISO 14644 4:2001 Cleanrooms and associated controlled environments—Design, construction, and start up, Case Postale 56, CH 1211 Geneve 20, Switzerland, tel. +41 22 749 01 11.~~

⁵ ~~By definition (IEST RP CC 001.4), HEPA filters are a minimum of 99.97% efficient when tested using 0.3 µm thermally generated particles and a photometer or rated at their most penetrating particle size using a particle counter.~~

⁶ ~~Sample procedures are detailed in CETA Applications Guide CAG 002 2006 section 2.09.~~

⁷ ~~Controlled Environment Testing Association, 1500 Sunday Drive, Ste. 102, Raleigh, NC 27607; www.CETAinternational.org.~~

⁸ ~~Agalloco J, Akers JE. Aseptic Processing: A Vision of the Future. *Pharmaceutical Technology*, 2005. Aseptic Processing supplement, s16.~~

⁹ ~~Eaton T. Microbial Risk Assessment for Aseptically Prepared Products. *Am Pharm Rev.* 2005; 8 (5, Sep/Oct): 46–51.~~

¹⁰ ~~Guideline for Hand Hygiene in Health-care Settings, MMWR, October 25, 2002, vol. 51, No. RR 16 available on the Internet at <http://www.cdc.gov/handhygiene/>.~~

¹¹ ~~The use of additional resources, such as the Accreditation Manual for Home Care from the Joint Commission on Accreditation of Healthcare Organizations, may prove helpful in the development of a QA plan.~~

¹² ~~See *American Society of Heating, Refrigerating and Air Conditioning Engineers, Inc. (ASHRAE), Laboratory Design Guide*.~~

¹³ ~~CETA Applications Guide for the Use of Compounding Isolators in Compounding Sterile Preparations in Healthcare Facilities, CAG 001 2005, Controlled Environment Testing Association (CETA), November 8, 2005.~~

▲¹ Centers for Disease Control and Prevention and the Safe Injection Practices Coalition, One & Only Campaign, http://www.oneandonlycampaign.org/safe_injection_practices. ▲*USP40*

▲² Agalloco J, Akers JE. Aseptic processing: a vision of the future. *Pharm Technol.* 2005; Aseptic Processing supplement, s16.

³ Eaton T. Microbial risk assessment for aseptically prepared products. *Am Pharm Rev.* 2005;8(5):46–51. ▲*USP40*

BRIEFING

(1039) **Chemometrics.** The purpose of this new general chapter is to summarize and incorporate, into one document, the concepts and practices of chemometrics, which are emerging in the pharmaceutical industry and the relevant fields. The concepts of chemometrics span the areas of chemical analysis and physical analysis of drugs, excipients, dietary supplements, and food ingredients. This chapter is an above-1000 guidance that describes the best practices for incorporation of chemometric models into the lifecycle of the analytical procedure. Key concepts such as method development, validation, and maintenance of these models are described, and an overview of relevant tools and applications is provided.

(GCCA: H. Pappa.)

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Comment deadline: January 31, 2016

Add the following:

▲ (1039) **CHEMOMETRICS**

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1. INTRODUCTION

1.1 Scope and Purpose

This chapter provides guidance regarding scientifically sound practices for the chemometric analysis and interpretation of typical multivariate data for compendial and industrial applications. Established chemometric practices, including calibration and validation, and applications within different analytical technologies (e.g., spectroscopic, chromatographic, and others) and for different purposes (e.g., fingerprinting, identification, classification, properties prediction, and others) are discussed in some detail and under a lifecycle approach. Both qualitative and quantitative applications are described.

The chapter discusses how method quality and performance are ensured through the proper lifecycle management of a chemometrics-based model, including the selection of appropriate algorithms, calibration, validation, verification, transfer, and ongoing maintenance steps. This chapter may be viewed as a supplement to other guidance chapters such as *Analytical Data—Interpretation and Treatment* (1010), which are mainly concerned with the analysis and interpretation of univariate data.

Note—It should not be inferred that the multivariate analysis tools mentioned in this chapter form an exhaustive list. Other equally valid models may be used at the discretion of the manufacturer and other users of this chapter.

1.2 Content Summary of Document

The mind map below (*Figure 1*) provides a visual representation of the content of this chapter. This diagram is meant to assist the reader by showing how the various concepts and practices of chemometrics relate to each other.

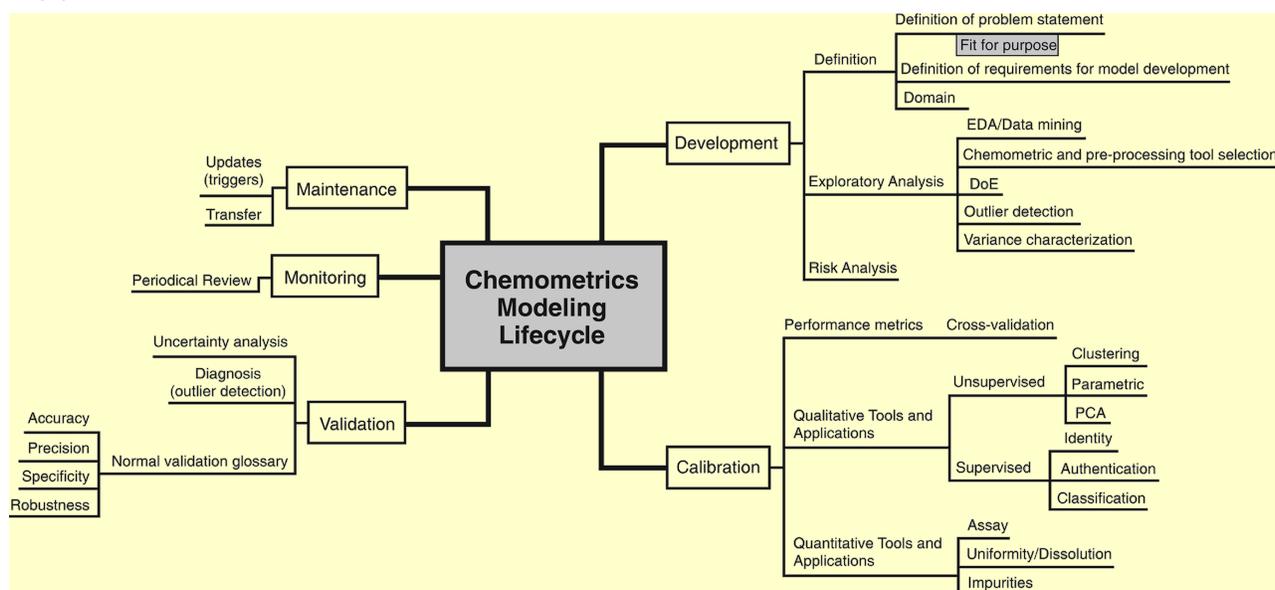


Figure 1. Content summary of document.

1.3 Audience

This chapter provides direction for both the chemometrician applying chemometric techniques to develop a model for a given application and the analyst that runs the model within an analytical procedure. The chemometrician will find support regarding algorithm selection for a given application and guidance for ensuring model performance throughout its lifecycle. The analyst will gain insight regarding the strengths and limitations of the chemometrics techniques as applied to support their applications.

2. WHAT IS CHEMOMETRICS?

Chemometrics was originally defined as the chemical discipline that uses mathematical, statistical, and other methods that employ formal logic to accomplish two objectives: 1) to design or select optimal measurement procedures and experiments, and 2) to provide the maximum amount of relevant chemical information by analyzing chemical data (1,2). More specifically, "chemometrics" has come to mean the application of multivariate methods for the analysis of chemical or related data, although the algorithms in question may be used to extract information out of almost any measured data, regardless of origin—chemical, physical, biological, pharmaceutical, or others. This chapter does not focus on the development of optimal procedures or methods and the applied design of experiments (DoE), but rather on the analysis of chemical sensor-based multidimensional data, such as spectroscopic and chromatographic data. A multivariate data set (i.e., multivariate data obtained for a number of samples or objects) forms an m by n data table or matrix. The matrix is represented by \mathbf{X} , with m as the number of samples or objects and n as the number of variables measured for each sample (Figure 2).

Consequently, the data analysis techniques considered in this chapter will be multivariate in nature. Depending on the purpose of the data treatment, different tools will be applied. Initially, the data handling techniques can be divided into two categories: unsupervised and supervised. The unsupervised tools use only the \mathbf{X} matrix to extract information, but in supervised data analysis, in addition to the \mathbf{X} matrix the samples are also described by a \mathbf{y} vector. This is an m

by 1 table containing property information for each sample (e.g., concentration, enzyme inhibition activity). The supervised data analysis techniques are used to build a model between the \mathbf{X} matrix and the \mathbf{y} vector. In chemometric modeling, the equations provided are data driven to empirically describe the underlying variance in the data for either unsupervised (i.e., qualitative) or supervised (i.e., quantitative) purposes. The different techniques or tools applied for the different purposes are discussed in more detail in 4. *Applications of Chemometrics*. The most commonly used unsupervised technique (principal component analysis, or PCA) and supervised technique (partial least squares regression, or PLS) are by nature projection approaches (see Figure 2), which transform a large number of potentially correlated \mathbf{X} variables, such as intensities at different retention times or wavelengths, into a possibly smaller number of uncorrelated variables (principal components, or PCs; latent variables). As can be seen in Figure 2, the original n -dimensional space was transformed to a two-PC space. When samples from the original data space are projected onto this lower-dimensionality space, the resulting sample coordinates are called scores (\mathbf{T}). Visualization of the scores along two or more PCs forms a score plot that contains information on the relationships among different samples. The loadings (\mathbf{P}) are a linear combination of the original variables and the coefficients or weights used in this linear combination. The loadings for specific latent variables also can be plotted in what is called a loading plot. The loading plot contains information on the relative importance among the original variables. Both scores and loading plots enable visualization and understanding of the underlying data structure (i.e., the presence of groups/clusters and/or outliers) within a reduced dimensional space. The variable matrix (\mathbf{E}) produced by the model is defined as the residual error. The sample information along axes of common variance is captured by the model's PCs. Variance unaccounted for by these PCs (residual error) is left for each sample at each variable, forming the residual matrix (\mathbf{E}).

$$\mathbf{X} = \mathbf{T}\mathbf{P}^T + \mathbf{E}$$

Data decomposition by projection:

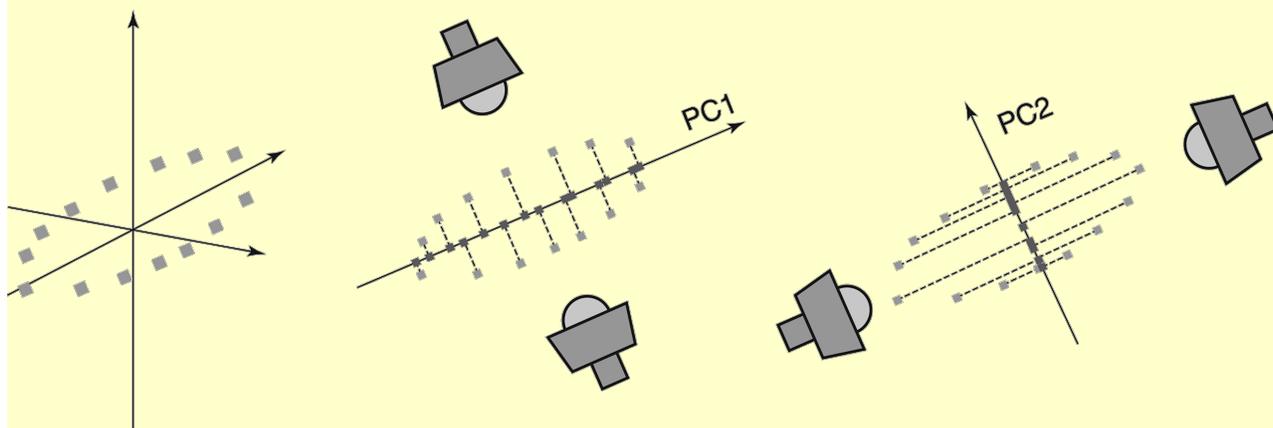


Figure 2. Schematic representation of chemometrics.

3. MODEL LIFECYCLE

The development of a chemometric model, as part of an analytical procedure, aims to fulfill a predefined, intended purpose. The intended purpose is typically a statement describing what to measure, the output format, and the level of performance needed with the result, and should be in accordance with the analytical target profile (ATP). The ATP may specify performance criteria for key characteristics of the analytical procedure output and/or decision risk probabilities expected in routine use of the analytical procedure.

Calibration of the model encompasses an iterative process that involves selection of the sample set to be used to develop the chemometric model, tuning of an appropriate chemometric algorithm with the necessary preprocessing algorithm, and evaluation of model performance according to predefined metrics for the ATP. During the validation stage, the method performance is demonstrated to fulfill the intended purpose and ATP. Both the knowledge gained during calibration and the assessment of specific metrics and corresponding limits are used to evaluate performance and define a method maintenance protocol that will be used for the monitoring stage, before deployment to routine use. Changes that may have an impact on model performance should trigger a defined set of activities to update it. Model update may also be triggered by the necessity of performing model transfer, in the course of its lifecycle, to different equipment or another site. The extent of the revalidation will be defined by the magnitude of the model update. *Figure 3* shows a schematic representation of the described workflow.

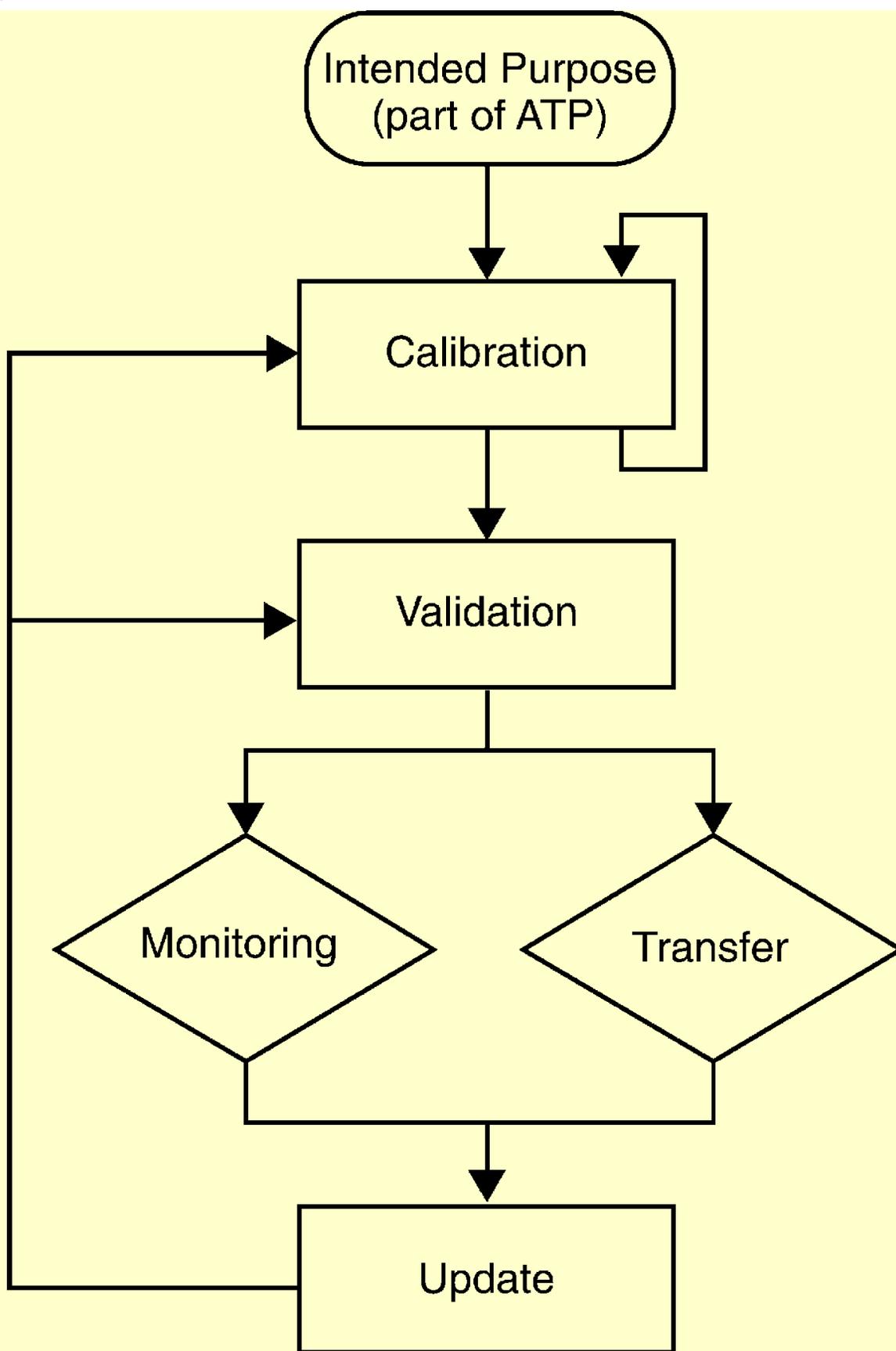


Figure 3. Schematic representation of the lifecycle workflow of a chemometric model.

3.1 Model Development: Calibration

The goal of multivariate calibration is to develop a statistical model that links predictor variables (e.g., absorbance values for the range of spectral wavelengths) with response variables such as concentrations of certain chemical compounds. Response variables are typically more difficult or costly to obtain than predictor variables. Calibration is a critical step because details of the final model are influenced by many factors including the selection of samples, variables, an algorithm, and preprocessing options, as well as the quality of the reference method initially used to measure the response variable(s). Thus, the order of individual components of model calibration presented in this section does not always represent the order of operation, and individual steps might be repeated in an iterative fashion depending upon the outcome of subsequent steps. To perform well, the calibration model should be robust to intended variations.

SAMPLE SELECTION

Selection of samples for the calibration model is a critical step in model development. Scientifically sound rationales must be used to select representative calibration samples with respect to two criteria: the type/range of variability, and the number of samples. The range of response values in selected samples should cover the entire expected range of response variation for the intended application. The range and type of variation in predictors may include relevant variability in factors other than the property being predicted, such as particle size, different lots of ingredients used for sample preparation, analyst-to-analyst and day-to-day variation, and other sources of variation. In the manufacturing setting, batches of samples representing within and outside specification should be included. A risk-based approach is recommended for deciding which factors and variability will be included in the calibration samples. Performing a failure mode and effect analysis (FMEA) or a feasibility study to evaluate the effects of such factors is one of the commonly used approaches.

The required number of samples increases as the complexity of the calibration model (i.e., the type and the range of variability in both response and predictor variables) increases. In general, the larger the number of samples, the higher the probability that correct results can be achieved given a uniform distribution of the samples throughout the range of calibration. However, obtaining new samples can be costly. The DoE and/or historical database approaches are commonly used to build calibration models.

Data obtained from selected samples should undergo further triage. Exploratory data analysis (see *Qualitative Application Examples*) may be valuable for understanding data structure, detecting potential clusters or groups of samples, identifying outliers, and selecting representative samples. Outliers may be detected using metrics that describe how well a given observation fits within model space (e.g., Hotelling's T^2 for latent variable methods) and how far it is from the model hyperplane (e.g., residual for latent variable methods).

PREPROCESSING

The goal of preprocessing is to amplify variation of interest within the data set and attenuate extraneous variation for both variables and observations. Preprocessing is an important initial step for most data analyses, and can be applied iteratively with variable selection. The initial selection of preprocessing options should be guided by an understanding of the sample data and/or the underlying analytical technique. Guidance may also be obtained through exploratory multivariate data analyses (see *Qualitative Application Examples*). Initial selections can be

modified or refined within the context of subsequent model performance optimization. This process is almost always cyclic in practice; comparison across different preprocessing strategies leads to a better understanding of the data, which can further refine the preprocessing to maximize the signal-to-noise ratio.

However, there are two points of caution. Preprocessing should be used judiciously because: 1) overprocessing can attenuate the signal and inflate undesirable variations; and 2) many chemometric methods can accommodate noisy and visually unappealing data. Furthermore, in some instances the preprocessing has been accomplished on the instrument; many algorithms used by instrument vendors are proprietary, and it may be impossible to know exactly what modifications have been made to the data. The chemometrician must be aware of, and cautious about, any preprocessing already applied in the reported raw data.

Preprocessing may consist of transformation, normalization, and/or other mathematical treatment of data. Preprocessing of samples (rows of data matrix) may include mean or median centering, scaling, and other procedures. Variables (columns of data matrix) can also be transformed, aligned (e.g., time warping), or scaled (e.g., autoscaling). Mean centering is probably the most common preprocessing method. For example, the use of mean-centered data in the regression model removes the intercept term, resulting in a parsimonious model. It is also common to remove the linear baseline (e.g., bias/offset correction) or polynomial interferences or to apply digital filtering techniques such as derivatives, wavelets, Fourier filter, or a Savitzky-Golay filter to remove or attenuate linear offsets and noise contribution. Normalization is the scaling of observations, giving objects similar impact on model creation by: 1) accounting for factors other than the analyte of interest that impact measurement (e.g., sample path length variability caused by particle size, sample volume, or tablet thickness); 2) correcting for variation in concentration or mass of an analyte for qualitative models; and 3) attenuating measurement artifacts (e.g., instrument performance drift). When variables are highly correlated, such as in spectroscopic measurements, normalization is typically performed via standard normal variate (SNV) and multiplicative scatter correction (MSC). These normalizations are typically conducted on each sample (row).

In cases where variables have different units, sensitivities, or signal-to-noise ratios, variable scaling is typically applied to correct for that; autoscaling is typically used, but the analyst may consider other alternatives, such as using the pooled standard deviations of measurement replicates as scaling/weight parameters. These normalizations are typically conducted on each variable (column). No guidelines exist for deciding which preprocessing approach is the best for a given data set. It is often a trial-and-error process as the analyst applies different preprocessing techniques to find the best one. Combinations of preprocessing techniques can be applied to a given data set as well.

VARIABLE SELECTION

The intended purpose of variable selection is to identify a subset of variables that can improve the performance of the model. The underlying rationale for variable selection in chemometrics is twofold. First, certain predictor variables could be irrelevant to the intended purpose, and ideally, these variables should be minimized or removed from the model. For example, only specific wavelengths of the whole range generated by the spectrophotometer may bear information relevant to the variation in levels of the response variable. Variable selection for this purpose should be based on first principles and experience. Inclusion of unrelated predictors, such as irrelevant spectral regions, could potentially degrade performance of the model. A smaller number of variables from the preprocessed data can be used to achieve

superior performance.

The second part of the rationale for variable selection is to avoid overfitting. Overfitting is the situation where the model is not only describing the intended variability in the data but also includes the modeling of the noise. The latter has a negative influence on the predictive properties of the model. Typically in chemometrics, the number of predictor variables (hundreds or thousands) is comparable to or larger than the number of observations (dozens to hundreds). There are two general strategies for handling the issue of overfitting. One strategy is to manually or computationally select only a subset of predictor variables and use them for model development. Predictor variables can be selected manually (e.g., choosing certain spectral wavelengths characteristic of a given compound) or by using a variety of statistical selection methods such as stepwise regression and using simple univariate statistical measures such as the *F*-test, *t*-test, correlation coefficient, signal-to-noise ratios, intensity thresholds, variable importance in projection metric, or genetic algorithms. Multivariate approaches may use PC variable loadings, linear discriminants, or regression coefficients to define the key features. In the second strategy for avoiding overfitting, variables are not selected at all. The latent variables methods—PLS and principal components regression (PCR)—avoid overfitting by generating a smaller number of new variables. These new latent variables, or components, are constructed as linear combinations of the observed variables. If combined with variable selection, the performance of latent variable models could potentially be improved, because some measurement channels contain only noise, thus perturbing the model-building process. Selecting a subset of the measurement channels that are informative will reduce the size of the data set, provide computational efficiency, and obtain better models for postprocessing. One caveat for applications such as authentication is that eliminating measurement channels that are devoid of information may prevent the recognition of adulterants or novel objects that have features in these excluded measurement channels.

ALGORITHM SELECTION

Many different algorithms have been tried with varying degrees of success in chemometrics applications. The methods include relatively simple ones such as multivariate linear regression and its modifications (robust or weighted regression); the widely used latent variables approaches such as PCR and PLS; local methods such as *k*-nearest neighbors (kNN); and the more sophisticated methods such as support vector machines (SVM) and artificial neural networks. More details on commonly used algorithmic tools can be found in 4. *Applications of Chemometrics*. In general, it is difficult to predict which algorithm will produce the best results for a particular data set. The multitude of choices for sample selection, variable selection, and data normalization and preprocessing—as well as the combination of tuning parameters for each algorithm—could significantly affect the performance of the calibration model. The choice of algorithm may depend on the task at hand, software availability, and the subject matter expert's familiarity with the method. Another consideration is that some algorithms provide useful tools for diagnostics and interpretation—such as PLS scores, loadings, and coefficients plots, whereas others are sometimes referred to as “black boxes” because their inner workings can be difficult to interpret (e.g., neural networks).

It is important to keep in mind that many algorithms are empirical in nature. Almost always, some kind of model can be developed. Thus, the results should be evaluated critically to ensure that results generated by the model are relevant and correct, and that model performance meets the requirements of the ATP. This can be accomplished by cross-validation, and ultimately, by validation with the independent test data set that was not used to develop the

model.

CROSS-VALIDATION

In practice, cross-validation is used to obtain an estimate of the model performance and to fine-tune an algorithm's parameters (e.g., the number of components for PLS). This is accomplished by repetitive splitting of the data into a calibration set, which is used to develop the model, and a testing set (or internal validation set), which is used to make predictions and to compare actual and predicted values.

There are several options for performing cross-validation. The n -fold cross-validation is used to split the data into n subsets. In each of the n iterations, $n-1$ subsets are used to develop the model, which is used to predict the remaining n^{th} data split. The procedure is repeated until all subsets are predicted. The error between reference values and predicted values during cross-validation is then recorded as root-mean-squared error of cross-validation (RMSECV). Multiple approaches exist for splitting the data into n segments. The most straightforward form is called leave-one-out cross-validation (LOOCV), where samples are removed one at a time. The LOOCV can involve intensive computations; its results can be heavily affected by outliers and are less consistent than the results of other forms of cross-validation. It is also possible to split the data according to prior information, such as one subset per batch given available historical data set across multiple batches. Another option for cross-validation is bootstrapping, where in each iteration a certain proportion of data is randomly sampled to create a calibration set, while the remainder of the data are used as a testing set. The procedure is repeated many times (typically 100 or more cycles) to obtain a stable estimate of prediction error.

The measure of error is the most common figure of merit used to characterize model performance during cross-validation. The intended purpose of the method determines the nature of the errors, such as the misclassification rate for qualitative methods and prediction error for quantitative methods. Irrespective of qualitative or quantitative applications, two metrics are commonly used to characterize error within cross-validation: root-mean-squared error of calibration (RMSEC) and RMSECV. The RMSEC is calculated for the samples when left in the regression, which monotonically decreases with each additional factor (i.e., PC) incorporated into the model. In comparison, the RMSECV that is calculated during cross-validation will decrease until the last meaningful (i.e., relevant signal-containing) factor is incorporated. Then, as each additional factor is incorporated into the model, the RMSECV will increase, indicating that the calibration data are being overfit. The plot of the RMSEC and/or the RMSECV versus the number of factors in the model is referred to as a predicted residual error sum-of-squares plot. In general, the best practice is to avoid inclusion of factors beyond where the minimum of the RMSECV plot line occurs. In addition, correlation between observed and predicted values (e.g., R^2) is also commonly used to assess performance of quantitative methods. Finally, the cross-validating results are only meaningful when the calibration and testing sets are comparable (i.e., drawn from the same population). Otherwise, extrapolation may lead to incorrect predictions.

3.2 Method Validation

The objective of validation is to demonstrate that the performance of a method is suitable for its intended purpose. Validation of a model and validation of a method are two different activities. Model validation routinely involves the use of an internal validation set or cross-validation to assess the appropriate parameters of a model via identification or quantification

error and uncertainty. Parameters often include the range of variables, the type of preprocessing, the model rank, the choice of the algorithm, and others. These activities have been addressed in detail in *3.1 Model Development: Calibration*. In comparison, method validation must be based upon a fully independent external validation set and must follow the validation requirement described in *Validation of Compendial Procedures* (1225), according to the method type category. The acceptance criteria should be justified for the intended purpose. During the lifecycle, method revalidation is necessary after a model transfer or model update. The method validation and revalidation strategy should be risk- and science-based and appropriate to its impact level.

The typical performance characteristics for method validation are specificity, accuracy, precision, linearity, range, and robustness for quantitative models, and specificity and robustness for qualitative models. The metrics and their descriptions are discussed below. In addition to those typical performance metrics, metrics such as limit of detection (LOD), limit of quantitation (LOQ), sensitivity, analytical sensitivity, and effective resolution may not be required for validation purposes but could be useful for understanding the boundary of the method performance for a specific analytical application and the analytical technique that a model is associated with.

PERFORMANCE CHARACTERISTICS FOR CHEMOMETRICS METHOD VALIDATION

The sections that follow provide chemometrics method validation attribute descriptions and metrics.

Accuracy: Statistical comparison between predicted and reference values is recommended. For quantitative applications, root mean-squared error of prediction (RMSEP), squared error of prediction (SEP), and bias are the typical measures of method accuracy. For qualitative applications such as classification, sometimes misclassification rate or positive prediction rate could be used to characterize method accuracy.

Precision: The routine metrics of RMSEP and SEP encompass both accuracy and precision. Assessment of precision could involve a determination of the uncertainty associated with the reportable result and a variance component analysis that determines an "error budget" that quantifies the sources of variation that contribute to the uncertainty. For an estimate of precision alone, the standard deviation across results for replicate analyses of the same sample within method, across days, across analysts, across instruments, and across laboratories could be used.

Specificity: For both qualitative and quantitative methods, whenever possible the underlying chemical or physical meaning of the chemometric model should be demonstrated and validated. For example, the scientific meaning of the variables (such as spectral range) used for model construction, data preprocessing, regression vectors, and loading vectors [(for PLS, PCR, PLS discriminant analysis (PLS-DA), and PCA] should be demonstrated. Specificity could also be validated by the tendency of sample components (matrix or other nonanalyte compounds present in the sample) to adversely affect the ability of the chemometric method to report results accurately and/or precisely. The level of specificity may be assessed by accuracy and precision in the presence of varying amounts of potentially interfering substances. Substances to be tested can be identified by considering the underlying physical/chemical methodology and modeling approach.

For qualitative methods, the method should demonstrate the capability to correctly identify or classify the samples. The receiver operating characteristic (ROC) curve and/or the probability

of identification (POI) are commonly used metrics. The ROC approach is intended to illustrate true-positive rate (TPR) and false-positive rate (FPR) over a range of decision thresholds. A good identification method should generate a ROC curve with the area under the curve (AUC) close to 1. For most well-designed identification methods, the probability of a positive identification is near zero when the analyte is not present, and the probability should approach 1 as the analyte concentration or mass increases.

Linearity: The algorithm used for chemometric method construction can be linear or nonlinear, as long as it is appropriate for modeling the relationship between the analytical signal and the analyte. The measures commonly used to assess either the model fit or its predictive properties are the correlation coefficient, y -intercept, and residual sum of squares of the plot between the predicted versus observed results. Note that the plot between the residual versus observed results across the analytical range is expected to show no pattern.

Range: The range of a method should be appropriate for its intended use (e.g., specification).

Robustness: Typical factors to be considered include the normal variability of materials (e.g., lot-to-lot variability); operating environment variability; and instrument variability, such as minor instrument maintenance. In conjunction with the validation results, the method development strategy—such as the design of the calibration set or library and the choice of model parameters—can be taken into account to demonstrate the method robustness. Other general aspects to be considered for chemometric method validation include the validation samples. The validation samples should be independent of the calibration samples to demonstrate the ability of the model to predict. Being independent means that the validation samples were not used in the calibration set or used for model optimization. Internal validation or cross-validation is typically used during calibration for model parameter optimization, but is not considered sufficient for final method validation. The validation samples should be selected based upon the ATP and the desired performance characteristics of the model. Method robustness is based on the evaluation of authentic samples with typical sources of variance. For pharmaceutical or dietary supplement products, validation samples of nominal production scale, such as those routinely manufactured for in-process, release, and/or stability testing, may be included. For botanical articles, taxonomic identity, geographic origin, season of collection, and other variants may be included. For naturally sourced materials such as many food ingredients and excipients, variables such as the geographic or microbiological source of the material, processing conditions, impurity composition, and other relevant attributes may be included in the validation sample set.

Specificity may be evaluated by including adulterated, substandard, or nonauthentic samples. Authentic samples, both within and out of the target criteria, may not be available for practical or economic reasons. For example, it may not be possible, due to cost, to obtain out-of-target samples for a controlled, large-scale process. Where possible, an exclusion panel of out-of-target samples that may be closely related to the target samples should be considered to increase the confidence in the specificity of the measurement. Simulated off-target samples (such as small-scale samples) may be used to validate the procedure's suitability for the intended purpose and range. In all cases, the suitability of validation samples must be justified with appropriate inclusion and exclusion criteria.

As with any other analytical procedure, the acceptance criteria for a chemometric method should be defined before execution of the validation protocol. If the chemometric model was developed using data from a secondary technique [e.g., near-infrared (NIR) or Raman] with reference values from a primary analytical procedure [e.g., nuclear magnetic resonance (NMR)

or high-performance liquid chromatography (HPLC)], the ATP for validation may not exceed that which is obtainable by the primary method. Some exceptions may occur, for example, it may be possible to achieve superior precision using a secondary procedure, although the accuracy will be limited to that of the reference technique.

In addition to those attributes addressed in *Performance Characteristics for Chemometrics Method Validation*, chemometrics method validation must also take into consideration the setting of diagnostics limits for a multivariate model before deployment for routine use. Samples that are out of model space are considered outliers and not suitable for obtaining a reportable model prediction. The model diagnostic should have a demonstrated capability to flag any of the out-of-model-space samples. To set up the diagnostics limits, two cases must be considered. The first is to determine the statistical distribution of leverage and residual within-the-calibration data set. The second is to prepare intended in- and out-of-model-space validation samples to test the limit. For instance, for an NIR spectroscopy-based content uniformity method, the in- versus out-of-model-space samples could be samples at target label claim versus samples containing active pharmaceutical ingredient (API) concentrations outside of the intended range for the method.

3.3 Model Monitoring

Throughout the model lifecycle, changes that can affect model performance may occur. Procedures should be in place for continuous performance verification of the model and for model update and procedure revalidation if necessary.

A control strategy for checking model performance over its lifecycle should be developed and documented as part of model development and procedure validation. The strategy should identify the necessary elements for ongoing monitoring and evaluation of model performance. In addition, a plan for monitoring, analyzing, and adjusting the model should be in place with a measurement frequency that allows identification of excursions related to critical aspects of the model. The level of maintenance should be considered part of the risk assessment activities and should be adequate for the model criticality. If applicable, analytical instrumentation used to generate the inputs for the model should be qualified and also subjected to a continuous verification plan (for relevant guidance, see general chapters related to the applicable analytical instrumentation).

Ongoing assurance of performance of the model throughout its lifecycle should include:

- Ongoing monitoring and review of the model
- Evaluation of risk assessment
- Evaluation of post-implementation changes and predefined model maintenance
- Model update and procedure revalidation as needed

The ongoing review of a model should occur at predefined intervals and also should be triggered by events that may have an impact on model performance. Examples of such events include changes in raw materials variability or manufacturer; changes in the upstream process that may alter the sample matrix (e.g., process equipment or operation settings); drifts in model prediction; and out-of-specification (OOS) or out-of-trend (OOT) results given by the model (dependent upon the root cause of the OOS or OOT). In addition to triggers that are based on the prediction output of the model, triggers based on model diagnostics metrics and corresponding action rules should be included. Multivariate models may be more strongly affected by aberrant data signatures than are univariate models. Special care is needed to: 1) justify the multivariate model diagnostics statistically, 2) verify with data from the model

development and procedure validation process, and 3) implement multivariate diagnostics for monitoring as part of the control strategy. Comparison of model predictions and reference or orthogonal procedures should take place on a periodic basis or as part of the investigation triggered by the review process.

The use of model diagnostics when applied to a new sample ensures that the model prediction is valid with regard to the calibration and validation sets used during model development and procedure validation, and also ensures that the result does not constitute an outlier. The observation of an outlier means that the result is invalid, but it is not a reliable indication of an OOS result; an OOS result is an observation produced by a model when the prediction falls outside the acceptance criteria and the model diagnostics are within the thresholds. In the case of qualitative models, nonconforming results should be treated as outliers and should trigger an investigation; the output of such an investigation will indicate whether the result is OOS.

The review process for a model should produce a decision regarding the need for an extension of model maintenance; such a decision may be the result of a risk-assessment exercise or the outcome of a predefined decision workflow. Model criticality and usage will define the extension of model maintenance, which can include restraining the model conditions; adjusting the calibration set (samples can be added, replaced, or removed); or even completely rebuilding the model. The decision, and corresponding rationale, must be scientifically sound and documented.

3.4 Model Update and Transfer

Model updating must be considered part of the analytical procedure verification, and both the justification and the activities must be documented as a part of the analytical procedure lifecycle. Before performing a model update, it is critical to understand the underlying causal factor that has prompted the update. The reasons for model updating can be roughly divided into two categories.

The first category is when the calibration set simply needs to be expanded. In this case, nothing has actually changed in terms of the response of the instrument to specific analytes. Instead, the original model is no longer valid because of the expanded range of original calibration, the addition of new analytes, or the occurrence of other, previously unseen variations (e.g., changes in particle size distribution, or the drift of a chemical process to a new steady state). Thus, the calibration space must be expanded with samples exhibiting this variation.

The second category is when the samples are the same but the measurement system response function has changed. This is often due to changes in the measurement components (new light source, clouding of optics, wavelength registration shift) or can be due to method transfer across different instruments. This, in essence, is an instrument standardization problem. Changes in instrument or measurement procedures over time can render a calibration model unusable, in which case a model update becomes necessary or a new model should be developed.

In practice, there are multiple model updating techniques that could be applied to each category of model update. Some updating techniques are relatively straightforward and simple to implement, whereas others are technically complex. Selection of an appropriate model updating approach must be based on a full understanding of the underlying causal factor. As a general rule, simple updating methods (e.g., slope and bias adjustment, as described below) should be considered first.

Before any model maintenance work is initiated, it is good practice to confirm that the

fundamental construct of the original model—such as data preprocessing and variable selection—remains sound. For both qualitative and quantitative applications-based model updates, a straightforward preprocessing approach and a scientifically sound variable selection approach are recommended as the initial attempt to address challenges associated with updating the model. These approaches are equally applicable to differences caused by instruments, measurement conditions, or sample matrices.

SLOPE AND BIAS ADJUSTMENT

One of the simplest model-updating methods is to postprocess the predictions with a bias adjustment, slope adjustment, or both. This approach is often used for quantitative applications. For some qualitative applications, this approach may also be useful, depending on the nature of the method. In a limited set of circumstances, bias/slope adjustments are expected to work. For example, if the matrix of the samples being predicted were systematically different from the calibration/validation sample set, the model predictions would be in error by a constant value. Bias/slope adjustments, however, would not correct for any new variation in the data, such as variation that would result from a different matrix (e.g., new analytes/interferents). Therefore, applying slope/bias adjustments without a full understanding of the underlying causal factor is not recommended. Guidance may be obtained via inspection of residuals corresponding to the new data obtained using the existing model or, alternately, from supplemental exploratory data analyses using techniques such as PCA (see 4. *Applications of Chemometrics*).

CALIBRATION EXPANSION

When expanding the original calibration set, one should consider the number of new samples to be added, the impact (leverage) of the added samples on the overall composition of the new calibration set, and how to partition any new samples between the calibration and validation sets. It may be appropriate to simply augment the original calibration and validation sets with all the new samples, or it may be advisable to use a subset of the new and original samples. Multiple approaches, such as the Kennard-Stone algorithm and nearest-neighbor approach, are available to aid in the selection of new samples to add to an existing calibration set, but in general, all the approaches use methods to identify new samples on the basis of high leverage (i.e., model influence). Hotelling's T^2 or multivariate score plots are also effective approaches for accomplishing this goal.

CALIBRATION TRANSFER

Instrument standardization and calibration transfer methods are used to transform the response function of a measurement system so that it matches that of a reference measurement system. The reference measurement system could consist of a completely different analyzer, or it could be the same analyzer before it experienced a response-function shift. The vast majority of these methods generally requires the use of stable transfer samples that are measured on the original instrument and require the instrument to be standardized at the same time. In addition, the approaches commonly used for instrument standardization could also be applied effectively to address the challenges resulting from changes in sample matrix and measurement conditions.

3.5 Revalidation

Before the redeployment of a multivariate model, appropriate procedure revalidation should be established using criteria equivalent to those used in the original validation protocol. This revalidation is necessary to document the validity of the model as part of the analytical procedure verification. The nature and extent of the revalidation procedure, including aspects such as scientific justification and experimental approaches, must be based on the cause of the update and the nature of the corrective action required for establishing suitable performance. Revalidation should be documented as part of the analytical procedure lifecycle.

4. APPLICATIONS OF CHEMOMETRICS

As discussed in 2. *What is Chemometrics?*, chemometric analyses may be performed in either a supervised or unsupervised manner depending on the availability of data and the specifics of a given application. This section provides an explanation of these different analysis scenarios, as well as the chemometric tools (i.e., algorithms) that are commonly used. Additionally, several specific applications will be described in detail.

4.1 Qualitative

GENERAL ASPECTS

Qualitative chemometric analyses may be performed by using supervised and/or unsupervised approaches. However, to be incorporated into analytical procedures that are alternatives to compendial methods, the performance of any chemometric model must be verified as described in 3. *Model Lifecycle*. This may not be possible if very little is known about the samples being analyzed. Nevertheless, unsupervised analyses play an important supporting role during the development of chemometric alternatives to compendial procedures within the lifecycle framework, and are recommended for use before the development of subsequent supervised approaches (examples are provided in *Qualitative Application Examples*).

Qualitative compendial procedures are those that seek to supply the value of some categorical descriptor for an unknown sample. Examples of categorical properties include (among others):

- Chemical identity: microcrystalline cellulose versus API
- Morphology: polymorph A versus B; monoclinic versus triclinic
- Sample authenticity: authentic versus nonauthentic and/or adulterated
- Sample origin: facility A versus B; lot ABC-001 versus lot XYZ-002

Categorical properties can be modeled effectively using supervised chemometric algorithms that leverage either proximity or distance in multivariate space, resulting in a qualitative assignment of samples to one or more classes depending on the application and the technique used.

According to (1225), procedures based on models of this type are suitable for incorporation into Category IV methods for identification and should be validated to demonstrate adequate specificity for the intended use. "Identification" is a term that is generally used to describe a range of analysis scenarios; several common ones are described in *Qualitative Tools*.

Supervised techniques used for classification purposes, besides the analytical sensor information, make use of discrete information related to the samples in the data set (e.g., class labels). It is intended to define the borders of the clusters (or classes), providing statistical criteria, in addition to the visualization techniques (e.g., scores plot of PCs from a PCA). The borders then can be used as acceptance criteria for inclusion or exclusion of new samples into

a given class. Several useful tools are available to the analyst for building the model, such as linear discriminant analysis (LDA) and quadratic discriminant analysis (QDA), soft independent modeling of class analogy (SIMCA), kNN, and PLS-DA. Additional, more complex techniques also may be used with justification. Each type of model has its own strengths and weaknesses. If a particular algorithm does not give the desired level of performance, a different algorithm may be selected. These aspects are discussed further in *Qualitative Tools*.

For model development, optimization, and validation, the classification and discrimination techniques follow the same process described in 3. *Model Lifecycle*. These techniques develop a threshold or limits to make class assignments. The simplest form of classification is a two-class system (e.g., good/bad or A/B). When a new sample is classified as good or bad (A or B) by the model, this results from what is called a discrimination technique. Classification is the technique of assigning a new sample as A, B, either A or B, or neither A nor B. The error rate of the model is the total number of incorrect classifications divided by the total number of samples. The specific details of the application will determine whether the assignment of a sample to more than one class would be an acceptable result. Where this must be avoided, classification thresholds should be set appropriately during model development to guarantee single-class results.

QUALITATIVE TOOLS

PCA: PCA is a commonly used exploratory analysis tool that was briefly introduced in 2. *What is Chemometrics?*. PCA is a variable reduction technique and acts on the data by defining new variables, so-called latent variables, or PCs. PCs are orthogonal and are defined in the direction of the largest (remaining) variance in the data. The results of PCA are discussed in terms of PC scores and loadings, which may be plotted graphically in two (and sometimes three) dimensions for visualizing clusters of samples or outlying samples (score plots, see *Figure 4*), whereas the loadings plots provide information on the original variables. The scores are the projections of the samples on the PCs, whereas the loadings provide the weights (coefficients) by which the values of the original variables are multiplied to generate the component score for a particular sample. Loadings are rich in information regarding which variables in the matrix are prominent and can be used to "understand" the information captured by the latent variables that provide the basis for the observed sample distribution in the scores plot (*Figure 4*). The PCs may also be used as the basis for quantitative models via PCR (see 4.2 *Quantitative*).

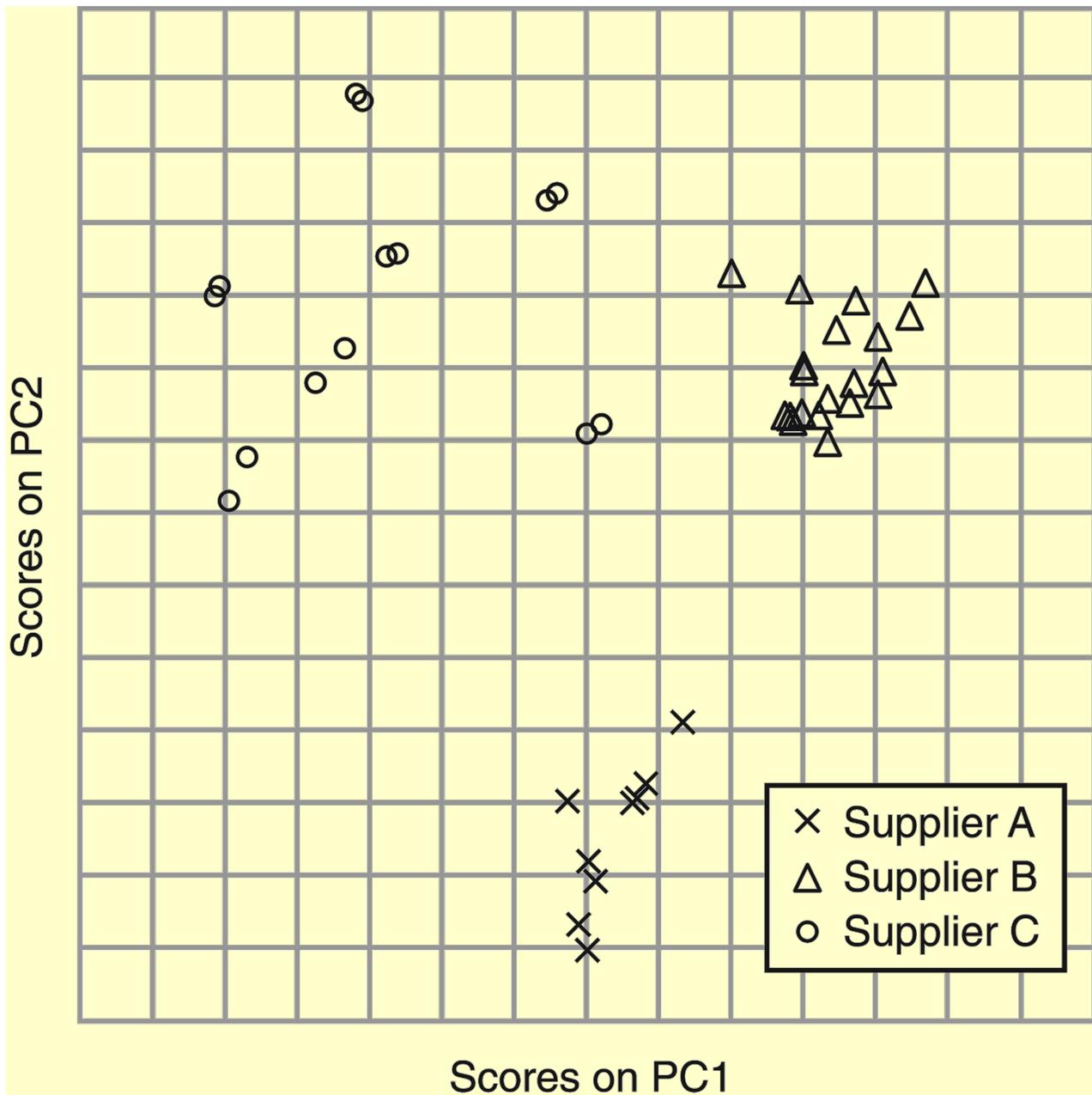


Figure 4. PCA scores plot: projection of PC1 versus PC2.

Clustering algorithms: The aim of clustering analyses is to partition data into different groups. Some methods, such as hierarchical cluster analysis (HCA), result in tree-like structures called dendrograms (*Figure 5*) that provide information on groups of samples or outlying objects occurring in the data set. Dendrograms can be built in many different ways and thus may show different aspects of the data set. A first possibility, called divisive or top-down methods, starts from the entire data set, which is split in consecutive steps until each object is in an individual cluster or all elements of a cluster fulfill a given similarity criterion. In the second scenario, called agglomerative or bottom-up methods, the opposite is done. Starting from individual objects/clusters, those most similar are merged until everything is in one cluster. Of the two options, bottom-up approaches tend to be computationally less intensive, are part of most computer packages, and are more frequently used.

The parameters used to express (dis)similarity between objects or clusters (see y-axis on

Figure 5) can be either correlation-based (e.g., correlation coefficient, r , for similarity or $1-|r|$ for dissimilarity) or distance-based (e.g., Euclidian distance) measures. Two clusters are linked in the dendrogram at the height related to this (dis)similarity measure. The parameter applied is expressed in such a way that clusters/objects linked low (i.e., close to the x-axis) are similar, whereas those linked high are dissimilar. Many different methods or criteria exist for use in deciding which objects/clusters are consecutively merged, e.g., single linkage, complete linkage, (weighted) average linkage, and centroid linkage. Depending on the applied criterion, the dendrograms may look very different. Drawing arbitrary horizontal lines (see Figure 5) splits the data set into different groups, which occasionally may be linked to sample properties. Outlying samples are linked very high in the dendrograms, most often as one-object clusters. The stability of clustering results can be affected by many factors, such as noise in the data, sample size, choice of algorithm, distance measure, and others. Divisive methods tend to produce more stable results than agglomerative methods, even though they are hardly ever used.

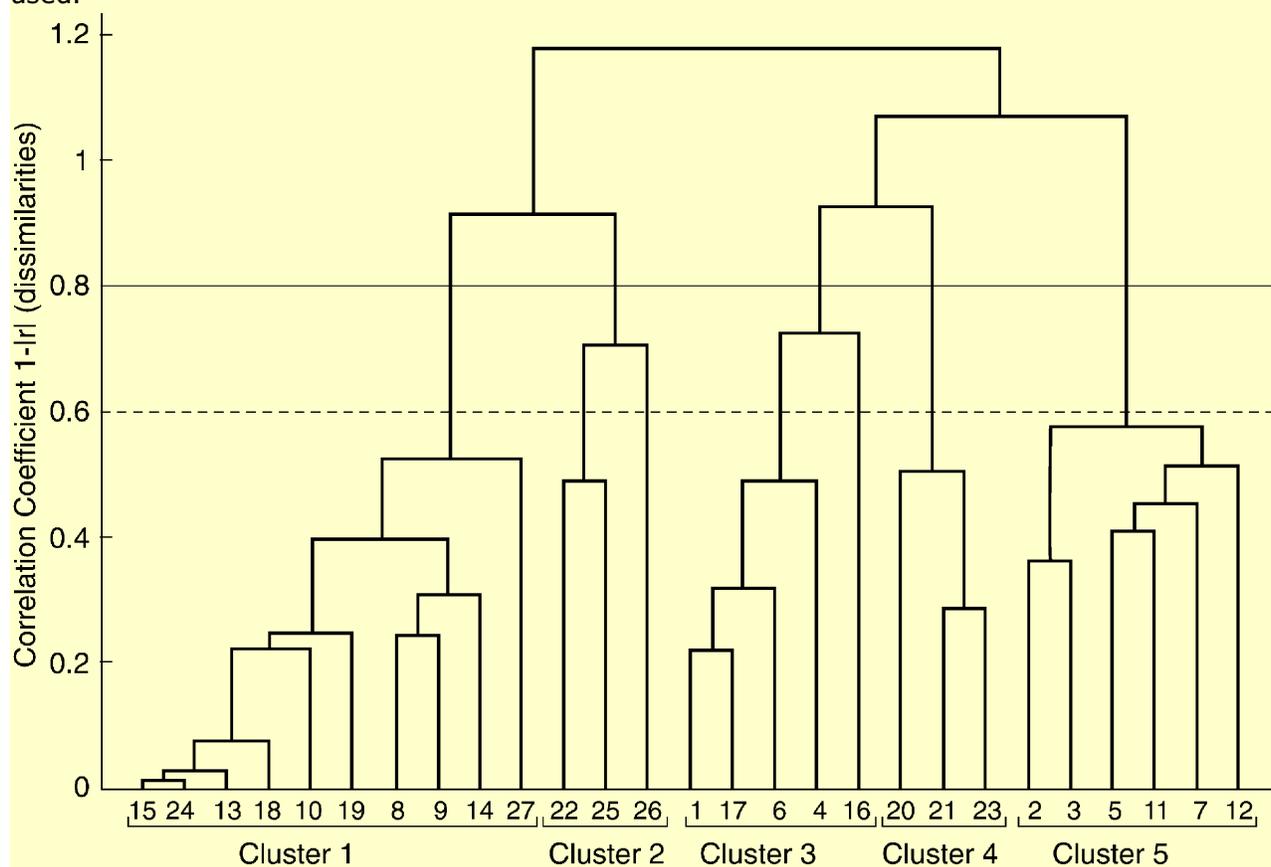


Figure 5. Hierarchical clustering based dendrogram for 27 objects. Abscissa: object numbers.

Parametric modeling: Authentication of a material or formulated product for quality assurance purposes may be achieved via multivariate parametric modeling of the chemical profile (e.g., spectrum or chromatogram). These methods are sometimes referred to as "one-class classifiers". Basically, a parametric model is developed statistically or empirically with a confidence boundary around the data from known authentic samples. Parametric models describe the behavior or structure of the majority of the data from a single class, assuming that a distribution (typically, a multivariate normal one) exists in those data. Predictions against a parametric model identify deviating points outside the significant boundaries of that distribution. Whereas classification or discrimination approaches provide identification (as

previously described) of the unknown sample, parametric models will designate the unknown sample as either belonging to the authentic class or as an outlier, or exhibiting features other than those defined by the distribution. Some discrimination techniques will also optimize class separation, and may have greater selectivity, but will tend to fail when presented with an object from none of the classes. In contrast, parametric modeling methods will reject any sample that does not fall within the confidence boundary established for the model. Perhaps the most commonly used parametric modeling algorithm is SIMCA, where PCA is used to model the data and Hotelling's T^2 and Q residuals (DmodX) are used to define the limits. In some instances, parametric modeling approaches will result in "soft classifications" wherein a sample may be assigned to more than one class. To satisfy compendial usage requirements, appropriate action must be predefined for cases of ambiguous class assignment, and/or the α and β error rates must be established and justified according to risk.

QUALITATIVE APPLICATION EXAMPLES

Exploratory analysis: Unsupervised algorithms are routinely used for initial exploratory data analyses, which precede formal calibration and validation exercises. Unsupervised data exploration can rapidly indicate, in the absence of any prior knowledge, whether distinct classes or outliers exist within the pool of available data. For instance, in *Figure 4* a graphical example is shown in which PCA applied to spectroscopic data was used to differentiate between samples from different suppliers. Exploratory models yield latent variables that may be inspected to identify the original variables with the greatest amounts of relevant analyte signal to carry forward into subsequent models or to guide and/or verify the results of variable selection approaches. Additionally, exploratory analyses may be used to test and empirically optimize various signal preprocessing options to maximize the relative contribution of desired analyte signal versus signals from sources of interference (e.g., scattering effects, optical path length differences, sample physical properties, and instrumental drift).

Analysis of DoE data: Unsupervised techniques provide a means of rapidly assessing the distribution, range, and precision of responses in latent variable space before submission of samples for reference data collection. This approach can be used as a feedback mechanism to optimize the experimental design before investing time in reference data collection (e.g., NMR, HPLC, bioassays). This concept can also be leveraged in chemometric model development to optimize various aspects of the overall analytical procedure (e.g., sampling procedure, sample physical properties, and other aspects) based on the responses from unsupervised algorithms during the design of the calibration strategy.

Material identity testing: Identification testing is routinely performed for all pharmaceutical active ingredients, excipients, drug products, and packaging materials to authenticate them and verify that the correct materials are used in manufacturing, release testing, and packaging operations. *Spectrophotometric Identification Tests* (197) illustrates one of the most widely used analytical techniques for identification, Fourier transform IR spectroscopy. Typically, identification testing involves comparing the sample spectrum to the spectrum of a reference sample and assessing the level of agreement between the two. Different chemometric algorithms can be applied for identification, and the general model development and maintenance should follow the guidance in *3. Model Lifecycle*. All methods for identity testing are supervised classification methods because either a single reference spectrum or a set of reference spectra from material(s) with known identity is utilized by these algorithms. Algorithms can be applied in either original variable space or transformed variable space (such

as latent variable space) after data preprocessing.

According to (1225), models of this type are suitable for incorporation into Category IV methods for identification and should, at a minimum, be validated to demonstrate adequate specificity for the intended use. An example approach for performance verification is the documentation of TPR and FPR. TPR is the percentage of samples which are correctly identified, whereas FPR is the percentage of samples which are incorrectly identified. TPR is also referred to as sensitivity, and FPR is referred to as specificity. Regardless of which approach is taken, there is always a trade-off between FPR and TPR, and this trade-off behavior and overall procedure performance are best visualized in a ROC curve plot (see *Figure 6*).

A classical ROC curve is generated by plotting the TPR on the ordinate and the FPR on the abscissa. Each point on a ROC curve represents a specific TPR and FPR pair obtained at a specific threshold. The threshold is typically a number generated by the algorithm, and when it is exceeded, this corresponds to a positive identification. This threshold value is determined during the procedure development process. The threshold could be the *P* value from hypothesis testing, hit quality index (HQI) threshold, Mahalanobis distance threshold, PLS discriminant score threshold, or some other threshold.

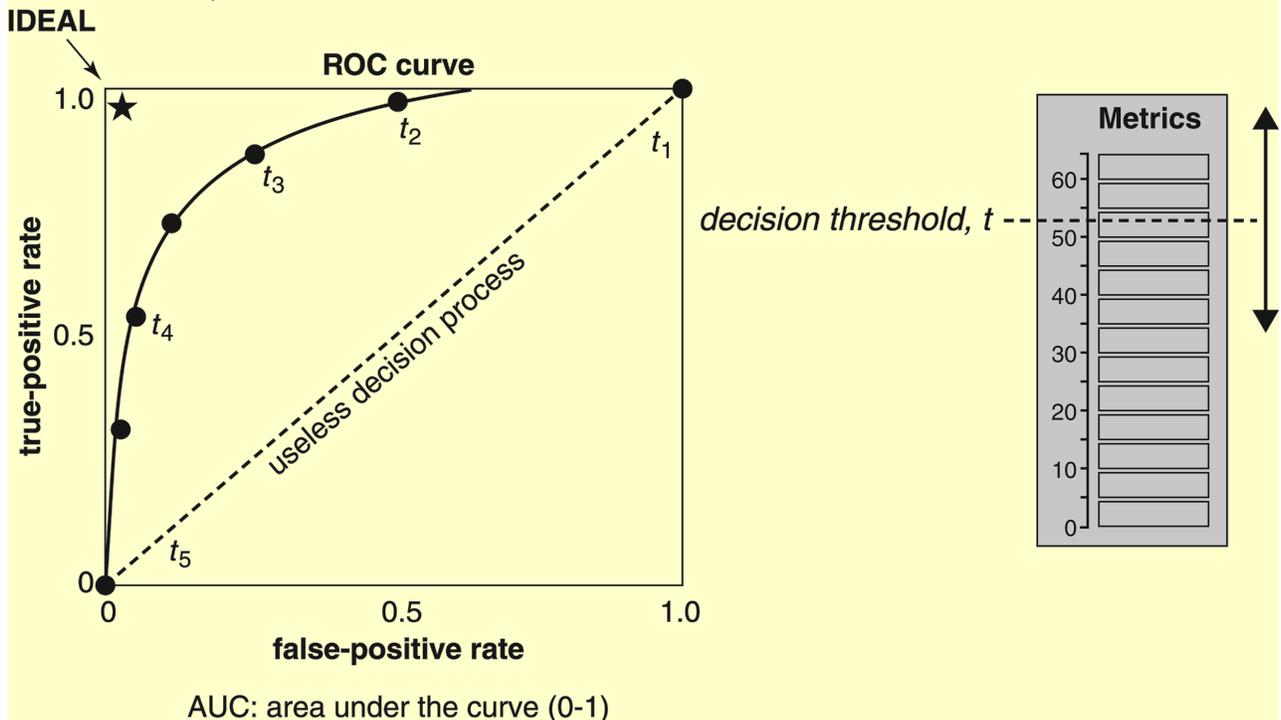


Figure 6. ROC curve plot showing the AUC (3).

During procedure development, a number of known positive and negative samples are examined, and the algorithm output for each sample is recorded. The performance of the method when operating at a particular threshold *t* is characterized by a single TPR/FPR pair, and the full operating characteristic curve can be produced by plotting TPR/FPR pairs over a range of decision thresholds. A false-positive identification corresponds to a decision threshold that is too low. False positives can be minimized by raising the decision threshold. However, excessively high decision thresholds can produce a false-negative identification.

A good identification method should generate a ROC curve that produces one or more TPR/FPR pairs in the top-left corner of the plot, as shown in *Figure 6*. This characteristic is best captured by the AUC. A random decision method is represented by the diagonal line with an

AUC of 0.5, whereas a good identification method should have an AUC closer to 1. The shape of ROC curve depends upon a combination of spectral properties of the sample and the chemometric algorithm used for identification. Characterization of the ROC curve is a fundamental task associated with method development and validation.

For a two-class model, false positives and false negatives are the incorrectly assigned samples. Together with the true positives and true negatives, they make up the success rate of the model. The analyst may wish to care about one type of error (α or β) more than another, and therefore could change the model to be more conservative when choosing a threshold. Thresholds can be used as action criteria or evaluation criteria.

Complex material authentication testing: Nutraceuticals and samples of herbal origin may be complex mixtures of numerous components. Authentication relies upon assessment of the presence of multiple analytes and their relative concentrations as well as potentially demonstrating the absence of adulterants and/or related materials. Thus, authentication in this scenario is a multivariate decision process that is appropriate for chemometric approaches. Typically, a characteristic "fingerprint" pattern incorporating information from the various analytes (and adulterants, potentially) that are present in the sample is obtained by applying a combination of separation and characterization techniques or spectroscopic measurements. The chemical composition information in the sample fingerprint provides a basis for multivariate pattern recognition. In particular, techniques with exceptional analytical selectivity, such as mass spectrometry and NMR spectroscopy, have emerged as powerful fingerprinting tools, especially when operated as detectors for chromatographic separations of multicomponent samples. Chromatographic fingerprinting is recognized as a viable identification procedure for herbal medicines by both the World Health Organization and the U.S. Food and Drug Administration (4,5).

Depending on the origin and purity of the sample, it may be appropriate to develop a multivariate classification model using either the entire fingerprint or a selected subset of peaks. Variable selection approaches may be useful in determining the optimal approach, depending on the nature of the samples and/or the ATP. It is crucial that the samples have an associated class label or quantitative property (activity) obtained by an objective reference technique (separate from that used to generate the fingerprint) to enable supervised modeling and subsequent model performance verification. Care should be taken to ensure that appropriate sources and levels of variability are included in model development and performance verification exercises based on the intended use of the test.

Several useful tools are available to the analyst for building the model, such as LDA and QDA, SIMCA, kNN, and PLS-DA. Additional, more complex techniques may also be used with justification (e.g., SVM).

Chemometric methods for multicomponent sample authentication are consistent with *Validation of Compendial Procedures* (1225), *Validation, Data Elements Required for Validation, Category IV*. Various numerical methods may be used to verify predictive performance. As discussed in *Qualitative Tools* for pure material identification, predictive performance may be verified by comparison of the TPR and FPR once a classification threshold appropriate to the application has been established. It is crucial to finalize this threshold before formal validation of the overall analytical method.

Classification: This is a supervised learning technique. The goal of calibration is to construct a classification rule based on a calibration set where both **X** and **Y** (class labels) are known. Once

obtained, the classification rule can then be used for the prediction of new objects whose **X** data are available.

Classification models may be divided into two main groups: hard and soft. Hard classification directly targets the classification decision boundary without producing a probability estimation. Examples of hard classification techniques are kNN and expert systems. Soft classification estimates the class conditional probabilities explicitly and then makes the class assignment based on the largest estimated probability. SIMCA is a commonly used soft classification technique. In either scenario, the thresholds to determine the class boundaries are important and should be established during method development based on the resulting TPR and FPR, the requirements for which are determined by the ATP. These rates should also be verified as part of the full procedure validation.

In many applications, it is unacceptable to have a sample classified into two classes. Further, if a sample is not classified into any class, the sample is deemed an outlier to the calibration set. For example, a classification model built from NIR spectra of four materials (A, B, C, D) has four classes. Any material that has a source of variance (e.g., different water content) not previously seen and included in the model calibration may not be classified, and therefore the material is an outlier. Similarly, when a spectrum of another material (E) is applied to the model, the model should not be able to classify it into a given class. If similar samples are expected in the future, then the model should be revised to include the outlier samples in the calibration set, either to supplement the calibration data for existing classes or to create an entirely new class, depending on the nature of the outlier(s) and the specific requirements of the application.

4.2 Quantitative

GENERAL ASPECTS

When the intended use of an analytical procedure is the determination of one or more sample components or properties that have values that can vary continuously rather than categorically, then a quantitative model is required. Similar to the process for qualitative modeling, quantitative chemometric models are produced in a supervised manner using both the independent **X**-block and dependent **y** variables to ensure optimal predictive performance. The difference arises in the nature of the **y** values, which are continuous/numerical (rather than categorical) for quantitative applications. Per (1225), quantitative chemometric models are suitable for incorporation into Category I, II, or III methods for determination of the content of drug substance or finished drug product, impurity levels, or performance characteristics, respectively. In-process tests applied in an offline manner or an in/on/at-line manner that produce the value for a continuous variable would also represent appropriate uses of quantitative chemometric models. The specific requirements of the analysis per the ATP (see 3. *Model Lifecycle*) should be used to guide the validation metrics used to demonstrate performance.

QUANTITATIVE TOOLS

The algorithmic tools needed for quantitative applications are wide ranging; for more complete descriptions see 6. *Appendix: Additional Sources of Information*. Tools that are commonly used are briefly described here. As is the case with any chemometric model, the tool must be matched to the ATP, and vice versa. Certain advantages and disadvantages are characteristic of each tool, and the chemometrician must be mindful of these when justifying their use.

Multi-linear regression (MLR): This tool establishes a correlation between a dependent variable (or \mathbf{y} , response variable) and multiple independent variables (or \mathbf{X} , explanatory variables) by fitting a linear equation to observed data. MLR has two broad applications, as follows.

The first broad application of MLR is to establish a model that explains the importance or magnitude of effect on the response variable, and this is typically used in the DoE screening or response analysis. Typically the input variables are mutually independent and by design represent large magnitudes. Often, the higher order of terms such as square terms and interaction terms are included, and a step-wise procedure is used to exclude those that make a smaller contribution to the model. Caution is advised to avoid having too many variables in the model, because including too many terms will overfit the model. The adjusted R^2 statistic is typically used to estimate whether there are too many factors in the model. Adjusted R^2 is a modification of the standard coefficient of determination that takes into account the number of model terms and the number of samples, resulting in a plot of R^2 (adjusted) that has a maximum value beyond which additional terms result in overfitting.

The second broad application of MLR is to establish a prediction model for analytical purpose. MLR can be used to fit a predictive model to an observed data set of \mathbf{y} and \mathbf{x} values. The established model can be used to predict the value \mathbf{y} from the \mathbf{x} of new samples. The model for prediction purpose created from correlated \mathbf{x} inputs is suitable to explore the relationship between \mathbf{y} and individual \mathbf{x} s.

The prediction MLR model can be used in a procedure or method for a pharmaceutical compendial test. For example, a filter NIR, which is designed for a specific use such as water testing in the product, produces a "spectrum" of only a few data points.

The development and validation can follow the same procedures as are used with latent variable models such as a PLS. During the development and validation of an MLR model: 1) appropriate data preprocessing needs to be used; 2) cross-validation is used to estimate the model performance; and 3) an independent data set is used to assess the model.

A limitation of MLR is the inability to model collinear variables. Thus, performance verification using independent samples is crucial to avoid erroneous models. Another disadvantage is that MLR models do not have diagnostic metrics (such as Hotelling's T^2 and \mathbf{X} residual test) that PLS models offer. Therefore, when the model is used for prediction on new data, it is important to include some prescreening steps to ensure that only valid data are processed; such prescreening tools could include individual variable range check, spectral similarity comparison, and others.

PCR: This is a two-step modeling approach in which a PCA is first performed on the \mathbf{X} -block data to obtain a basis set of PCs. The PCs are then used in combination with the \mathbf{y} data to develop a regression model to predict the \mathbf{y} values.

PLS: This is one of the most commonly used chemometric algorithms for both quantitative and qualitative (PLS-DA, see *Qualitative Tools*) modeling scenarios. The computational advantage that PLS offers over MLR and PCR is that the PCs are derived in the PLS algorithm using the \mathbf{X} and \mathbf{y} data simultaneously. This often results in predictive models that require fewer latent variables compared to PCR, thereby improving robustness. PLS is capable of handling mildly nonlinear relationships, whereas PCR and MLR are linear modeling techniques. Further, more than one \mathbf{y} variable can be modeled using the same \mathbf{X} -block data via different algorithmic approaches. PLS-1 results in a separate model for each \mathbf{y} variable. PLS-2 will produce a single model capable of simultaneously predicting the values of two or more \mathbf{y} variables. The relative

advantages of each approach may vary from one application to another. For instance, if different mathematical preprocessing is necessary to resolve each component to be predicted, separate PLS-1 models may provide improved performance over a single PLS-2 model.

QUANTITATIVE APPLICATION EXAMPLES

Pharmaceutical dosage form assay and/or uniformity of content: Assay of the API content in the final dosage form is commonly performed via measurement of a sample drawn from a homogenous composite of a number of individual dosage units (e.g., tablets or capsules). The quantitative result is then reported as an "average content" (expressed as % of target API amount) for the batch tested. Uniformity of content may be tested using a method similar to the assay, but measurements are performed on individual samples. The reported result is primarily based on the variability in assay results across the individual dosage units analyzed. Assay and uniformity of content methods are intended to characterize the "major" component in the sample(s) in question and are considered Category I tests per the definitions established in (1225). In the majority of cases, the target analyte will be the active ingredient in the sample, although quantitative assays for other components exist throughout the compendia as well.

For pharmaceutical solid dosage forms, the majority of assay procedures are based upon HPLC. HPLC analysis is time consuming and often involves the use of large volumes of solvent for the mobile phase. However, HPLC is highly linear and is typically calibrated using univariate mathematics, i.e., one measured variable is directly and uniquely proportional to sample concentration.

In contrast, alternative methods for assay that are based on spectrophotometry (e.g., NIR and transmission Raman spectroscopies) offer the advantage of increased analysis speed and are nondestructive. However, in many cases the analyte signal may not display the same degree of linearity or signal-to-noise as with the corresponding HPLC method. Moreover, no single variable is directly and uniquely related to the concentration of interest. For this reason, multivariate models, often based on PLS regression, are commonly used in spectrophotometric procedures for assay and uniformity of content.

For Category I tests, it is crucial to demonstrate the accuracy, precision, specificity, linearity, and range of results during the validation of the overall analytical procedure. Each of these performance aspects involves considerations during multivariate model development or validation that are distinct from those related to univariate techniques.

Most spectrophotometric methods are not quantitative in an absolute sense. For example, models developed using NIR or Raman data must be calibrated relative to a primary reference technique such as HPLC or NMR spectroscopy. Given the nondestructive nature of NIR and Raman, it is straightforward to analyze a given set of samples using one of these techniques and then subsequently analyze the same set of samples via HPLC or NMR spectroscopy to obtain the reference content values. The chemometric models are developed via a multivariate calibration using the NIR or Raman spectral data and the content values from the reference technique. Accuracy is determined by using the absolute difference between the model predictions and the reference values. Precision is determined by using the standard deviation of procedure results alone, such as from replicate analyses of the same sample. The model itself will be absolutely precise. That is, given the same spectral input, the same numeric output will always be generated. Thus, precision is a measure of performance for the analytical procedure as a whole. That said, precision may be influenced by the specifics of the chemometric modeling approach (e.g., preprocessing, modeling algorithm, number of latent variables) and therefore precision should be evaluated during model development as well as during validation.

It is important to note that the accuracy of chemometric models will not be able to exceed the accuracy of the reference analytical technique used to calibrate the model. However, it may in certain cases be possible to exceed the precision of the reference technique.

The range of the method will be determined by the calibration samples that are used to develop the multivariate model. According to (1225), it is recommended that assay calibration samples should have analyte contents ranging from 80%–120% of the target amount. For evaluating uniformity of content, the recommended range is 70%–130% of the target amount. In some cases, both the assay results and uniformity results may be obtained from the same set of sample measurements. Averaging of a requisite number of individual dosage-unit assay results will provide a value equivalent to the “average content” parameter. Calculation of the “acceptance value” (see *Uniformity of Dosage Units* (905)) using this mean result combined with the standard deviation for the same set of individual dosage unit results will provide a uniformity of content result. In this scenario, a single calibration set that spans the wider of the two recommended ranges (70%–130%) should be used.

If it is not practical to obtain a sufficiently wide range of content values in calibration samples produced at commercial manufacturing scale, calibration samples may also be produced in a smaller scale or in the laboratory. In this scenario, all attempts should be made to replicate the physical properties representative of the commercial scale. Additionally, care should be taken to verify that minimal bias exists between samples from different scales. Commercial-scale samples may need to be incorporated into the model (and/or procedure) validation process to verify the accuracy and precision of the model (and/or procedure) results against any differences and/or variability in physical properties. Mathematical processing algorithms (e.g., normalization, second derivatives) should be optimized as much as possible during initial exploratory data analysis (see *Qualitative Application Examples*) to mitigate prediction bias resulting from these factors. For instance, it is known that thickness, particle size, and density differences can lead to spectral slope changes and baseline offsets in NIR spectra. Depending on which specific effect is present, improperly selected preprocessing may not fully correct for spectral differences, leading to errors in prediction.

Fit and predictive performance of chemometric models should be demonstrated via the linearity of a plot of the model outputs versus the reference or nominal values of the target analyte(s), ideally using results from a set of independent test samples. The raw analytical signal may demonstrate a nonlinear relationship with the reference analyte concentrations. Likewise, multiple latent variables may be utilized for the chemometric model building. However, the key aspect of the model that must be demonstrated to vary linearly with analyte concentration is the model outputs, not the inputs. A plot of the prediction residuals versus concentration may assist in revealing any systematic lack of fit. Any observations of patterns in this residuals plot may indicate a need for revision of either 1) the number of latent variables included in the model, 2) the preprocessing mathematics, or 3) the algorithm type used for modeling.

Demonstration of the specificity of the procedure for assay may be based on likely sources of interference or material substitution based on an understanding of the material properties of the sample components and the manufacturing process and/or supply chain. One approach might be to verify that low assay results that are OOS are consistently obtained for a placebo version of the dosage unit. Another approach might be to verify that dosage units of a different product (especially one manufactured in the same facility and/or tested in the same laboratory) result in assay values that are consistently OOS.

Impurity limit tests: Routine testing is required for determination of impurities and/or degradants in intermediates, bulk drug substances, and finished pharmaceutical products.

Testing for this purpose typically takes the form of a limit test. This type of test is considered a Category II procedure per the definitions established in (1225).

Traditionally, these impurities tests are based upon HPLC or other methods (e.g., Karl Fischer titration for water). These analytical procedures are highly linear and are typically calibrated using univariate mathematics. However, these methods are often time consuming and involve manual sampling, which is disadvantageous, especially for products having high potency or for those with toxic intermediates produced during API synthesis.

In contrast, alternative methods for assay based on spectrophotometry (e.g., NIR and Raman spectroscopy) offer the advantage of increased analysis speed, and more importantly, total elimination of human sampling due to the noninvasive nature of the measurement. However, these advantages are typically offset by decreased sensitivity (LOD) and potentially nonlinear sensor response across the required analyte concentration range as the corresponding reference method. For this reason, multivariate approaches combining spectral preprocessing and latent variable models are often employed in spectrophotometric methods for these limit tests.(6)

For a limit test in Category II procedures, a calibration set containing varied concentrations of the analyte of interest is often used to characterize the performance of such an analytical procedure. Although it is not required, these performance metrics could include accuracy, range, linearity, and others. The use of the performance metrics depends on the nature of the limit test.

In contrast, it is critical to illustrate specificity and LOD during validation of a limit test according to (1225). For specificity, a comparison between loading/regression vector and a spectrum representative of the pure component of interest is often used. Additionally, measuring matrix effects on the determination of the analyte within the specified range is another useful approach to demonstrate that the analytical procedure is specific to the analyte of interest without impacts from other variables introduced in the calibration set. Moreover, evidence of method specificity can be demonstrated by calculation of a selectivity ratio from the calibration data set, defined as the variance of model-reconstructed spectra divided by that of residual spectra. The selectivity ratio at the analyte absorption band is expected to be higher relative to other spectral regions.

For LOD, the traditional approach to determine LOD involves calculating a spectral signal-to-noise ratio. The instrument response on a blank sample may be used to represent the noise. Alternatively, the noise may be approximated using the standard error of a multivariate model. For example, the LOD of a PLS model could be estimated using three times the RMSEP (or RMSECV). With either approach, the estimated LOD should be verified experimentally using samples with analyte levels at or near that concentration. Because the LOD obtainable via a spectrophotometric method will likely be higher than that obtainable via a traditional chromatographic approach, it becomes crucial to demonstrate that the LOD based on the chemometric model meets the requirements of the ATP.

Dissolution testing: A performance test based on dissolution is required for drugs with solid oral dosage forms to provide critical in vitro drug-release information. Dissolution results are used to predict in vivo drug-release profiles during drug development and to assess batch-to-batch consistency for quality control purposes. Chemometric models can be used to predict the drug product dissolution from relevant measured product properties, and the method that uses such a model may be established as an alternative procedure.

Dissolution data may be collected as a profile (i.e., a series of values obtained at various time points), even though the acceptance criteria may rely on a single time point. When building a

chemometric model, it is essential for the model to be able to predict the entire profile. The number, and spacing in time, of points in the dissolution profile will vary depending upon the product type (e.g., immediate versus modified/extended release). A common approach is to transform the profile into a single value, which then serves as a dependent variable (or y , response variable). This variable, combined with another measured variable, can be used to restore the dissolution profile. The transformation can be accomplished by fitting the dissolution profile data into a mechanistic model (such as a first-order rate equation or its variants) or an empirical model (such as a Weibull function). After transformation, a few variables (typically, two or three variables) can be used to represent the dissolution profile. Generally, the two variables are a dissolution rate factor and a plateau factor; a third variable such as lag time may be necessary. The dissolution rate factor, which represents the rate of dissolution, will be the dependent variable for the modeling. The plateau factor represents the final amount of the drug in the solution, which should be equal to the drug content for most products. These two factors are used to restore the profile. Therefore, the model for dissolution prediction is used to predict the dissolution rate.

The key to designing a successful model is defining the input variables. The plateau factor may be obtained from drug content data, whereas the dissolution rate factor may be modeled from a data set of chemical and physical attributes of the product or intermediate product. The necessary knowledge of physicochemical properties and engineering concepts should be used to identify and justify the relevant inputs that have potential impact on the dissolution of the product. This typically involves risk assessment and an additional data collection step such as a small-scale DoE study. The inputs typically include the measured material attributes at various processing stages (such as particle size data for granules or blend; NIR measurements for blend or tablets; and physical tablet properties).

There are two approaches for defining samples in the model calibration: batch samples and individual unit dose samples. For the batch-sample approach, one product batch is treated as a sample, and the input variables are the attribute means of the batch. The individual unit dose approach is to measure the attributes of individual unit doses (such as NIR measurement on the individual tablets). The input variables from both approaches may be supplemented with other raw material properties as justified by the critical quality attributes.

The validation of the model-based dissolution prediction method could be different from the traditional HPLC dissolution method validation, depending on the specific approach used. The HPLC dissolution method puts more emphasis on precision, as the dissolution is treated as a Category III method in (1225).

In the batch-sample approach, the model-based method treats dissolution as a batch property instead of a property of individual tablets, and accuracy is the focus of method validation or verification. For example, each batch forms a sample, and then it is not realistic to have a large validation sample set. This approach generates a single profile for a batch, and the result at a certain time point can be used to assess the product batch quality. Evaluation of variability must be aligned with the criteria in *Dissolution* (711). The variability of the final dissolution result can be evaluated by analyzing the variations of inputs. Possible ways to evaluate the variation of the sample (batch) include the analysis of variation of inputs; simulations; and dividing a batch into multiple sub-batches.

In the individual unit dose approach, the model-based method evaluates individual dosage units. If many tablets are tested, this becomes a large n situation in which the acceptance criteria in (711) should be used with caution. The chemometrician may propose acceptance criteria as long as these criteria are demonstrated (by simulation or other means) to have

equivalent or tighter passing criteria than those in (711). In the method validation or verification, it is desirable to have at least one sample that shows low dissolution (near or below the specification), and the chemometric model-based method should demonstrate the capability to distinguish this low-dissolution sample from normal samples.

GLOSSARY

Many common chemometric terms have been defined within the text of this chapter. Some additional terms are defined here and serve as a reference to their usage in the text. For a more complete description, consult the texts listed in *Appendix: Additional Sources of Information*.

Calibration model: A mathematical expression used to relate the response from an analytical instrument to the properties of samples, or to capture the underlying structure of a calibration data set.

Calibration set: Collection of data used to develop a chemometric classification or model.

Derivatives: The change in intensity with respect to the measurement variable (i.e., abscissa). Derivatives are useful for removing baseline offsets (constant or linear) due to sample properties, or for highlighting small changes in a signal, helping to enhance selectivity and sensitivity (e.g., one spectroscopy and chromatographic peak with a shoulder peak adjacent).

Internal validation: The application of resampling statistics such as cross-validation. Subsets of the calibration data set are subjected to a variety of statistical processes to identify which calibration model best fits the available data. Each model is characterized by a statistical parameter. For cross-validation, the entire data set of samples is split into individual samples or groups of samples, which are removed individually from the rest of the samples and tested as unknowns against a calibration model constructed using the rest of the samples. The characteristic statistic is the standard error of cross validation (SECV).

Matrix: A two-dimensional data structure comprised of columns and rows used to organize inputs or outputs during chemometric analyses. Common practice is for each row to correspond to an individual sample and each column to correspond to an individual variable.

Validation set: The data set that challenges the performance attributes of the model. The validation data set is independent of the training data set, although testing on the training data set gives an optimistic view of performance and thus allows for iteration of preprocessing and model tweaking until this optimistic view meets the expectations. Validation or cross-validation is then necessary to adequately gauge the performance of the model. The model performance with the validation data set will always be equal to or worse than its performance with the training data set. If the performance attributes are not met, then the chemometrician must assess whether the model underfits or overfits the data and whether iterations of the model are needed to meet the acceptable error rate. If these efforts fail, it is recommended to back up one step, alter the preprocessing conditions, and perform the same task again.

APPENDIX

Additional Sources of Information

Many books have been written on the subject of chemometrics and multivariate analysis. Various terms not found in the glossary may be found in this short list of additional sources of information.

- Massart DL, Vandeginste BGM, Buydens LMC, De Jong P, Lewi PJ, Smeyers-Verbeke J, eds. Handbook of chemometrics and qualimetrics: part A. 1st ed. Amsterdam: Elsevier Science B.V.; 1997.
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▲USP40

BRIEFING

(1790) **Visual Inspection of Injections.** The General Chapters—Dosage Forms Expert Committee proposes this new chapter to provide guidance on the inspection of injectable drug products for visible particles. The methods discussed are also applicable to detection of other visible defects that may affect container integrity or cosmetic appearance of the product.

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Comment deadline: January 31, 2016

Add the following:

▲ (1790) VISUAL INSPECTION OF INJECTIONS

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1. SCOPE

1.1 Introduction

This chapter provides guidance on the inspection of injections for visible particles. The terms particle, particulates, and particulate matter are equivalent and do not have different meaning when used in this chapter. Particulate matter is defined in *Particulate Matter in Injections* (788) as “mobile undissolved particles, other than gas bubbles, unintentionally present in the solutions.” Visual inspection is a probabilistic process and the specific detection probability observed for a given product for visible particles will vary with differences in product formulation, particle characteristics, and package design. The methods discussed in this chapter are also applicable to the detection of other visible defects not the subject of *Visible Particulates in Injections* (790), but critical to a qualified, comprehensive inspection process. These include, but are not limited to, container integrity defects such as cracks, misplaced stoppers, or incomplete seals, any of which may compromise the sterility of the product. Additional container defects (1), as well as other product characteristics such as fill level, discoloration, or clarity may also be detected during visual inspection, and non-conforming units should be rejected using the methods described in this chapter. Inspection for these other quality attributes often occurs at the same time as the inspection for particles. The primary focus of this chapter is a manual reference inspection method; however, semi-automated and automated methods are also discussed and permitted by the pharmacopeia.

1.2 Related Chapters

Injections and Implanted Drug Products (1) provides an overview of injectable dosage forms and the quality tests associated with them. Another chapter, (790), has been added to the *USP–NF* to provide a clear definition of routine inspection procedures for injectable products; the goal is to comply with the expectation that products be essentially free of visible particulate matter. Additionally, information on the detection of subvisible particulates is provided in *Subvisible Particulate Matter in Therapeutic Protein Injections* (787), (788), and *Particulate Matter in Ophthalmic Solutions* (789). *Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections* (1787) and *Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions* (1788) provide additional supporting information on measurement methods for subvisible particles.

1.3 Defect Prevention

Although this chapter focuses on detection and removal of product units that show evidence of visible particles, the need for preventing such contamination should not be overlooked. No inspection process, manual or automated, can guarantee complete removal of all visible particulate matter or other visible defects; thus, prevention of such defects is an important consideration. Good process and product design, along with environmental control, are necessary to ensure the reliable production of products with a low particle burden. To ensure

the control of defects throughout the process, manufacturers should consider an inspection life-cycle approach (2). This approach begins with developing quality attributes based on incoming component specifications, followed by component-level acceptance testing. It extends to component preparation and product-filling procedures, followed by 100% in-process inspection of filled product, and concluding with final acceptance sampling and testing of the finished product. The approach must extend to purchased, ready-to-use components such as containers or closures, where there is no opportunity for subsequent particle removal after receipt and before filling. Stability and retention sample inspection, customer complaint evaluation, and in-house investigative procedures support this integrated approach. The inspection life-cycle is composed of, and supported by, sub-cycles involving qualification, maintenance, personnel training, defect characterization by forensic analytical methods, and the use of standards within each of the critical areas. The final element of the life-cycle is a feedback loop of trending and data review from each of these process areas, resulting in a mechanism that supports continuous process improvement.

2. INTRODUCTION

2.1 Inspection Process Capability

Visual inspection of injections is necessary to minimize the introduction of unintended particles to patients during the delivery of injectable medications. Such inspection also offers the opportunity to reject containers whose integrity has been compromised, such as those with cracks or incomplete seals, which pose a risk to the sterility of the product. The desire to detect these defects, despite their very low frequency and the randomness of their occurrence, has resulted in the long standing expectation that each finished unit will be inspected (100% inspection). Although zero defects is the goal and this should drive continuous process improvement, zero defects is not a feasible specification for visible particles given current packaging components, processing capability and the probabilistic nature of the inspection process.

The detection process is probabilistic: the likelihood of detection is a cumulative function of visible attributes such as particle size, shape, color, density, and reflectivity. Understanding human performance is therefore critical to establishing visual inspection criteria. Individual receptors in the eye have a theoretical resolution of 11 μm , but typical resolving power is reported as 85–100 μm (3). Analysis of inspection results pooled from several studies (4–6) conducted with standards prepared with single spherical particles show that the probability of detection for a seeded sample with a single 50- μm particle in a clear solution contained in a clear 10-mL vial utilizing diffuse illumination between 2,000 and 3,000 lux is only slightly greater than 0%. The detection probability increases to approximately 40% for a seeded standard with a 100- μm particle and the threshold for routine, reliable detection ($\geq 70\%$ probability of detection) of individual visible particles is often near 150 μm in diameter (4) and typically exceeds 95% for particles that are 200 μm and larger. Thus, in a qualified visual inspection system, the vast majority of particles that might go undetected and be introduced into the pharmaceutical supply chain will be smaller than 200 μm . Changes to the container (e.g., increasing size and opacity), formulation (e.g., color and clarity), fill level, and particle characteristics beyond size (e.g., color, shape, and density) will all affect the probability of detection which can be achieved for a specific product and package (6).

2.2 Patient Risk

A complete review of the medical literature is beyond the scope of this chapter, but the effect of extraneous particles on the patient must be considered. A number of reviews on this subject are available (7–13). The clinical implications of extraneous particulate matter in injections are determined by many factors, including the size and number of particles, the composition of the material, the potential for microbiological contamination, the route of administration, the intended patient population, and the clinical condition of the patient. For example, an otherwise healthy individual receiving a subcutaneous or intramuscular injection containing sterile, inert particulates would likely experience no adverse effect or at worst would develop a small granuloma. On the other hand, a critically ill premature infant receiving a particle-laden infusion directly through an umbilical catheter might suffer considerable pathophysiologic sequelae (14,15).

Garvin and Gunner were among the first to report a concern about the effects of particles in human patients (16,17). For obvious ethical reasons, there is a lack of controlled clinical studies on the effects of particles in human patients. Some anecdotal information about human patient safety may be obtained by examining case reports of intravenous drug abusers (18–20). In these cases, solid oral dosages are often ground up and injected as a slurry; pulmonary foreign body emboli and granulomas were observed in these patients (21). Unfortunately, the clinical risks to human patients posed by small numbers of particles are difficult to infer from these observations due to the extreme number of insoluble particles and the uncontrolled conditions in which they were administered.

Numerous animal studies have been conducted to determine the fate of intravenous particles with different sizes and composition (22–25). Most studies have focused on subvisible particles with a diameter of <50 µm. In these studies, a massive infusion of particles has been accompanied by histologic evidence of injury to pulmonary capillary endothelial cells (26), microscopic thrombi in the pulmonary capillaries (27), pulmonary microscopic granulomata (28), and hepatic inflammatory effects (29). Although useful for understanding the pathophysiologic response to particulate matter, the large number of particles used in these studies (e.g., 109 particles/kg/injection) provides little insight into the risk to humans posed by small numbers of macroscopic particles. Arterial embolization using materials such as polyvinyl alcohol (PVA), collagen-coated acrylic microspheres, and gelatin spheres also provides some insight into the potential human pathophysiologic implications of non-target embolization of extraneous-particle intravenous infusions. In these cases, massive particle loads moving from the arterial injection site into the venous circulation were also reported (30–34).

In a review of the hazards of particle injection, it has been found that the primary contributor of particulate matter in vial presentations is the rubber closure, a risk that is present with almost every injection. In addition, case reports have documented injury associated with infusion of significant quantities of precipitated admixtures or therapeutic use of particles for embolization (14,15,35). Despite the administration of an estimated 15 billion doses of injectable medicines each year (36), no reports of adverse events associated with the injection of individual visible particles have been found.

Ultimately, the safety considerations related to particulate matter in injections must be assessed for each drug product, intended patient population, and method of administration. No single set of inspection criteria can adequately anticipate all of the potential risks to the patient. The methods outlined in (790), should serve as essential requirements when assessing the adequacy of the visual inspection procedure, but alternative acceptance criteria (for example, the use of tightened sampling plans) should be implemented when the patient population and intended use of the product warrant these additional measures.

2.3 History of Compendial Inspection Standards

The requirement for injections to be “true solutions” appeared in *USP IX* in 1915, and the first appearance of “solution clarity” for parenterals occurred in 1936 in *NF IV*. Since then, there have been numerous modifications to the compendia in this regard. A comprehensive history of compendial inspection standards is available in the *Pharmaceutical Forum* (37).

3. TYPICAL INSPECTION PROCESS FLOW

3.1 100% Inspection

Chapter (790) establishes the expectation that each unit of injectable product will be inspected as part of the routine manufacturing process. This inspection should take place at a point when defects are most easily detected; for example, prior to labeling or insertion into a device or combination product. Each unit may be examined manually with the unaided eye, or by using a conveyor to transport and present the containers to a human inspector (semi-automated inspection), or by means of light obscuration or electronic image analysis (automated inspection). Manual and semi-automated inspection should only be performed by trained, qualified inspectors. Inspection may also be enhanced by means of a device that holds more than a single unit at one time for examination. This inspection may be performed in-line with filling or packaging or in a separate, off-line inspection department. The intent of this inspection is the detection and removal of any observed defect. When in doubt, units should be removed (see *Figure 1*).

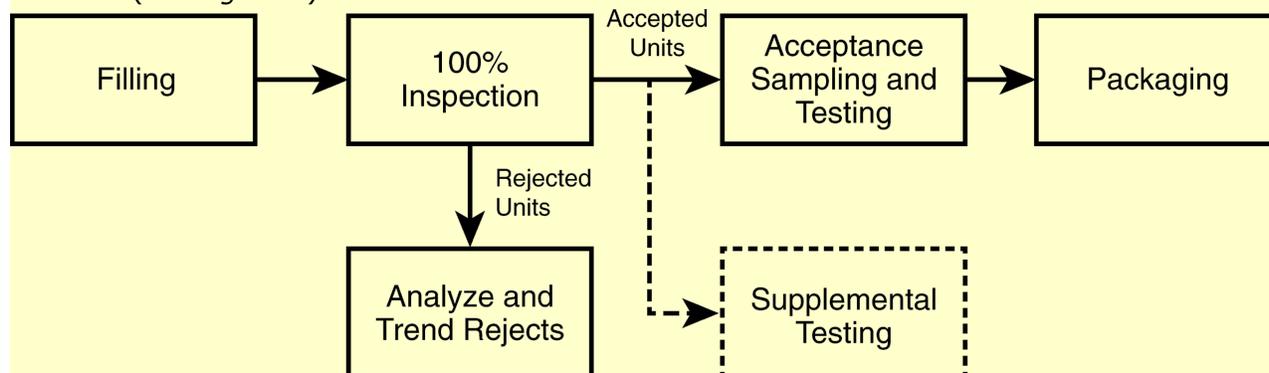


Figure 1. Typical process flow chart.

[Note—100% inspection refers to the complete inspection of the container–closure system and its contents. Inspection may be accomplished in a single operation or in multiple steps using a combination of technologies. See additional discussion in 3.3 *Remediation and Alternative Practices* and 6. *Inspection Methods and Technologies*.][Note—Supplemental testing is required when the nature of the product or container limits visual inspection of the contents (e.g., with a lyophilized cake or powder or with an amber glass or opaque container). See additional discussion in 5.2 *Unique Product and Container Considerations*. Samples for supplemental testing may be taken from any point in the process after 100% inspection.]

During 100% inspection, limits on typical rejection rates should be established to identify atypical lots (38). These limits may be established for categories of defects (e.g., critical, major, and minor) or for specific types of defects (e.g., particles). A review of historical performance is useful in establishing these limits, and the review may include grouping products similar in appearance and manufacture. Periodic reassessment of these limits is recommended to

account for expected process improvements and/or normal fluctuations in process baseline (39). If a limit is exceeded, it should trigger an investigation. The investigation may include additional inspection or it may determine whether additional inspection is necessary.

3.2 Acceptance Sampling and Testing

After 100% inspection, a statistically valid sample is taken from the units accepted by the inspection process. This may be a random sample or a representative sample (e.g., at fixed time intervals or a fixed number per tray). Defects may not be distributed equally over the lot, and therefore a sampling process that represents the whole lot is required. Typical sampling plans used for this purpose can be found in the ANSI/ASQ Z1.4 standard (40). Equivalent plans may also be found in the ISO 2859 (41) or JIS Z9015 (42) standards. For batch release, the sampling plans listed as Normal II are typically used. Tightened sampling plans may be appropriate when an atypical result is observed or reinspection is performed. These plans specify a sample size for a range of batch sizes and require selection of an acceptable quality limit (AQL). The AQL is the defect rate at which 95% of the lots examined will be accepted and is a measure of falsely rejecting good batches. Critical defects (those that pose the greatest risk to the patient) should be assigned an AQL with a very low value. Often, the accept number (the number of defective units allowed in the sample) for a critical defect is zero. Major and minor defects, which pose less risk to the patient, will have increasing (less stringent) AQL values and accept numbers greater than zero. *Table 1* shows the range of AQL values typically used for visual inspection processes (43).

Table 1. Typical AQL Values for Visual Inspection Processes

Defect Category	AQL Range (%)
Critical	0.010–0.10
Major	0.10–0.65
Minor	1.0–4.0
[Note—When selecting a sampling plan for AQL testing after 100% inspection using ANSI/ASQ Z1.4, ISO 2859 or JIS Z9015, choose the sample size to satisfy the AQL value for the most critical category (e.g., critical) of defects being evaluated. Then use the accept numbers for this sample size for the AQL values chosen for the other defect categories (e.g., major and minor). This assures that the sample size will produce a statistically valid result for all defect categories examined. The defect categories shown here represent a common basic approach to grouping defects by risk; however additional categories may be added to these for more detailed analysis.]	

The unacceptable quality limit (UQL) for the sampling plan used should also be known. The UQL is the defect rate at which 90% of the lots examined will be rejected and is a better measure of the customer or patient risk. The protection afforded by any sampling plan is represented by its operational characteristic (OC) curve. This is a plot of the probability of lot acceptance versus the defect rate in the lot. The AQL and UQL are two points on this curve. Sampled units should be manually inspected under controlled conditions by trained inspectors. Inspection conditions should be aligned with the 100% inspection process.

Acceptance sampling should be performed after any type of 100% inspection process, including manual, semi-automated, and automated inspection processes. It provides a measure of the performance of the overall inspection process and the quality of a specific lot, compared with predefined acceptance criteria. Although automated systems are validated before use and are

routinely challenged to ensure acceptable performance, the use of acceptance sampling detects unexpected defects that were not included in the development and training of the automated system by the manual inspection process.

Acceptance criteria are comprised of the product specifications and acceptance/rejection criteria, such as the AQL and UQL values, with an associated sampling plan that are necessary for making a decision to accept or reject a lot or batch (or any other convenient subgroups of manufactured units) as described in 21 CFR 210.3 (44). If the acceptance criteria of the sampling plan are not met, an investigation should be conducted. Depending on the nature of the failure, this investigation should include examinations of the manufacturing process, the raw materials, and the packaging materials, as well as the inspection process. If, after investigation, the inspection process is deemed capable of detecting the defect(s) in question, the batch may be reinspected. An alternative inspection process, better suited to detection of a specific defect may also be chosen for reinspection. After reinspection, a new sample of the accepted units is taken and compared against established acceptance criteria. It is a good practice to use a tightened sampling plan and acceptance criteria under these circumstances because of the atypical nature of this process step.

3.3 Remediation and Alternative Practices

REINSPECTION

As discussed in the preceding section, reinspection may be appropriate if the initial 100% inspection is not successful. This includes instances when the established 100% inspection failure rate(s) and/or the accept/reject number(s) associated with the chosen AQL values have been exceeded. Reinspection should only be conducted using a prior-approved procedure that addresses key parameters such as the inspection conditions (e.g., same as primary inspection or modified to enhance detection of a specific defect type), the number of times reinspection may be performed (this should be limited, and justified), and the acceptance criteria (e.g., same as primary inspection or tightened). If reinspection is required often, consideration should be given to improving the sensitivity of the primary inspection process. Frequent and routine reinspection is not recommended. Reinspection is not considered rework and is more closely associated with reprocessing as defined in 21 CFR 211.115 (45), where a qualified or validated processing step is repeated.

TWO-STAGE INSPECTION

In cases where an assignable cause, such as formation of air bubbles or specific container or closure variation, results in a high false-rejection rate (rejection of acceptable units), the use of a second inspection step may be considered. This is more common with automated inspection systems, where there is less ability to tolerate normal variation in product or container. Under these circumstances, the inspection system is adjusted to ensure acceptance of good units. Those not accepted are considered of uncertain disposition until inspected by another means (e.g., manual inspection following automated inspection). Inspection conditions may be adjusted to provide greater sensitivity in this second inspection step (e.g., additional inspection time) to ensure a high probability that true defective units will be rejected. The limitations of the first inspection and the reason for conducting a second stage of inspection should be clearly defined and documented. The second inspection of these units by the same method (e.g., automated inspection after automated inspection) is generally not recommended, because the same limitation in inspection method is present for both inspections. However, it

may be suitable when the root cause is air bubbles in the solution and a study has been performed to establish an appropriate holding time to allow the bubbles to dissipate before performing the second inspection. It is recommended that each inspection stream (those accepted by the first stage and those accepted by the second stage) be sampled separately and evaluated against the sampling plan acceptance criteria before they are confirmed as accepted and recombined into a single batch.

4. INSPECTION LIFE-CYCLE

4.1 Extrinsic, Intrinsic or Inherent Particles

Particles may originate from many sources. These are discussed here, as well as in other chapters in the *USP* (e.g., (1787)). Those that are foreign to the manufacturing process are considered to be exogenous or "extrinsic" in origin; these include hair, non-process-related fibers, starch, minerals, insect parts, and similar inorganic and organic materials. Extrinsic material is generally a one-time occurrence and should result in the rejection of the affected container in which it is seen; however, elevated levels in the lot may implicate a broader contribution from the same source. These particles may carry an increased risk of microbiological or extractable contamination, because less is known about their path prior to deposition in the product container or their interaction with the product.

Other particles are considered "intrinsic", from within the process, or "inherent", which are known to be or intended to be associated with specific product formulations. The determination of whether the particulate is inherent or intrinsic to the process is based upon appropriate characterization of the particle's physicochemical properties. Intrinsic particles may come from processing equipment or primary packaging materials that were either added during processing or not removed during container preparation. These primary product-contact materials may include stainless steel, seals, gaskets, packaging glass and elastomers, fluid transport tubing, and silicone lubricant. Such particles still pose the risk of a foreign body, but generally come from sterile or sanitized materials and more is known about their interactions when in contact with the product. Any process-related intrinsic particles should have controls established based on the use of a life-cycle approach as outlined in *1.3 Defect Prevention*. Another group of particles considered intrinsic is interrelated with the stability of the product. These product stability-related particles come from container-closure interaction, changes to the drug formulation (insoluble degradation products), or temperature sensitivity over time. Stability-related intrinsic particles should be identified and addressed as early in the product development process as possible.

The physical form or nature of inherent particles varies from product to product and includes solutions, suspensions, emulsions, and other drug delivery systems that are designed as particle assemblies (agglomerates, aggregates). Product formulation-related particulate formation should be studied in the development phase and in samples placed on stability to determine the normal characteristics and time-based changes that can occur. Use of automated particle counting or image analysis in the subvisible (for particle sizes $\geq 2 \mu\text{m}$) and visible ranges may be required to fully characterize inherent formulation-related particles. In biologics, protein particles are considered inherent when their presence may be measured, characterized, and determined to be part of the clinical profile. Inherent particles may be accepted if the drug product has a control strategy showing that this particulate category is part of the product clinical profile. The manufacturer may allow inherent particles if the product appearance

specification also allows their presence or if the product is an emulsion or suspension.

An evaluation of the potential impact of particles identified from any of these sources may be enhanced by incorporating a clinical risk assessment. This assessment may include factors such as the intended patient population, route of administration, source of the particles, and implications for product sterility. For intrinsic or inherent particulate matter sources, a risk assessment may be useful in developing product-specific control strategies. Given the probabilistic nature of particle detection, it is important to assess the possible implications of particles identified through the product life-cycle to better ensure the product's safe use.

4.2 Prevention of Particulates

The manufacturing process is designed to keep the final container and its contents clean within the control parameters established for process-related particulates. Once the container is filled, the stability of the product needs to be maintained throughout its shelf life. Changes that occur as the product ages during its normal shelf life must be characterized. Avoidance of intrinsic particle sources that may affect final product stability depends on careful consideration of the entire product system. If these intrinsically sourced changes occur, and they affect stability, particles ranging from sub-visible to visible may develop. Typically, these particles result from change mechanisms that slowly affect the on-shelf product.

ROBUST DESIGN DURING DEVELOPMENT

To anticipate potential sources of instability that yield intrinsic particles, the product design is evaluated from many perspectives, beginning with a literature review of similar formulae/packages. Points to consider include the reported sensitivities of the active, the formulation type, and the final container-closure system needed for delivery. Knowledge of how glass containers are fabricated, controlled, sterilized, and tested is important as this may affect the tendency to form glass lamellae (46,47). Obtaining further information on residual extracts, possible leachates, metals, or solubility-edge conditions is important as these factors may promote formation of solid material in the aging solution. Several additional key factors for successful product design are the product concentration, solution pH, critical micelle concentration, oligomerization content/potential, package effects (large surface area, product volume, head space, light/oxygen transmission), and compatibility of the formulation with the package. Some key formulation design factors include the formula components chosen and their purity; the solubilities of the active ingredient(s) and excipients, and consideration of potential salt forms. Finally, to maximize product stability, consider the final product preparation for delivery, product dilutions, and shelf stability of the commercial product or its therapeutic preparations.

To examine the appropriateness of the product design for maintaining product stability, there are two levels of evaluation. Both levels examine retained containers for visible changes using methods described in this chapter, but neither level dwells on low percentage defects. For the first level of stability study, bench trials consisting of visual inspection of trial containers in the formulation lab will show general compatibility of the chosen components over time with regard to clarity, color, and particle formation. Careful product assembly in clean containers, with consideration of the container type, headspace, and sealing, will yield a beneficial first-pass trial of stability over several months' time. Detection of extrinsic particles at this stage of development is generally not significant, as the particles do not reflect on the formulation under development.

The second, more refined level of stability study involves conducting visual inspections of the injection in defined, International Conference on Harmonisation (ICH)-relevant trials. This may include periodic inspection of the same containers over time if the product does not require reconstitution or is not affected by frequent temperature changes. Detection of minor or subtle differences in these containers is not the goal at this stage of development. Catastrophic change and the occurrence of intrinsic product-related visible particles should be the focus. Typically, a set of containers is carefully prepared to exclude extrinsic particles and is then inspected to cull out any units with visible defects. Next, a numbered set of containers appropriate for the batch size is placed on trial and visually inspected periodically; a typical sample size is 80–100 units. Additional sets of containers stored at selected extremes of ICH temperatures can be followed to aid discovery of solubility-edge phenomena. When unwanted changes are detected, such as particle formation, solution color change, solution haze, and package changes, the process of isolation, characterization, and identification can commence. Identification of the material making up the changes aids in determination of the cause, as well as development of improvements for future use.

COMMON SOURCES OF INTRINSIC PARTICULATES

Process-related intrinsic particles originating from product contact materials tend to be stable and unchanging (e.g., glass, rubber, or metal). In contrast, there may also be particles resulting from product stability-related change mechanisms within the final product. It is very important to understand that these changes only have to be slight in certain cases, far below the detection limit of most release or stability assays, to result in visible changes to the product. The threshold levels for the formation of visible change for certain substances may be only 10–100 ppm (0.001%–0.01%). However, if all of this insoluble material were contained in a single visible particle, it would likely cause rejection of the container.

FORMULATION COMPONENTS

The active ingredient may also contribute to the presence of stability indicating intrinsic particles. For example, significant haze and particles have manifested in aqueous formulations due to extraction of plasticizers from filtration media during bulk drug production (5). Metal content in the active ingredient has contributed to organometallic salt formation and has also been observed as precipitated inorganic salts, blooming long after product release. The active ingredient and related degradation products may also be relatively insoluble and may grow to form visible particles. The particulate material must be analyzed to determine its chemical nature and possible identification.

Monomers or single molecules may join together through chemical processes to form dimers, trimers, and oligomers (a limited assemblage of monomers, short of polymerization). Such changes are not unexpected (48). In high-concentration and/or saturated formulations, and especially for micellar drug associations, the solubility of related forms is significant when the aging formulations contain progressively higher concentrations of these substances. Larger molecules may have a greater effect on solution integrity due to their inherent insolubility, especially if the active drug is in a micellar formulation.

Polymorphs are unique crystalline forms of identical chemical entities. Although uncommon in solutions that have been mixed homogeneously and filtered, small seed crystals of a relatively stable polymorph may form over time, especially at nucleation sites such as container-surface defects. More common than formation of polymorphs is formation of a modified crystal lattice containing an integral liquid, typically water or solvent. The lattice may form slowly, promoted

by evaporation, nucleation, and temperature extremes (49,50).

PACKAGING COMPONENTS

Extractables and leachables are terms commonly used to describe the potential for primary packaging materials to contribute unwanted agents to the product. Extractables represent all of the materials that could be contributed, and leachables represent the practical contribution upon contact between packaging components and drug formulation (51). These substances can also contribute to the formation of subvisible and visible particles.

Formulation attack of the container is a dramatic change and most often occurs in glass container systems. Glass containers undergo corrosion that is 25 times greater at pH 8 than at pH 4 (52). A formulation pH above 7, especially with high-ionic strength solutions, promotes attack of the inner glass surface, resulting in particle generation.

Silicone oil is added to pre-filled glass syringe systems to enhance lubricity for closure insertion and/or syringe movement. Silicone may also come from tubing used for fluid transfer and a variety of polymeric fittings and seals that are used in the processing equipment. All of these components must be compatible with the formulation to minimize leachates. Although silicones are processed to be sterile and are widely used, their use must still be controlled. Silicone can cause container sidewall droplets and a variety of visible semi-solid forms. No more than the minimum quantity should be used during processing. Silicone and other hydrophobic substances have the capacity to coalesce and agglomerate with other particles, reaching a visible size.

4.3 Particulate Removal by Component Washing

GLASS CONTAINERS

Each step of the glass-container washing and rinsing process should be evaluated for particle-reduction capability. The washer validation studies should demonstrate a reduction in naturally occurring particles or should use seeded containers to demonstrate such reduction capability. The use of statistical sampling plans with light obscuration and/or membrane microscopic particle-counting methods can provide a means to demonstrate reduction of both subvisible and visible particles during washing cycle development and validation. During process development, validation, and routine use, container-washing procedures should include periodic visual operational checks. This routine verification ensures that effective draining of all containers is occurring during all washing and rinsing steps. Review the wash-water recirculating filter maintenance procedures to ensure that particle overloading or breakthrough is being prevented.

Glass breakage that occurs during the component washing process could affect surrounding containers and the washing cycle should be evaluated for possible glass particle generation and distribution. Effective, written container-clearance procedures following these occurrences should specify the number of containers to be removed from the affected portion of the line. Removing units that could potentially contain glass particles aids in minimizing particle transfer to the downstream process.

ELASTOMERIC CLOSURES

Each step of the elastomeric-component washing and rinsing process should be evaluated for particle-reduction opportunities. Utilize statistical sampling plans to collect meaningful test units. Light obscuration or other automated particle counting and membrane microscopic particle-counting methods may be used to demonstrate reduction of both subvisible and visible

particles during washing validation. During process development and validation and in routine use, container-washing procedures should include visual checks to ensure that stoppers are not routinely sticking together. Such sticking surfaces reduce cleaning efficacy and entrap particles. Periodic assessment of component cleanliness and supplier washing capabilities should be included as part of the supplier qualification program when using purchased, ready-to-sterilize, or ready-to-use components.

Evaluate any current siliconization process used, whether in-house or by the supplier, to minimize excess silicone levels while maintaining machinability of the stoppers. Light obscuration or other automated particle-counting method may be used to compare overall particle level reduction (background silicone oil droplets) during process development or validation. The level of residual silicone oil will affect the particulate quality of the final filled product, observed as dispersed droplets and particle-forming matrices.

GLASS HANDLING

Processes that use racks or trays for transporting and holding samples, as are typically used in batch ovens, should be monitored for metal particle generation. The racks or trays should have a formal maintenance program associated with their routine use. Trays should be inspected for wear and scoring, which can be sources of particulates. Periodic cleaning, polishing, and/or resurfacing may be warranted to effectively control particles. Tunnels used for depyrogenation should also have a routine maintenance program for periodic cleaning, inspection, and replacement of parts that may wear and generate particles. Routine process observation for glass breakage allows for clearance of any potentially affected surrounding containers and minimizes the occurrence of glass particles being carried downstream to filling. Glass-to-glass and glass-to-metal contact should be minimized where possible to reduce weakening of the glass surface and increasing the risk of subsequent fracture. The use of polymeric facing on guides can help in reducing such damage.

EQUIPMENT PREPARATION

It is important to minimize redeposition of particles on product contact surfaces after cleaning. Cleaned and sterilized equipment should be protected by HEPA-filtered, unidirectional airflow until transferred to, and installed on, the filling line. For cleaned equipment that needs to be wrapped or bagged prior to sterilization, utilize low-shedding, non-cellulose (synthetic) wrapping materials. Cellulose fibers are one of the most common particles found in the injections-manufacturing environment and injectable products and their origin will be a prime concern (43).

FILLING LINE

The transfer of open containers should be evaluated and reviewed to mitigate particle contamination. For example, for aseptically filled products the transfer should be conducted in Grade A (ISO 5, Class 100), unidirectional air flow to minimize particle contamination. The air in critical zones should be monitored continuously during operation to confirm compliance. Routine checks to detect particles and potential particle-generation locations should be explained in the procedures. Effective, written container-clearance procedures to be used after glass breakage should specify the number of containers to remove from the affected portion of the line. Note that improper set-up and adjustment of the filler can lead to "needle strikes" where the filling needles make contact with the container being filled. This can generate either

stainless steel or glass particles.

Filling pump design and the pump's compatibility with the filling solution are important considerations. Metal-on-metal piston pumps have a greater potential for generating metal particles, compared with other types of piston pumps. Pump maintenance is essential and includes a requirement to resurface the cylinders and pistons periodically. Peristaltic-action pumps must be monitored for generation of silicone tubing particles, especially with aggressive, near-saturated solutions or suspensions. Friction in the peristaltic roller area can break down the tubing, resulting in the generation of particles.

Stopper bowl surfaces should have a formal maintenance program, and stopper handling or replenishment by operators should be specifically designed to minimize particle transfer to the stoppers. Proper operator positioning and avoidance of open containers is important in good, aseptic filling practices, to avoid microbial contamination. These same principles help reduce particle transfer to the open containers and exposed elastomeric closures.

Careful selection of cleaning and gowning materials will help reduce contamination from extrinsic particles and fibers. These clean-room materials should be selected for their superior non-shedding and low-particle properties.

4.4 Trending

Data obtained from the inspection process are used for batch release. These data should also be analyzed for adverse trends on a periodic basis, typically at least once per year. High-volume products may generate sufficient data to allow quarterly analysis, whereas a longer period of time may be necessary to accumulate data for products that are produced infrequently. Data from component inspection, production 100% inspection, and the AQL inspections should be evaluated based upon sound statistical principles to determine whether the current action levels are accurately reflecting the current process capability. Alert levels may be introduced and/or adjusted accordingly if the statistical analyses indicate that lower defect levels are being observed consistently.

When establishing new action or alert levels, a preliminary value may be used until sufficient production experience is obtained. Consideration should be given to planned improvements in the manufacturing and inspection processes. If significant improvements are planned, the reduction of the action/alert level should not be instituted until the impact of the improvement is measured over sufficient time to establish the validity of the new value.

5. INTERPRETATION OF INSPECTION RESULTS

5.1 Defect Classification

Defects are commonly grouped into classifications based on patient and compliance risk (1). The most common system uses three groups: critical, major, and minor. Critical defects are those that may cause serious adverse reaction or death of the patient if the product is used. This classification includes any nonconformity that compromises the integrity of the container and thereby risks microbiological contamination of the sterile product. Major defects carry the risk of a temporary impairment or medically reversible reaction, or involve a remote probability of a serious adverse reaction. This classification is also assigned to any defect which causes impairment to the use of the product. These may result in a malfunction that makes the product unusable. Minor defects do not impact product performance or compliance; they are

often cosmetic in nature, affecting only product appearance or pharmaceutical elegance. For visible particles, particle motion aids in detection. Stationary particles are difficult to detect. Upon 100% inspection, visible extrinsic and intrinsic particles should be reliably removed. The test method allows inherent particles to be accepted if the product appearance specification allows inherent particle types. The size of particles reliably detected ($\geq 70\%$ probability of detection) is generally 150 μm or larger (4). This Probability of Detection (POD) is dependent on the container characteristics (e.g., size, shape, transparency), inspection conditions (lighting and duration), formulation characteristics (color and clarity), and particle characteristics (size, shape, color, and density). The POD at 70% or greater is known as the Reject Zone described in Knapp's methodology (53,54) which is used worldwide as an industry common practice for rejecting particle defects. Test sets characterized by repeated inspections, as described in 7.4 *Rejection Probability Determination*, are used to "calibrate" the inspection method's POD, inspector performance or automated inspection systems, and to demonstrate the sensitivity to threshold particle size at the Reject Zone of $>70\%$ POD. It should be understood that the limitation of the Reject Zone at 70% detection is that at this size threshold particles of the same size may routinely be missed or go undetected up to 30% of the time. These undetected units may contain some amount of threshold sized particles or sub-visible particles at a lower POD. It is therefore important to characterize any particles recovered from AQL testing, retention sample inspection and product returned from distribution to understand how it could have gone undetected originally during the initial 100% in-process inspection.

5.2 Unique Product and Container Considerations

LYOPHILIZED PRODUCT

Lyophilized products receive 100% inspection after the freeze-drying step has been completed and each unit has been sealed. However, the solid, lyophilized cake can mask the presence of visible particles because they cannot be seen within the solid matrix. The cake surface is visible during inspection but accounts for only a small fraction of the cake volume. Because of these challenges in evaluating acceptability, a small sample of units is reconstituted and inspected for visible particles in addition to the 100% inspection of the cakes for visible particles. Care must be taken during reconstitution of these samples to avoid contamination that can lead to false-positive results. Sample preparation should be done in a clean environment with appropriate particle-control measures. Reconstituted samples should be inspected using the same conditions as those for visible particles. The destructive nature of this test limits the size of the sample; however, the resultant fluid allows visible particles to be more readily detected. Typical sampling plans for this type of test can be found in the special sampling plans S-3 and S-4 in ANSI/ASQ Z1.4 (40). The S-plans offer a practical compromise between sample size and statistical power and for most batch sizes between 3,201 and 150,000 suggest a sample size of 20 with an accept number of 0 (based on an AQL of 0.65%). Alternative plans are acceptable, but care should be taken to examine the UQL of such plans to assess their sensitivity. Once inspection of these reconstituted samples has been performed, they may be used for other required testing, such as that for subvisible particles, potency, impurities, or other specified tests. If particles are detected in this relatively small sample, additional units may be reconstituted as part of an investigation and to assess the compliance of the entire batch.

POWDER PRODUCT

Sterile powders are difficult to inspect for particles due to powder flow and the occlusion of white or light-colored particles by the drug product itself. Sterile powders should be reconstituted and inspected for visible foreign particles using an approach similar to that for lyophilized products, as discussed above.

EMULSION AND SUSPENSION PRODUCT

The manufacturer may allow inherent particles if the product is an emulsion or suspension. For suspension products, a test dissolving the suspension or disruption of the emulsion that provides for extrinsic and intrinsic particle detection is also recommended as part of destructive supplemental testing of a small sample as described above for lyophilized products.

AMBER CONTAINERS

Inspecting amber containers is challenging because selected elements have been added to mask UV light penetration into the Type I glass container. Light transmission is blocked below 500 nm, and thus increased light intensity (e.g., 8,000–10,000 lux) may be required to observe visible particles during inspection. Directional lighting from behind the container may also be beneficial. At the extreme, filled solution in practically opaque containers may be audited via sampling and transfer to clear, clean containers.

TRANSLUCENT PLASTIC CONTAINERS

Plastic or translucent containers are chosen for break resistance or other properties that glass cannot offer, such as injection molding into shapes that minimize hold-up volume or for use in a combination product. Plastic containers may have optical properties that require significantly more light (e.g., 8,000–10,000 lux) to illuminate any visible particles against black and white backgrounds. Directional lighting from behind the container may also be beneficial.

LARGE-VOLUME CONTAINERS

Large-volume containers (>100 mL) may require additional time to complete a thorough inspection. For flexible bags, the semi-transparent nature of the PVC film used to manufacture these containers may require the use of additional light intensity to enhance the visibility of particles. Directional lighting from behind the container may also be beneficial.

COMBINATION PRODUCTS

When inspecting the unlabeled primary drug container for a combination product, the inspection considerations should be the same as those specified for a conventional drug product in a vial or syringe. This inspection should be performed before assembly into the device. Where there are critical attributes that are only visible after assembly (such as alignment with a fill-level window), a second inspection after assembly may also be required.

6. INSPECTION METHODS AND TECHNOLOGIES

6.1 Manual Visual Inspection

Manual visual inspection (MVI) is the reference inspection method described in all of the major pharmacopeias (55,56). It consists of viewing filled and sealed containers under controlled

conditions. This process may be aided by the use of a tool to allow consistent examination of more than one container at a time. The quality decision, to either accept or reject the container, is made by a trained person. Inspection is a probabilistic process, and detection rates <100% are to be expected, especially for smaller or low-contrast defects.

CRITICAL PROCESS PARAMETERS IN MVI

Light intensity: The results of the manual inspection process are influenced by the intensity of the light in the inspection zone. In general, increasing the intensity of the light that illuminates the container being inspected will improve inspection performance; (790) recommends light levels NLT 2,000–3,750 lux at the point of inspection for routine inspection of clear glass containers. Special attention should be given to assure that inspection is not performed below the lower limit of 2,000 lux. Increased light levels are recommended for plastic containers or those made from amber glass. Under these circumstances, light levels as high as 10,000 lux may prove beneficial. The final inspection condition will depend on measured performance. Light should be diffuse and even across the inspection zone, and it is a good practice to clearly identify this zone within the inspection station where the intensity meets the required levels. Fluorescent lamps have often been used as the light source for inspection. When fluorescent lamps are used, high-frequency ballasts are recommended to reduce visible flicker (and associated inspector fatigue). Incandescent lamps have also been used successfully for this purpose, but they generate significant heat during use. Light-emitting diodes (LED) offer an energy efficient, stable source of light without the added heat of incandescent lamps. Light intensity in each inspection station should be measured periodically to ensure continued compliance within the specified range. The frequency of monitoring should be based on historical experience with the type of light source in use. A lower light-intensity action limit should be established to trigger corrective action before inspection is performed below the lower limit of the range.

Background and contrast: Contrast between the defect of interest and the surrounding background is required for detection, and increased contrast improves detection. The use of both black and white backgrounds is described in (790), as well as other global pharmacopeias. The use of both backgrounds provides good contrast for a wide range of particulate and container defects, which can be light or dark in appearance.

Inspection rate: Sufficient time must be provided to allow for thorough inspection of each container; chapter (790) specifies a reference time of 10 s/container (5 s each against both black and white backgrounds). Larger or more complex containers may require additional time for inspecting all attributes. Increased time may facilitate detection of defects near the threshold of detection, but studies by Wolfe, et al. (57,58) suggest that there are diminishing gains with increasing inspection time. Time spent per container may be controlled through the use of a pacing device such as a light or tone, or these may be used during training only, much as a musician uses a metronome during practice to learn the tempo of a musical piece for later performance. Recording the time spent inspecting each batch and then calculating a nominal inspection rate is a good way to confirm that the rate of inspection was within established limits. Correction can be made for non-inspection activities performed during this time by the inspectors to better document the nominal inspection rate.

Container handling and movement: When observing objects, the human eye is very sensitive to movement. Good techniques for manual inspection include a careful swirl or inversion of the liquid product within the container. This rinses any particles from the upper inner surfaces of the container and the closure and puts them into motion. A technique that

minimizes the introduction of air bubbles is important, as air bubbles can appear as particles and interfere with detection of offending particles. A tool that holds multiple containers for consistent presentation can be useful when performing inspection. Holding many containers by hand at once should be avoided, as it is difficult to obtain a complete view of all container surfaces and contents. Container motion is also helpful for identifying small container defects such as cracks or chips.

Magnification: Some inspection processes use a large magnifier to increase image size and thus increase the probability of detecting and rejecting containers with defects near the threshold of detection. Although magnification can be useful for critical examination of a portion of the container, it does not often lead to increased overall detection rates for defects of interest. This may be due, in part, to the added eye strain that often results from use of magnification. As such, it is not recommended as part of the reference inspection method described in (790) or in other global pharmacopeias (55,56). Although not recommended for use during routine inspections, magnification can be helpful for critical examination of a small number of units, as may be needed during an investigation.

INSPECTOR FATIGUE AND ERGONOMIC CONSIDERATIONS

Inspecting for extended periods of time can cause inspector fatigue and a decrease in inspection performance. Based on industry experience (43), it is recommended that inspectors be given a break from performing inspection at least every hour. This break should allow time to rest the eyes and mind, and may be achieved with a short rest (e.g., 5 min) or a longer meal break. This need for regular breaks may also be met through rotation to a non-inspection function, such as material handling or documentation.

Inspection stations should be designed and operated in a manner that minimizes the inspector's risk of repetitive-motion injury. Adjustable chairs and careful positioning of light sources as well as incoming and inspected product can reduce the risk of such injury. These adjustments can also reduce inspector fatigue and discomfort, both of which can be distracting and thus can decrease performance.

The inspection room environment should also be considered. Temperature and humidity should be controlled for inspector comfort. Reduced ambient lighting is recommended to focus the inspection process and to reduce distraction from extraneous reflections. Special care should be given to inspection rooms with exterior windows that allow daylight into the room and thus changing ambient lighting throughout the day and with changing seasons.

6.2 Semi-Automated Visual Inspection

Semi-automated visual inspection combines automated material handling of the containers to be inspected with human vision and judgment to make the decision to accept or reject. These systems often use a conveyor equipped with rollers to transport the containers in front of the inspector inside an inspection booth or station. For inspection of liquids, the booth can be equipped with a high-speed spin station to set particles in motion. The rollers are also used to slowly rotate the containers in front of the inspector as they traverse the inspection zone. These systems offer a means to control the presentation of the vials and can offer additional lighting options, such as Tyndall lighting, which may enhance the appearance of some defects such as cracks or small particles. Mirrors may also be used to provide a clear view of the top and bottom of each container. Rejected units may be removed from the rollers by hand, and some systems are equipped with a remote rejection system that can be triggered by the

inspector. Care should be taken in the qualification and operation of these systems to ensure full rotation of vials in the inspection zone; this allows examination of all surfaces. In addition, studies should be conducted to ensure the detection of heavy particles, which may not be lifted from the bottom of the container, and to ensure that the rate of inspection produces an acceptable detection rate for defects of interest.

With semi-automated visual inspection, performance is similar to that with MVI. Some increase in throughput may be achieved because the inspector spends all of the available time viewing the containers, rather than splitting the time between inspection and material handling.

CRITICAL PROCESS PARAMETERS FROM SEMI-AUTOMATED INSPECTION

Light intensity must be controlled, as with MVI. The rate of inspection is controlled by the speed of the roller/conveyor. Spin speed for liquid products and rotation rate for all containers should be established during validation/qualification and maintained within the validated range for routine inspection. The background color is controlled by the color of the rollers selected and the color of the background seen through the spaces between the rollers. Qualification of inspectors and validation of the inspection equipment should be based on comparison with the compendial manual inspection process with an expectation that alternative methods such as semi-automated inspection demonstrate equivalent or better performance.

6.3 Automated Visual Inspection

Automated visual inspection (AVI) combines automated material handling of the containers with electronic sensing of product appearance. Containers that do not meet pre-programmed acceptance criteria are automatically rejected by the machine. Early machines performed inspection for particles and fill level, but manual or semi-automated inspection was required for the container and closure system. Newer models have the capability to inspect all attributes of the containers, along with the contents. As with MVI, machines often spin the containers to set particles in motion and make them easier to detect. Multiple cameras are used to image various regions on the container in great detail. Each camera is coupled with unique lighting to highlight specific defects in the region of interest. Light-field and dark-field lighting techniques offer the same benefits as white and black backgrounds as discussed above, offering contrast for a full range of light- and dark-colored defects. A defect found by any camera is tracked through the machine to allow accurate ejection by the reject system. These machines also offers detailed reporting of defects observed in a specific production lot.

AVI offers advantages in the areas of throughput and consistency, compared with MVI (4). AVI may also offer enhanced sensitivity for some defects, compared with MVI, but may suffer from higher false rejection rates due to the inability to tolerate normal variation in containers or product. This is especially true for molded glass containers and flexible bags.

Validation of the automated inspection equipment should be based on comparison with the compendial manual inspection process with an expectation that alternative inspection methods demonstrate equivalent or better performance.

LIGHT-OBSCURATION METHODS

Some systems use an optical sensor to detect the shadow of particles in solution products. This method requires particles to be in motion, typically using a high-speed spin and rapid braking of the container to achieve this motion. Spin conditions must be optimized to provide sensitivity for heavier particles while minimizing false rejections due to bubbles. Some biological

products experience shear-induced agglomeration, so care should be taken with regard to agitation of these products.

Light obscuration methods are optimized for sensitivity to moving particles, and can thus be made less sensitive to minor container imperfections. This technique can be used with both tubing and molded containers. Results are generally robust in detecting particles that are 100 μm in diameter and larger.

These systems can also detect fill height by detecting the shadow of the solution meniscus. Generally, this process is not sensitive enough to ensure compliance with dose or fill-weight specifications, but it can provide a secondary check of gross fill. Sensitivity is a function of the container shape, with greater sensitivity achieved in small-diameter containers.

IMAGING METHODS

Continuing advances in camera technology now allow the rapid capture of high-resolution images for inspection. When coupled with high-speed processors that have ever-increasing computational capability, a powerful inspection tool can result. Images are divided into inspection windows, and an array of tools such as image subtraction, pixel counting, intensity analysis, and others are used to assess the images against programmed quality attributes. Significant amounts of time are required to train inspectors to test the performance of such systems against a range of known defects, as well as acceptable containers.

Imaging systems can detect particles and fill level, as well as other container and closure attributes. Inspection in this manner can provide 100% inspection of all visual attributes. These systems can offer high sensitivity, but may also have high false-rejection rates if container and product attributes are not tightly controlled.

OTHER TECHNOLOGIES

Container-closure integrity can also be assessed using non-visual methods such as electrical conductivity and capacitance, vacuum decay, or mass extraction, for example (59). Laser-based gas headspace analysis can also be used if there is a modified headspace such as vacuum or inert gas. Generally, such nondestructive container-integrity inspection methods offer greater sensitivity than visual detection with the potential to reduce false rejection of acceptable product. See *Sterile Product Packaging—Integrity Evaluation* (1207) for further information regarding package integrity testing by these and other test methods.

X-ray imaging has also been explored as a means to detect particles within freeze-dried cakes, powders, or suspensions (60).

These technologies may be used alone or in combination with other inspection methods to provide a comprehensive assessment of product quality before labeling and packaging.

7. QUALIFICATION AND VALIDATION OF INSPECTION PROCESSES

7.1 Standards

The use of standards for visual inspection has been described by Melchore and Berdovich (61). Development of inspection standards begins with identification or characterization of the defect types that will be represented in the test set(s). This information typically comes from the manufacturing area, where naturally occurring defective units can be identified from rejected product. The defects are categorized as critical, major, or minor. These defects must be

further characterized to allow for 1) selection from naturally occurring particulate and physical or cosmetic production rejects removed from product lots, and/or 2) re-creation of equivalent defect types in a controlled laboratory environment. Characterization information on defects should include, where appropriate, the range of sizes typically observed and the specific location on the container. If feasible, a photograph of the defect should be included. All information that could support consistent re-creation of the defect standards should be included in the characterization description.

7.2 Preparing Defect Standards

Visual inspection standards may be identified from known production rejects, or may be created manually with characterized particulate material. A single particle/seeded container should be used when determining detection thresholds.

7.3 Particle Types

The primary packaging materials that directly contact the product and the potential environmental contaminants can be divided into specific particle groups such as glass, stainless steel, elastomeric closure, plastic, and fibers (synthetic or natural). Naturally occurring particles from rejects should be no smaller than the visible particle (measured in situ) in the container. Measurement can be accomplished with a wide field microscope or loupe with a calibrated reticle. Physically prepared particles can be sieved initially to target a specific size, and then the individual particles are measured using optical microscopy. These materials, or production defects, are preferred for inspector training and qualification, as well as machine validation as they better represent actual inspection performance. Spherical standard particles may be utilized as surrogates for naturally occurring particulates; however, these are best used for routine machine calibration rather than validation or inspector qualification, as they do not move or look like actual production defects.

7.4 Rejection Probability Determination

Once a well-defined defect standard is available, it is assigned a detection frequency or probability of detection (POD) by conducting a documented, manual human inspection qualification that is accomplished by repeated manual inspection. This repeated inspection is the basis for qualifying the defect standard. This approach has been described by Knapp and Kushner (53,54). The Knapp methodology recognizes that the detection of particles is probabilistic, and repeated inspections with strict controls on lighting and inspection pacing/sequencing generate the statistical confidence to assign a reject probability to each standard unit. A manual, visual inspection POD of ≥ 0.7 or 70%, is required to assign the container to the Reject Zone for subsequent calculation of the reject zone efficiency (RZE). Secure probabilistic data for particulate standards can be achieved with 30–50 inspections of each container. This is best achieved with multiple inspectors. Inspection reject probability is calculated for the defect as follows:

$$\text{POD} = (\text{Number of times rejected}) / (\text{Number of times inspected})$$

7.5 Test Sets

These qualified defect standard units are then assembled into test sets, which may be used to

specifically challenge the particle detection technique of human inspectors, used as part of a defect test set (including container–closure defects) for human qualification, or for comparison during automated equipment qualification and validation. When possible, the test set should be prepared with duplicate product units per particle type and size to ensure that backup units are available in the event that a standard container is broken or the particle is trapped or lost within the container. When using test sets, it is a good practice to verify the presence of particles before and after use, as particles may become lodged between the container and the closure. When a freely moving particle cannot be verified, the unit should not be used and the data should be excluded from subsequent calculations. When this happens, it may be possible to free the particle with the use of an ultrasonic bath. If this is not possible, the unit should be replaced. The number of defective units in each test set should be limited to approximately 10% to prevent rejection bias (57). The accept containers will be identified as having a pre-determined manual, visual inspection POD of <0.3 or 30%. Any particle standards found to fall within the acceptable “grey zone”, indicating a manual inspection rejection probability $\geq 30\%$ and $<70\%$, may be included as an “acceptable unit” in a test set, if desired.

It is important to prepare a written procedure for the creation and maintenance of standards. This procedure should define the qualification criteria, appropriate storage conditions, periodic examination and requalification, expiration, and sample custody during use. Test sets should be approved by the quality unit. The container in which the specific particle set is stored must be clearly labeled with the test set identification information.

7.6 Types of Test Sets

The particle detection threshold can be determined for a specific inspection method and product/package combination. It is a standard curve of detection probabilities at various particle types and sizes in an approximate range of 100–500 μm (with recommended increments of 100 μm). Fibers are typically observed in sizes $>500 \mu\text{m}$. The typical size range of particles used in threshold studies incorporates a variety of particle types and densities that are typically found in the manufacturing environment.

Threshold studies are conducted to determine the sensitivity of manual inspection methods, using a range of particle sizes, in a blinded study that yields the particle-size detection capabilities of a defined group or of an individual inspector. The threshold studies indicate that the method of inspection is valid and appropriate. For example, for clear solutions in 10-mL tubing glass vials, past thresholds studies indicate that particles within the range of 150–250 μm (500–2000 μm for fibers) can be detected with a POD of 70% or greater. Results can differ due to differences in product formulation as well as container type and size. Threshold studies are also useful as an assessment tool when evaluating or qualifying visual inspection staff on a specific method with fixed testing parameters. Detection threshold studies are typically the first step in evaluating the performance of any new inspection method.

Depending on product and/or presentation, rejects in the test set should represent all defects anticipated for a given container type or product family. For particles, use a bracketed range of types (densities) and sizes from near the lower limit of the visible range (100 μm) to the largest routinely observed in the pool of rejects. For an individual manual test set, it is important that all containers and closures are of the same type, and the samples are blinded. UV ink (invisible to the inspectors) may be used to mark all containers. Alternatively, bar codes or other coded labels may be used. Manual test sets can be used initially to qualify, or periodically to re-qualify, human inspectors. These test sets may also be used for direct comparison to semi-automated or automated inspection methods. If significantly different formulations (e.g., clear

solution, suspension, lyophilized) or packages (e.g., clear vials, amber vials, ampoules, syringes) are produced at the same facility, separate test sets should be prepared to represent each unique combination. A bracketing approach may be used with regard to different container sizes.

7.7 Training and Qualification of Human Inspectors

Before training, potential inspectors should be tested for visual acuity (62) and color perception. Near-vision performance should be the equivalent of 20/20 with no impairment of color vision. Both the Snellen and Jaeger charts are useful for verifying visual acuity; they test far and near vision, respectively. Training should include a phased approach with a specified number of training hours expected for each segment. Initially, train the potential inspectors with defect photographs or a video library and clear written descriptions. Utilize subject matter experts to mentor and provide hands-on training with defect standards for the specified method. Reinforce mental or silent counting and follow the paced sequence to achieve consistent inspection timing. Stress the importance of strict adherence to the inspection process (procedure, sequence, and timing). Inspector fatigue may be addressed in the qualification process by testing under worst case conditions (e.g., at the end of a typical inspection shift). Train all inspectors (QC, QA, and production) with common procedures used for 100% inspections and AQL inspections. All inspection practices should be standardized and consistently executed across all inspection groups.

Qualification should be performed for each product type and package that the inspector will encounter. A bracketed or matrix approach can be used to simplify qualification of products with similar physical or visual characteristics such as container type and size, formulation type, product viscosity, color, and others. It is common to initially train and qualify personnel on clear solutions in clear containers (if produced at the facility) and then expand their expertise to inspection of more difficult formulations or presentations.

7.8 Inspector Qualification Requirements

The qualification of all inspection personnel utilizes a manual test set to be inspected under normal operating conditions and inspection critical parameters, including inspection timing and sequence, physical environment, and inspection duration. Three successful inspections of the test set are recommended to demonstrate consistent performance for initial qualification of new inspectors. Acceptance criteria for each defect class should be based on the POD (or RZE) observed during test set qualification. A limit is also needed for false rejection, with a recommended target of <5% falsely rejected good units.

7.9 Requalification

Inspectors should be requalified at least annually. Requalification includes a test of visual acuity and testing with at least one product/test set configuration. A single successful inspection of the test set is sufficient for requalification. Requalification may also be necessary in the event that poor performance is observed during routine inspection or if the inspector has been away from the inspection operation for an extended period of time (e.g., 3 months).

If an inspector fails the requalification test, a retraining process should be initiated to identify the root cause and allow the inspector to receive additional instruction. After this process has been completed, the inspector may attempt to meet the acceptance criteria one additional

time. If the inspector fails, he or she may attempt to qualify again after a specified time period.

8. PRODUCTS IN DISTRIBUTION

Chapter (790) states, "If it becomes necessary to evaluate product that has been shipped to customers (e.g., because of a complaint or regulatory concern), sample and inspect 20 units. If no particles are observed in the sample, the batch is considered essentially free of visible particulates. If available, additional units may be inspected to gain further information on the risk of particulates in the batch."

For products in distribution, questions regarding batch quality will occasionally arise from customer complaints, observations in the field, customer use questions and from the use of non-standard (sensitive) conditions of inspection. As discussed in this chapter, the detection process is probabilistic and the likelihood of detection is a cumulative function of the particle's visible attributes, drug product and container characteristics, and the inspection method used. In an appropriately qualified manufacturing process, the batch is presumed to have been prepared according to robust processes and all containers with package defects and visible particles (non-conforming units) removed prior to labeling. In that regard, the evaluation outlined in general chapter *Visible Particulates in Injections (790), Introduction, Sampling at Batch Release (After 100% Manufacturing Inspection), Product in Distribution* is only permissible if both *Sampling at Batch Release* and a *100% Manufacturing Inspection* have been successfully completed.

The particle detection threshold should be determined for a specific inspection method and product/package combination incorporating a variety of particle types and densities that are typically found in the manufacturing environment. For example, the detection threshold for routine, reliable detection ($\geq 70\%$ probability) of a single spherical particle in a clear solution contained in a 10-mL vial utilizing diffuse illumination between 2,000 and 3,000 lux is often near 150 μm in diameter (4). Units returned from distribution may be false positive, may contain particles larger than the acceptance threshold that were missed, may contain particle(s) in the "grey zone", e.g., less than the detection threshold, or may have suffered a physicochemical change that resulted in a visible change. Ideally there were no visible particles in the containers released to market; however, there is always a low probability that this may occur. Upon receipt, suspect containers should be subjected to the same inspection conditions and methodology used in the release inspection. Particle(s) verified in the returned or re-evaluated supply must be carefully characterized by an analytical forensic process to determine their source and likely cause. Single particles of typical product-contact materials are unlikely to present a concern. Multiple particles, large particle sizes, and any particles indicative of physical or chemical change are significant events and should be subject to further investigation. Rare instances of particulate material falling into the "grey zone" should be expected given the probabilistic nature of the inspection process and should not routinely trigger further evaluation of retention samples. While (790) provides that zero particles found in the sampling and inspection of 20 units signifies that the batch is essentially free of visible particulates, if multiple suspect containers from the same batch are detected, additional units should be inspected and an appropriate rationale provided to support the batch's conformance to the registered specifications.

Overall batch quality using internal systems to control particulate matter and the means to investigate these occurrences is key to the life cycle approach for modern pharmaceutical production. Evaluation of retention and stability samples provides insight to batch quality, as do

the field-use effects for any medication. While the presence of particles or product or container defects discovered in retained or returned product do not necessarily incriminate the quality of the batch, careful investigation should be conducted to exclude systemic risks.

9. CONCLUSIONS AND RECOMMENDATIONS

Visual inspection for particles and other visible defects continues to be an important part of the manufacturing process for injections. Chapter <790> provides a useful reference method and acceptance criteria for visible particulates in injections. Successful execution of visual inspection requires an understanding of the inspection process and careful control of inspection conditions. Inspectors must be trained to ensure consistent, high-quality performance. Alternative inspection methods, either semi-automated or fully automated, may be used in place of manual inspection methods. Where machine methods are used, the equipment must be validated to demonstrate equivalent or better performance when compared to manual inspection. The use of test sets that contain standard defects is an important element in inspector training and qualification as well as machine validation. Good product development will lead to a stable product with a lower risk of particle formation. Identification of the type or types of particles found during product development and routine manufacturing is an important aid in source identification and reduction. Inspection results should be trended to further aid in continuous process improvement with the ultimate goal of defect prevention.

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BRIEFING

Reagents: Reagents, Indicators, and Solutions, page 7691 of the *Second Supplement* to *USP 38*. It is proposed to delete the entries under *4.7 Water* from the text. Each type of water is being added individually to the *Reagent Specifications* section.

Additionally, minor editorial changes have been made to update the text to current *USP* style.

(HDQ: M. Marques.)

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1. SCOPE

Reagents required in the tests and assay for *U.S. Pharmacopeia* and *National Formulary* articles and those required only in determining the quality of other reagents are listed in this section, with specifications appropriate to their intended uses.

As stated in *General Notices, 6.70 Reagents*, listing of reagents, indicators, and solutions in the *U.S. Pharmacopeia* in no way implies that they have therapeutic utility; thus, any reference to *USP* or *NF* in their labeling shall include also the term “reagent” or “reagent grade”.

Where a particular brand or source of a material, instrument, or piece of equipment, or the name and address of a manufacturer or distributor, is mentioned (ordinarily in a note or footnote), this identification is furnished solely for informational purposes as a matter of convenience, without implication of approval, endorsement, or certification.

1.1 ACS (American Chemical Society) Reagent Grade

Where it is directed to "Use ACS reagent grade", it is intended that a grade meeting the corresponding specifications of the current edition of *Reagent Chemicals*, published by the American Chemical Society (ACS), shall be used.

1.2 Suitable Grade

In the cases where no ACS reagent monograph exists or if the reagent is available in different quality grades, each one specific for a particular application, it is directed to "Use a suitable grade". The intent is that a suitable reagent grade available commercially shall be used. Occasionally, additional test(s) augment the designation "suitable grade", as indicated in the text. Listed also are some, but not all, reagents that are required only in determining the quality of other reagents. For those reagents that are not listed, satisfactory specifications are available in standard reference publications.

1.3 USP or NF or FCC Grade

In the instances in which a reagent required in a *U.S. Pharmacopeia* or *National Formulary* test or assay meets the requirements in the monograph for that article appearing in this *U.S. Pharmacopeia* or the *National Formulary* or the current edition of the *Food Chemicals Codex (FCC)*, it suffices to refer to the corresponding monograph in one of the these three compendia. In such cases it is to be understood that the specifications are minimum requirements and that any substance meeting more rigid specifications for chemical purity is suitable.

2. PACKAGING AND STORAGE

Reagents and solutions should be preserved in tight containers made of resistant glass or other suitable material. Directions for storage in light-resistant containers should be carefully observed.

Stoppers and stopcocks brought into contact with substances capable of attacking or penetrating their surfaces may be given a protective coating of a thin film of a suitable lubricant unless specifically interdicted.

3. METAL-ION STANDARD SOLUTIONS

Atomic absorption and flame photometry require the use of a number of metal-ion standard solutions. While the individual monographs usually provide directions for preparation of these solutions, use of commercially prepared standardized solutions of the appropriate ions is permissible, provided that the analyst confirms the suitability of the solutions and has data to support their use.

Change to read:

4. DEFINITIONS

4.1 Reagents: Reagents are substances used either as such or as constituents of solutions.

4.2 Indicators: Indicators are reagents used to determine the specified endpoint in a chemical reaction, to measure hydrogen-ion concentration (pH), or to indicate that a desired change in pH has been effected. They are listed together with indicator test papers.

4.3 Buffer Solutions: Buffer solutions resist changes in the activity of an ion on the addition of substances that are expected to change the activity of that ion.

4.4 Colorimetric Solutions (CS): Colorimetric solutions are solutions used in the preparation of colorimetric standards for comparison purposes.

4.5 Test Solutions (TS): Test solutions are solutions of reagents in such solvents and of such definite concentrations as to be suitable for the specified purposes.

4.6 Volumetric Solutions (VS): Volumetric solutions are solutions of reagents of known concentration intended primarily for use in quantitative determinations.

~~**4.7 Water:**—As elsewhere in the *U.S. Pharmacopeia*, where “water”, without qualification, is mentioned in the tests for reagents or in directions for preparing any solutions, *Purified Water* (USP monograph) is always to be used.~~

~~—4.7.1 CARBON DIOXIDE-FREE WATER:—It is *Purified Water* that has been boiled vigorously for 5 min or more and allowed to cool while protected from absorption of carbon dioxide from the atmosphere, or *Purified Water* that has a resistivity of NLT 18 Mohm-cm.~~

~~—4.7.2 DEAERATED WATER:—For purposes other than dissolution and drug release testing, it is *Purified Water* that has been treated to reduce the content of dissolved air by suitable means, such as by boiling vigorously for 5 min and cooling or by application of ultrasonic vibration.~~

~~—4.7.3 PARTICLE-FREE WATER:—It is water that has been passed through a suitable filter of 0.22- μ m pore size.~~

~~—4.7.4 ORGANIC-FREE WATER:—It is *Purified Water* that produces no significantly interfering peaks when chromatographed as indicated in *Residual Solvents* (467), *Identification, Control, and Quantification of Residual Solvents*.~~

▲▲USP40

5. CHROMATOGRAPHIC SOLVENTS AND CARRIER GASES

The chromatographic procedures set forth in the *U.S. Pharmacopeia* may require use of solvents and gases that have been especially purified for such use. The purpose may be (a) to exclude certain impurities that interfere with the proper conduct of the test procedure, or (b) to extend the life of a column by reducing the buildup of impurities on the column. Where solvents and gases are called for in chromatographic procedures, it is the responsibility of the analyst to ensure the suitability of the solvent or gas for the specific use. Solvents and gases suitable for specific high-pressure or other chromatographic uses are available as specialty products from various reagent supply houses, although there is no assurance that similar products from different suppliers are of equivalent suitability in any given procedure.

BRIEFING

Copper Sulfate Pentahydrate. It is proposed to add this new reagent.

(HDQ: M. Marques.)

Correspondence Number—C164326

Comment deadline: January 31, 2016

Add the following:

▲ Copper Sulfate Pentahydrate (*Cupric Sulfate Pentahydrate*), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ —**249.68** [7758-99-8]—Use ACS Cupric Sulfate Pentahydrate reagent grade. ▲USP40

BRIEFING

1,1-Cyclobutanedicarboxylic Acid. It is proposed to add this new reagent used in the *Limit of 1,1-Cyclobutanedicarboxylic Acid* test in the monographs for *Carboplatin* and *Carboplatin for Injection*.

(HDQ: M. Marques.)

Correspondence Number—C165298

Comment deadline: January 31, 2016

Add the following:

▲ 1,1-Cyclobutanedicarboxylic Acid (*Cyclobutane-1,1-dicarboxylic acid; 1,1-Dicarboxycyclobutane*), $C_6H_8O_4$ —**144.13** [5445-51-2]—Use a suitable grade with a content of NLT 99%.

[Note—A suitable grade is available as catalog number C95803 from www.sigmaaldrich.com.]

▲USP40

BRIEFING

Hydroxypropyl Cellulose. It is proposed to add this new reagent.

(HDQ: M. Marques.)

Correspondence Number—C163758

Comment deadline: January 31, 2016

Add the following:

▲ Hydroxypropyl Cellulose, Average molecular weight approximately **100,000** [9004-64-2]

VISCOSITY—Dissolve 5.0 g of Hydroxypropyl Cellulose with 95.0 mL of water by stirring. If necessary, centrifuge the solution to expel any entrapped air bubbles. Determine the viscosity of the solution as directed in *Viscosity—Rotational Methods* (912) at $25 \pm 0.1^\circ$. The viscosity is between 75 and 150 centipoises. ▲USP40

BRIEFING

Isonicotinic Acid, *USP* 38 page 1846. It is proposed to correct the CAS number of this reagent.

(HDQ: M. Marques.)

Correspondence Number—C165257

Comment deadline: January 31, 2016

Change to read:

Isonicotinic Acid, $C_6H_5NO_2$ —**123.11** [~~52-22-1~~

▲ 55-22-1 ▲USP40

]—Use a suitable grade.

BRIEFING

Lithium. It is proposed to add this new reagent.

(HDQ: M. Marques.)

Correspondence Number—C165112

Comment deadline: January 31, 2016

Add the following:

▲ Lithium, Li—**6.94** [7439-93-2]—Use a suitable grade (ribbon, granular, or wire) with a content of NLT 99.9%. ▲*USP40*

BRIEFING

Malonic Acid. It is proposed to add this new reagent used in the test for *Limit of Acetic Acid/Acetate* in the monograph for *Polyvinyl Acetate Dispersion*, published in *PF* 41(5) [Sept.–Oct. 2015].

(HDQ: M. Marques.)

Correspondence Number—C164754

Comment deadline: January 31, 2016

Add the following:

▲ Malonic Acid (*Propanedioic Acid*), $C_3H_4O_4$ —**104.06** [141-82-2]—Use a suitable grade with a content of NLT 98%. ▲*USP40*

BRIEFING

d-Mannitol. It is proposed to add this new reagent.

(HDQ: M. Marques.)

Correspondence Number—C164320

Comment deadline: January 31, 2016

Add the following:

▲ d-Mannitol—Use Mannitol USP. ▲*USP40*

BRIEFING

Marfey's Reagent. It is proposed to add this new reagent used in the *Enantiomeric Purity* test in the new monograph for *Pregabalin*, also appearing in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C136074

Comment deadline: January 31, 2016

Add the following:

▲ Marfey's Reagent [N_α -(2,4-dinitro-5-fluorophenyl)-l-alaninamide, FDAA], $C_9H_9FN_4O_5$ —**272.19** [95713-52-3]—Use a suitable grade with a content of NLT 99.0%. ▲*USP40*

BRIEFING

Methyl cis-11-eicosenoate. It is proposed to add this new reagent used in *Identification, A. Identity by Fatty Acid Composition* in the monograph for *Castor Oil* and in *Identification, C. Identity by Fatty Acid Composition* in the monograph for *Polyoxyl 35 Castor Oil*, both published in *PF* 41(5) [Sept.–Oct. 2015].

(HDQ: M. Marques.)

Correspondence Number—C164757

Comment deadline: January 31, 2016

Add the following:

▲ **Methyl cis-11-eicosenoate** (*cis-11-eicosenoic acid methyl ester*), $C_{21}H_{40}O_2$ —**324.54** [2390-09-2]—Use a suitable grade with a content of NLT 98%. ▲*USP40*

BRIEFING

Methyl 12-Ketostearate. It is proposed to add this new reagent used in *Identification, A. Identity by Fatty Acid Composition* in the monograph for *Hydrogenated Castor Oil* and in *Identification, A. Identity by Fatty Acid Composition* in the monograph for *Polyoxyl 40 Hydrogenated Castor Oil*, both published in *PF* 41(5) [Sept.–Oct. 2015].

(HDQ: M. Marques.)

Correspondence Number—C164755

Comment deadline: January 31, 2016

Add the following:

▲ **Methyl 12-Ketostearate** (*Methyl 12-oxooctadecanoate*), $C_{19}H_{36}O_3$ —**312.49** [2380-27-0]—Use a suitable grade with a content of NLT 97%. [Note—A suitable grade is available as catalog number sc-235651 from www.scbt.com or as catalog number M5306 from www.usbio.net.] ▲*USP40*

BRIEFING

Nickel Nitrate Hexahydrate. It is proposed to add this new reagent.

(HDQ: M. Marques.)

Correspondence Number—C164322

Comment deadline: January 31, 2016

Add the following:

▲ **Nickel Nitrate Hexahydrate** [*Nickel(III) Nitrate Hexahydrate, Nickelous Nitrate Hexahydrate*], $Ni(NO_3)_2 \cdot 6H_2O$ —**290.81** [13478-00-7]—Use ACS reagent grade. ▲*USP40*

BRIEFING

Propionic Acid. It is proposed to add this new reagent.

(HDQ: M. Marques.)

Correspondence Number—C164327

Comment deadline: January 31, 2016

Add the following:

▲ **Propionic Acid** (*Propanoic Acid*), $C_3H_6O_2$ —**74.08** [79-09-4]—Use ACS reagent grade. ▲*USP40*

BRIEFING

Sodium 1-Dodecanesulfonate. It is proposed to add this new reagent.

(HDQ: M. Marques.)

Correspondence Number—C163759

Comment deadline: January 31, 2016

Add the following:

▲ Sodium 1-Dodecanesulfonate (*1-Dodecanesulfonic Acid Sodium Salt*), $C_{12}H_{25}NaSO_3$ —**272.38**
[2386-53-0]—Use a suitable grade with a content of NLT 99%. ▲*USP40*

BRIEFING

Tetrahydrofuran, *USP 38* page 1874. It is proposed to add the specification for chromatographic applications.

Additionally, minor editorial changes have been made to update the reagent to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C163685

Comment deadline: January 31, 2016

Change to read:

Tetrahydrofuran, C_4H_8O —**72.11** [109-99-9]—Use ACS reagent grade.

▲ **For chromatographic application:** Use a suitable grade, inhibitor-free, with a content of NLT 99.9%.

▲*USP40*

BRIEFING

Water. It is proposed to add the specification of water for analytical purposes to the *Reagent Specifications* section.

(HDQ: M. Marques.)

Correspondence Number—C164477

Comment deadline: January 31, 2016

Add the following:

▲ Water—Use *USP Purified Water*. ▲*USP40*

BRIEFING

Water, Carbon Dioxide-Free, *USP 38* page 1880. It is proposed to add the specification of this type of water for analytical purposes to the *Reagent Specifications* section.

Additionally, minor editorial changes have been made to update this reagent to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C164564

Comment deadline: January 31, 2016

Change to read:

Water, Carbon Dioxide-Free—~~See *Water*, in the introductory section.~~

▲ It is *Purified Water* that has been boiled vigorously for 5 min or more and allowed to cool while protected from absorption of carbon dioxide (CO₂) from the atmosphere, or *Purified Water* that has a resistivity of NLT 18 Mohm-cm. ▲*USP40*

BRIEFING

Water, Deaerated, *USP 38* page 1880. It is proposed to add the specification of this type of water for analytical purposes to the *Reagent Specifications* section.

Additionally, minor editorial changes have been made to update this reagent to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C164563

Comment deadline: January 31, 2016

Change to read:

Water, Deaerated—~~See *Water*, in the introductory section.~~

▲ For purposes other than dissolution and drug release testing, it is *Purified Water* that has been treated to reduce the content of dissolved air by suitable means, such as by boiling vigorously for 5 min and cooling or by application of ultrasonic vibration. ▲*USP40*

BRIEFING

Water, Organic-Free. It is proposed to add the specification of this type of water for analytical purposes to the *Reagent Specifications* section.

(HDQ: M. Marques.)

Correspondence Number—C164481

Comment deadline: January 31, 2016

Add the following:

▲ Water, Organic-Free—It is *Purified Water* that produces no significantly interfering peaks when chromatographed as indicated in *Residual Solvents* (467), *Identification, Control, and Quantification of Residual Solvents*. ▲*USP40*

BRIEFING

Water, Particle-Free. It is proposed to add the specification of this type of water for analytical purposes to the *Reagent Specifications* section.

(HDQ: M. Marques.)

Correspondence Number—C164480

Comment deadline: January 31, 2016

Add the following:

▲ **Water, Particle-Free**—It is *Purified Water* that has been passed through a suitable filter of 0.22- μm pore size. ▲*USP40*

BRIEFING

2 N Sodium Hydroxide TS. It is proposed to add this new test solution used in the monograph for *Acamprosate Delayed-Release Tablets*, also appearing in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C141734

Add the following:

▲ **2 N Sodium Hydroxide TS**—Dissolve 80 g of *sodium hydroxide* in about 800 mL of water. Cool and dilute with water to 1000 mL. ▲*USP40*

BRIEFING

1 M Acetic Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C164761

Add the following:

▲ **1 M Acetic Acid TS**—Transfer 57.4 mL of *glacial acetic acid* to a 1000-mL volumetric flask. Dilute with *water* to volume. ▲*USP40*

BRIEFING

2 M Acetic Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C164830

Add the following:

▲ **2 M Acetic Acid TS**—Transfer 114.8 mL of *glacial acetic acid* to a 1000-mL volumetric flask. Dilute with *water* to volume. ▲*USP40*

BRIEFING

1 M Ammonium Hydroxide TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C164758

Add the following:

▲ **1 M Ammonium Hydroxide TS**—Transfer 67.6 mL of *ammonium hydroxide* to a 1000-mL volumetric flask. Dilute with *water* to volume. ▲*USP40*

BRIEFING

2 M Ammonium Hydroxide TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C164759

Add the following:

▲ **2 M Ammonium Hydroxide TS**—Transfer 135 mL of *ammonium hydroxide* to a 1000-mL

volumetric flask. Dilute with water to volume. ▲USP40

BRIEFING

0.05 N Hydrochloric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C163434

Add the following:

▲ **0.05 N Hydrochloric Acid TS**—Transfer 4.1 mL of hydrochloric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume. ▲USP40

BRIEFING

1 N Phosphoric Acid TS. It is proposed to add this new test solution used in the *Dissolution* test in the monograph for *Acamprosate Delayed-Release Tablets*, also appearing in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C141734

Add the following:

▲ **1 N Phosphoric Acid TS**—To a 1000-mL volumetric flask containing about 800 mL of water, slowly add 22.8 mL of phosphoric acid. Cool and dilute with water to volume. ▲USP40

BRIEFING

1.8 N Potassium Hydroxide TS. It is proposed to add this new test solution used in the *Dissolution* test in the monograph for *Acamprosate Delayed-Release Tablets*, also appearing in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C141734

Add the following:

▲ **1.8 N Potassium Hydroxide TS**—Dissolve 101 g of potassium hydroxide in about 800 mL of water. Cool and dilute with water to 1000 mL. ▲USP40

BRIEFING

0.02 M Monobasic Potassium Phosphate TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C163444

Add the following:

▲ **0.02 M Monobasic Potassium Phosphate TS**—Transfer 2.72 g of monobasic potassium phosphate to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. ▲USP40

BRIEFING

0.0025 N Sodium Hydroxide TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C164833

Add the following:

▲ **0.0025 N Sodium Hydroxide TS**—Transfer 12.5 mL of 0.2 N sodium hydroxide TS to a 1000-mL volumetric flask. Dilute with water to volume. ▲USP40

BRIEFING

2.5 N Sodium Hydroxide TS. It is proposed to add this new test solution used in the monograph for *Acamprosate Delayed-Release Tablets*, also appearing in this issue of *PF*.

(HDQ: M. Marques.)

Correspondence Number—C141734

Add the following:

▲ **2.5 N Sodium Hydroxide TS**—Dissolve 100 g of sodium hydroxide in about 800 mL of water. Cool and dilute with water to 1000 mL. ▲USP40

BRIEFING

10 N Sodium Hydroxide TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C163373

Add the following:

▲ **10 N Sodium Hydroxide TS**—Transfer 400 g of sodium hydroxide to a 1000-mL volumetric flask, and dissolve in about 800 mL of water. Cool and dilute with water to volume. ▲USP40

BRIEFING

0.2 N Sulfuric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C163636

Add the following:

▲ **0.2 N Sulfuric Acid TS**—Transfer 5.6 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume. ▲USP40

BRIEFING

0.5 N Sulfuric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C163637

Add the following:

▲ **0.5 N Sulfuric Acid TS**—Transfer 14 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume. ▲USP40

BRIEFING

2 N Sulfuric Acid TS. It is proposed to add this new test solution.

(HDQ: M. Marques.)

Correspondence Number—C163640

Add the following:

▲ 2 N Sulfuric Acid TS—Transfer 56.2 mL of *sulfuric acid* to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume. ▲*USP40*

BRIEFING

Acetic Acid, Double-Normal (2 N), *USP 38* page 1894. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C163372

Comment deadline: January 31, 2016

Change to read:

~~**Acetic Acid, Double-Normal (2 N)**~~

▲ 2 N Acetic Acid VS▲*USP40*

$C_2H_4O_2$, **60.05**

120.10 g in 1000 mL

Add 116 mL of *glacial acetic acid* to sufficient *water* to make 1000 mL after cooling to room temperature.

BRIEFING

Ammonium Thiocyanate, Tenth-Normal (0.1 N), *USP 38* page 1894. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C164484

Comment deadline: January 31, 2016

Change to read:

~~**Ammonium Thiocyanate, Tenth-Normal (0.1 N)**~~

▲ 0.1 N Ammonium Thiocyanate VS▲*USP40*

NH_4SCN , **76.12**

7.612 g in 1000 mL

Dissolve about 8 g of ammonium thiocyanate in 1000 mL of water. ~~and standardize the solution as follows:~~

▲ Standardization: ▲*USP40*

Accurately measure about 30 mL of 0.1 N silver nitrate VS into a glass-stoppered flask. Dilute with 50 mL of water, then add 2 mL of nitric acid and 2 mL of ferric ammonium sulfate TS, and titrate with the ammonium thiocyanate solution to the first appearance of a red-brown color.

$$N = \frac{\text{mL AgNO}_3 \times N \text{ AgNO}_3}{\text{mL NH}_4\text{SCN Solution}}$$

If desirable, 0.1 N ammonium thiocyanate may be replaced by 0.1 N potassium thiocyanate

where the former is directed in various tests and assays.

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Bismuth Nitrate, 0.01 M, *USP 38* page 1894. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C164565

Comment deadline: January 31, 2016

Change to read:

Bismuth Nitrate, 0.01 M

▲ **0.01 M Bismuth Nitrate VS**▲*USP40*

Bi(NO₃)₃·5H₂O, **485.07**

1000 mL of this solution contains 4.851 g of bismuth nitrate pentahydrate

Dissolve 4.86 g of *bismuth nitrate pentahydrate* in 60 mL of *diluted nitric acid*, and add 0.01 N *nitric acid* to make 1000 mL. ~~and standardize the solution as follows.~~

▲ **Standardization:** ▲*USP40*

Accurately measure 25 mL of the prepared bismuth nitrate solution, add 50 mL of *water* and 1 drop of *xylene orange TS*, and titrate the solution with 0.01 M edetate disodium VS until the red color changes to yellow. Calculate the molarity factor.

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Bromine, Tenth-Normal (0.1 N), *USP 38* page 1894. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C164566

Comment deadline: January 31, 2016

Change to read:

Bromine, Tenth-Normal (0.1 N)

▲ **0.1 N Bromine VS**▲*USP40*

Br, **79.90**

7.990 g in 1000 mL

Dissolve 3 g of *potassium bromate* and 15 g of *potassium bromide* in *water* to make 1000 mL. ~~and standardize the solution as follows~~

▲ **Standardization:** ▲*USP40*

Accurately measure about 25 mL of the solution into a 500-mL iodine flask, and dilute with 120 mL of *water*. Add 5 mL of *hydrochloric acid*, insert the stopper in the flask, and shake it gently. Then add 5 mL of *potassium iodide TS*, again insert the stopper, shake the mixture, allow it to

stand for 5 min, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached.

Preserve in dark amber-colored, glass-stoppered bottles.

$$N = \frac{\text{mL Na}_2\text{S}_2\text{O}_3 \times N\text{Na}_2\text{S}_2\text{O}_3}{\text{mL Br}_2 \text{ Solution}}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Ceric Ammonium Nitrate, Twentieth-Normal (0.05 N), *USP 38* page 1895. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C164569

Comment deadline: January 31, 2016

Change to read:

~~**Ceric Ammonium Nitrate, Twentieth-Normal (0.05 N)**~~

▲ **0.05 N Ceric Ammonium Nitrate VS**▲*USP40*

$\text{Ce}(\text{NO}_3)_4 \cdot 2\text{NH}_4\text{NO}_3$, **548.22**

2.741 g in 100 mL

Dissolve 2.75 g of *ceric ammonium nitrate* in 1 N *nitric acid* to obtain 100 mL of solution, and filter. ~~Standardize the solution as follows.~~

▲ **Standardization:** ▲*USP40*

Accurately measure 10 mL of freshly standardized 0.1 N *ferrous ammonium sulfate VS* into a flask, and dilute with *water* to about 100 mL. Add 1 drop of *nitrophenanthroline TS*, and titrate with the ceric ammonium nitrate solution to a colorless endpoint.

$$N = \frac{\text{mL Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \times N \text{ Fe}(\text{NH}_4)_2(\text{SO}_4)_2}{\text{mL Ce}(\text{NO}_3)_4 \cdot 2\text{NH}_4\text{NO}_3}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Ceric Sulfate, Tenth-Normal (0.1 N), *USP 38* page 1895. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C164570

Comment deadline: January 31, 2016**Change to read:****Ceric Sulfate, Tenth-Normal (0.1 N)****▲ 0.1 N Ceric Sulfate VS ▲^{USP40}**Ce(SO₄)₂, **332.24**

33.22 g in 1000 mL

Use commercially available volumetric standard solution. ~~Standardize the solution as follows:~~**▲ Standardization: ▲^{USP40}**

Accurately weigh about 0.2 g of *sodium oxalate*, primary standard, dried according to the instructions on its label, and dissolve in 75 mL of *water*. Add, with stirring, 2 mL of *sulfuric acid* that has previously been mixed with 5 mL of *water*, mix well, add 10 mL of *hydrochloric acid*, and heat to between 70° and 75°. Titrate with 0.1 N ceric sulfate to a permanent slight yellow color. Each 6.700 mg of sodium oxalate is equivalent to 1 mL of 0.1 N ceric sulfate.

$$N = \frac{\text{mg Na}_2\text{C}_2\text{O}_4}{67.00 \times \text{mL Ce(SO}_4)_2 \text{ solution}}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.] ▲^{USP40}

BRIEFING

Cupric Nitrate, Tenth-Normal (0.1 N), *USP 38* page 1895. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C164715

Comment deadline: January 31, 2016**Change to read:****Cupric Nitrate, Tenth-Normal (0.1 N)****▲ 0.1 N Cupric Nitrate VS ▲^{USP40}**Cu(NO₃)₂·2.5H₂O, **232.59**

23.26 g in 1000 mL

Cu(NO₃)₂·3H₂O, **241.60**

24.16 g in 1000 mL

Dissolve 23.3 g of *cupric nitrate 2.5 hydrate*, or 24.2 g of the *trihydrate*, in *water* to make 1000 mL. ~~Standardize the solution as follows:~~

▲ Standardization: ▲^{USP40}

Transfer 20.0 mL of the solution to a 250-mL beaker. Add 2 mL of 5 M sodium nitrate, 20 mL of *ammonium acetate TS*, and sufficient *water* to make 100 mL. Titrate with 0.05 M *edetate disodium VS*. Determine the endpoint potentiometrically using a cupric ion-double junction reference electrode system. Perform a blank determination, and make any necessary correction.

$$N = \frac{\text{mL edetate disodium (corrected for the blank)} \times M \text{ edetate disodium}}{20.0}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Cupric Tartrate, Alkaline, Solution (Fehling's Solution), *USP 38* page 1895. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C164716

Comment deadline: January 31, 2016

Change to read:

Cupric Tartrate, Alkaline, Solution (Fehling's Solution)

Copper solution (Solution A)—Transfer 34.639 g of *cupric sulfate* to a 500-mL volumetric flask, and dissolve in and dilute with *water* to volume. Filter, if necessary.

Alkaline tartrate solution (Solution B)—Transfer 173 g of *potassium sodium tartrate* and 50 g of *sodium hydroxide* to a 500-mL volumetric flask, and dissolve in and dilute with *water* to volume. Filter, if necessary.

Just before use, prepare *Cupric tartrate, alkaline, solution* by mixing equal volumes of *Solution A* and *Solution B*. ~~Standardize this solution as follows.~~

▲ **Standardization**▲*USP40*

Standard stock solution—Transfer 9.5 g of *sucrose* to a 1-L volumetric flask, dissolve in 100 mL of *water*, add 5 mL of *hydrochloric acid*, and store 3 days at 20°–25°. Dilute with *water* to volume. This solution is stable for several months.

Invert sugar solution—Immediately before use in standardizing the *Cupric tartrate, alkaline, solution*, transfer 25 mL of *Standard stock solution* to a 100-mL volumetric flask, and dilute with *water* to volume.

Procedure

Apparatus—Mount a ring support on a ring stand 1–2 inches above a gas burner, and mount a second ring 6–7 inches above the first. Place 6-inch open-wire gauze on the lower ring to support a 400-mL conical flask, and place a 4-inch watch glass with a center hole on the upper ring to deflect heat. Attach a 50-mL buret to the ring stand so that the tip just passes through the watch glass centered above the flask. Place an indirectly lighted white surface behind the assembly for observing the endpoint.

Standardization—Transfer 20.0 mL of the *Cupric tartrate, alkaline, solution* to a 400-mL flask containing a few boiling chips, and add 15 mL of *water* and 39.0 mL of *Invert sugar solution*. Mix by swirling at ambient temperature, and immediately place the flask on the wire gauze of the *Apparatus*. Adjust the burner so that the boiling point of the solution is reached in about 2 min. Boil gently but steadily for 2 min. As boiling continues, add 3–4 drops of *methylene blue* solution (1 in 100). Complete the titration within 1 min by adding the *Invert sugar solution* dropwise until the blue color disappears. Allow a 5-s reaction time between drops at the end of titration. Adjust the *Cupric tartrate, alkaline, solution* for the correct amount of copper (equivalent to 100 mg of invert sugar), and restandardize if the total volume of *Invert sugar solution* is more or less than 40.0 mL.

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Edetate Disodium, Twentieth-Molar (0.05 M), *USP 38* page 1895 and *PF 41(1)* [Jan.–Feb. 2015]. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C164719

Comment deadline: January 31, 2016

Change to read:

Edetate Disodium, Twentieth-Molar (0.05 M)

▲ **0.05 M Edetate Disodium VS**▲*USP40*

$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, **372.24**

18.61 g in 1000 mL

Dissolve 18.6 g of *edetate disodium* in *water* to make 1000 mL. ~~and standardize the solution as follows~~

▲ **Standardization:** ▲*USP40*

Accurately weigh about 200 mg of *chelometric standard calcium carbonate*, previously dried at ~~110° for 2 hours and cooled in a desiccator, or dried according to the label instructions,~~▲ according to the label instructions or, if this information is not available, at 110° for 2 h,▲*USP39* transfer to a 400-mL beaker, add 10 mL of *water*, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 2 mL of *diluted hydrochloric acid* from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass with *water*, and dilute with *water* to about 100 mL. While stirring the solution, preferably with a magnetic stirrer, add about 30 mL of the edetate disodium solution from a 50-mL buret. Add 15 mL of *sodium hydroxide TS* and 300 mg of *hydroxy naphthol blue*, and continue the titration with the edetate disodium solution to a blue endpoint.

$$M = \frac{(\text{g CaCO}_3)(1000)}{100.09 \times \text{mL EDTA}}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Edetate Disodium, Tenth-Molar (0.1 M), *PF 40(6)* [Nov.–Dec. 2014]. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C164718

Comment deadline: January 31, 2016

Change to read:

▲ **Edetate Disodium, Tenth-Molar (0.1 M)**

▲ **0.1 M Edetate Disodium VS**▲*USP40*

$\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$ **372.24**

37.22 g in 1000 mL

Dissolve 37.22 g of *edetate disodium* in *water* to make 1000 mL. ~~and standardize the solution as follows:~~

▲ **Standardization:** ▲*USP40*

Accurately weigh about 400 mg of *chelometric standard calcium carbonate*, previously dried at 110° for 2 h and cooled in a desiccator, or dried according to the label instructions, transfer to a 400-mL beaker, add 10 mL of *water*, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 4 mL of *diluted hydrochloric acid* from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass with *water*, and dilute with *water* to about 200 mL. While stirring the solution, preferably with a magnetic stirrer, add about 30 mL of the edetate disodium solution from a 50-mL buret. Adjust the solution to a pH of 12–13 with *sodium hydroxide TS*, add 300 mg of *hydroxy naphthol blue*, and continue the titration with the edetate disodium solution to a blue endpoint.

$$M = \frac{(\text{g CaCO}_3)(1000)}{100.09 \times \text{mL EDTA}}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

▲*USP39*

BRIEFING

Ferric Ammonium Sulfate, Tenth-Normal (0.1 N), *USP 38* page 1896. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C164932

Comment deadline: January 31, 2016

Change to read:

Ferric Ammonium Sulfate, Tenth-Normal (0.1 N)

▲ **0.1 N Ferric Ammonium Sulfate VS**▲*USP40*

$\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, **482.19**

48.22 g in 1000 mL

Dissolve 50 g of *ferric ammonium sulfate* in a mixture of 300 mL of *water* and 6 mL of *sulfuric acid*, dilute with *water* to 1000 mL, and mix. ~~Standardize the solution as follows.~~

▲ **Standardization:** ▲*USP40*

Accurately measure about 40 mL of the solution into a glass-stoppered flask, add 5 mL of *hydrochloric acid*, mix, and add a solution of 3 g of *potassium iodide* in 10 mL of *water*. Insert the stopper, allow to stand for 10 min, and then titrate the liberated iodine with *0.1 N sodium thiosulfate VS*, adding 3 mL of *starch TS* as the endpoint is approached. Correct for a blank run on the same quantities of the same reagents.

Store in tight containers, protected from light.

$$N = \frac{\text{mL Na}_2\text{S}_2\text{O}_3 \times N \text{ Na}_2\text{S}_2\text{O}_3}{\text{mL FeNH}_4(\text{SO}_4)_2}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Ferrous Ammonium Sulfate, Tenth-Normal (0.1 N), *USP 38* page 1896. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165039

Comment deadline: January 31, 2016

Change to read:**Ferrous Ammonium Sulfate, Tenth-Normal (0.1 N)****▲ 0.1 N Ferrous Ammonium Sulfate VS**▲USP40Fe(NH₄)₂(SO₄)₂·6H₂O, **392.14**

39.21 g in 1000 mL

Dissolve 40 g of *ferrous ammonium sulfate* in a previously cooled mixture of 40 mL of *sulfuric acid* and 200 mL of *water*, dilute with *water* to 1000 mL, and mix. ~~On the day of use, standardize the solution as follows:~~

▲ Standardization (carried out on the day of use): ▲USP40

Accurately measure 25–30 mL of the solution into a flask, add 2 drops of *orthophenanthroline TS*, and titrate with *0.1 N ceric sulfate VS* until the red color is changed to pale blue.

$$N = \frac{\text{mL Ce}^{\text{IV}} \times N \text{ Ce}^{\text{IV}}}{\text{mL Fe}^{\text{II}} \text{ Solution}}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲USP40

BRIEFING

0.02 N Hydrochloric Acid VS. It is proposed to add this new volumetric solution.

(HDQ: M. Marques.)

Correspondence Number—C161803

Comment deadline: January 31, 2016**Add the following:****▲ 0.02 N Hydrochloric Acid VS**HCl, **36.46**

729.2 mg in 1000 mL

Transfer 1.6 mL of *hydrochloric acid* to a 1000 mL volumetric flask containing about 250 mL of *water*. Cool and dilute to volume with *water*.

Alternatively, dilute 20 mL of *1 N Hydrochloric Acid VS* to 1000 mL with *water*.

Standardization: Accurately weigh about 50 mg of *tromethamine*, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h, and dissolve in 50 mL of *water*. Determine the endpoint potentiometrically. Each 2.4228 mg of *tromethamine* is equivalent to 1 mL of 0.02 N hydrochloric acid.

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL HCl}}$$

[Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Hydrochloric Acid, Alcoholic, Tenth-Molar (0.1 M), *USP 38* page 1896. It is proposed to revise this volumetric solution to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C165040

Comment deadline: January 31, 2016

Change to read:

Hydrochloric Acid, Alcoholic, Tenth-Molar (0.1 M)

▲ **0.1 M Alcoholic Hydrochloric Acid VS**▲*USP40*

HCl, **36.46**

Dilute 9.0 mL of *hydrochloric acid* to 1000 mL with *aldehyde-free alcohol*.

BRIEFING

Hydrochloric Acid, Half-Normal (0.5 N), *USP 38* page 1896. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165042

Comment deadline: January 31, 2016

Change to read:

Hydrochloric Acid, Half-Normal (0.5 N)

▲ **0.5 N Hydrochloric Acid VS**▲*USP40*

HCl, **36.46**

18.23 g in 1000 mL

To a 1000-mL volumetric flask containing 40 mL of *water* slowly add 43 mL of *hydrochloric acid*. Cool, and add *water* to volume. ~~Standardize the solution as follows.~~

▲ **Standardization:** ▲*USP40*

Accurately weigh about 2.5 g of *tromethamine*, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. Dissolve in 50 mL of *water*, and add 2 drops of *bromocresol green TS*. Titrate with 0.5 N hydrochloric acid to a pale yellow endpoint. Each 60.57 mg of *tromethamine* is equivalent to 1 mL of 0.5 N hydrochloric acid.

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL HCl}}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Hydrochloric Acid, Half-Normal (0.5 N) in Methanol, *USP 38* page 1896. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165041

Comment deadline: January 31, 2016

Change to read:

Hydrochloric Acid, Half-Normal (0.5 N) in Methanol

▲ **0.5 N Hydrochloric Acid in Methanol VS**▲*USP40*

HCl, **36.46**

18.23 g in 1000 mL

To a 1000-mL volumetric flask containing 40 mL of *water* slowly add 43 mL of *hydrochloric acid*. Cool, and add *methanol* to volume. ~~Standardize the solution as follows:~~

▲ **Standardization:** ▲*USP40*

Accurately weigh about 2.5 g of *tromethamine*, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. ~~Proceed as directed under *Hydrochloric Acid, Normal (1 N)*, beginning with "Dissolve in 50 mL of water."~~

▲ Dissolve in 50 mL of *water* and add 2 drops of *bromocresol green TS*. Titrate with 0.5 N hydrochloric acid in methanol to a pale yellow endpoint. Each 60.57 mg of tromethamine is equivalent to 1 mL of 0.5 N hydrochloric acid in methanol.▲*USP40*

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL HCl}}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment,

dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Hydrochloric Acid, Normal (1 N), *USP 38* page 1896. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165043

Comment deadline: January 31, 2016

Change to read:

~~Hydrochloric Acid, Normal (1 N)~~

▲ **1 N Hydrochloric Acid VS**▲*USP40*

HCl, **36.46**

36.46 g in 1000 mL

Dilute 85 mL of *hydrochloric acid* with *water* to 1000 mL. ~~Standardize the solution as follows.~~

▲ **Standardization:** ▲*USP40*

Accurately weigh about 5.0 g of *tromethamine*, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. Dissolve in 50 mL of *water*, and add 2 drops of *bromocresol green TS*. Titrate with 1 N hydrochloric acid to a pale yellow endpoint. Each 121.14 mg of *tromethamine* is equivalent to 1 mL of 1 N hydrochloric acid.

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL HCl}}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Iodine, Tenth-Normal (0.1 N), *USP 38* page 1896. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165045

Comment deadline: January 31, 2016

Change to read:

~~Iodine, Tenth-Normal (0.1 N)~~

▲ **0.1 N Iodine VS**▲*USP40*

I, **126.90**

12.69 g in 1000 mL

Dissolve about 14 g of *iodine* in a solution of 36 g of *potassium iodide* in 100 mL of *water*, add 3 drops of *hydrochloric acid*, dilute with *water* to 1000 mL. ~~and standardize the solution as follows.~~

▲ Standardization: ▲*USP40*

Transfer 25.0 mL of the iodine solution to a 250-mL flask, dilute with *water* to 100 mL, add 1 mL of 1 N *hydrochloric acid*, swirl gently to mix, and titrate with 0.1 N *sodium thiosulfate VS* until the solution has a pale yellow color. Add 2 mL of *starch TS* and continue titrating until the solution is colorless.

Preserve in amber-colored, glass-stoppered bottles.

$$N = \frac{\text{mL Na}_2\text{S}_2\text{O}_3 \times N \text{ Na}_2\text{S}_2\text{O}_3}{25}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.] ▲*USP40*

BRIEFING

Iodine, Twentieth-Normal (0.05 N), *USP 38* page 1896. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165046

Comment deadline: January 31, 2016

Change to read:

~~**Iodine, Twentieth-Normal (0.05 N)**~~

▲ 0.05 N Iodine VS ▲*USP40*

I, **126.90**

6.33 g in 1000 mL

Dissolve about 6.5 g of *iodine* in a solution of 18 g of *potassium iodide* in 100 mL of *water*, add 3 drops of *hydrochloric acid*, and dilute with *water* to 1000 mL. ~~and standardize the solution as follows~~

▲ Standardization: ▲*USP40*

Transfer 50.0 mL of the iodine solution to a 250-mL flask, dilute with *water* to 100 mL, add 1 mL of 1 N *hydrochloric acid*, swirl gently to mix, and titrate with 0.1 N *sodium thiosulfate VS* until the solution has a pale yellow color. Add 2 mL of *starch TS*, and continue titrating until the solution is colorless.

$$N = \frac{\text{mL Na}_2\text{S}_2\text{O}_3 \times N \text{ Na}_2\text{S}_2\text{O}_3}{50}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Iodine, Hundredth-Normal (0.01 N), *USP 38* page 1896. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165044

Comment deadline: January 31, 2016

Change to read:

Iodine, Hundredth-Normal (0.01 N)

▲ **0.01 N Iodine VS**▲*USP40*

I, **126.90**

1.269 g in 1000 mL

Dissolve about 1.4 g of *iodine* in a solution of 3.6 g of *potassium iodide* in 100 mL of *water*, add 3 drops of *hydrochloric acid*, dilute with *water* to 1000 mL. ~~and standardize the solution as follows.~~

▲ **Standardization:** ▲*USP40*

Transfer 100.0 mL of iodine solution to a 250-mL flask, add 1 mL of 1 N *hydrochloric acid*, swirl gently to mix, and titrate with 0.1 N *sodium thiosulfate VS* until the solution has a pale yellow color. Add 2 mL of *starch TS*, and continue titrating until the solution is colorless.

Preserve in amber-colored, glass-stoppered bottles.

$$N = \frac{\text{mL Na}_2\text{S}_2\text{O}_3 \times N \text{ Na}_2\text{S}_2\text{O}_3}{100}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Lead Nitrate, Hundredth-Molar (0.01 M), *USP 38* page 1897. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165047

Comment deadline: January 31, 2016

Change to read:

Lead Nitrate, Hundredth-Molar (0.01 M)

▲ 0.01 M Lead Nitrate VS ▲^{USP40}

Pb(NO₃)₂, **331.21**

3.312 g in 1000 mL

Xylenol Orange Triturate—Triturate 1 part of *xylenol orange* with 99 parts of *potassium nitrate*.

0.1 M Lead Nitrate—Dissolve 33 g of *lead nitrate* in 1000 mL of *water*. ~~Standardize the solution as follows.~~

▲ Standardization: ▲^{USP40}

To 20.0 mL of the lead nitrate solution add 300 mL of *water*. Add about 50 mg of *Xylenol Orange Triturate*, and add *methenamine* until the solution becomes violet-pink. Titrate with 0.1 M *edetate disodium VS* to the yellow endpoint. Calculate the molarity.

Dilute 50.0 mL of 0.1 M *Lead Nitrate* to 500.0 mL with *water*.

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.] ▲^{USP40}

BRIEFING

Lead Perchlorate, Hundredth-Molar (0.01 M), *USP 38* page 1897. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165099

Comment deadline: January 31, 2016

Change to read:

Lead Perchlorate, Hundredth-Molar (0.01 M)

▲ 0.01 M Lead Perchlorate VS ▲^{USP40}

Pb(ClO₄)₂, **406.10**

Accurately pipet 100 mL of commercially available 0.1 M lead perchlorate solution into a 1000-mL volumetric flask, ~~add a sufficient quantity of water to make 1000 mL, and standardize the solution as follows:~~

▲ and dilute to volume with *water*.

Standardization: ▲^{USP40}

Accurately pipet 50 mL of 0.01 M lead perchlorate solution, ~~as prepared above,~~ into a 250-mL conical flask. Add 3 mL of aqueous *hexamethylenetetramine* solution (2.0 g per 100 mL) and 4 drops of 0.5% *xylenol orange* indicator prepared by adding 500 mg of *xylenol orange* to 10 mL of *alcohol* and diluting with *water* to 100 mL. ~~(Omit the alcohol if the sodium salt of the indicator is used).~~

▲ ▲^{USP40}

Titrate with 0.05 M edetate disodium VS to a yellow endpoint.

$$M = \frac{\text{mL edetate disodium} \times M \text{ edetate disodium}}{50.0}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Lead Perchlorate, Tenth-Molar (0.1 M), *USP 38* page 1897. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165100

Comment deadline: January 31, 2016

Change to read:

Lead Perchlorate, Tenth-Molar (0.1 M)

▲ **0.1 M Lead Perchlorate VS**▲*USP40*

$\text{Pb}(\text{ClO}_4)_2 \cdot 3\text{H}_2\text{O}$, **460.15**

46.01 g in 1000 mL

Dissolve 46 g of *lead perchlorate* in *water*, and dilute with *water* to 1000.0 mL.

▲ **Standardization:** ▲*USP40*

Accurately weigh about 150 mg of *sodium sulfate*, previously dried at 105° for 4 h, and dissolve in 50 mL of *water*. Add 50 mL of a mixture of *water* and *formaldehyde* (1:1), and stir for about 1 min. Determine the endpoint potentiometrically using a lead ion selective electrode. Perform a blank determination, and make any necessary corrections. Each 14.204 mg of sodium sulfate is equivalent to 1 mL of 0.1 M lead perchlorate.

$$M = \frac{\text{mg sodium sulfate}}{142.04 \times \text{mL lead perchlorate}}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Lithium Methoxide, Fiftieth-Normal (0.02 N) in Methanol, *USP 38* page 1897. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165101

Comment deadline: January 31, 2016

Change to read:

Lithium Methoxide, Fiftieth-Normal (0.02 N) in Methanol

▲ 0.02 N Lithium Methoxide in Methanol VS ▲^{USP40}

CH₃LiO, **37.97**

759.6 mg in 1000 mL

Dissolve 0.12 g of freshly cut *lithium* metal in 150 mL of *methanol*, cooling the flask during addition of the metal. When the reaction is complete, add 850 mL of *methanol*, and mix. Store the solution preferably in the reservoir of an automatic delivery buret suitably protected from carbon dioxide and moisture. ~~Standardize the solution by titration against *benzoic acid* as described under *Sodium Methoxide, Tenth-Normal (0.1 N) (in Toluene)*, but use only 100 mg of *benzoic acid*.~~

▲ Standardization: Accurately weigh about 100 mg of primary standard *benzoic acid*, and dissolve in 80 mL of *dimethylformamide* in a flask. Add 3 drops of a 1 in 100 solution of *thymol blue* in *dimethylformamide*, and titrate with the lithium methoxide solution to a blue endpoint. Correct for the volume of the lithium methoxide solution consumed by 80 mL of the *dimethylformamide*. ▲^{USP40}

Each 2.442 mg of benzoic acid is equivalent to 1 mL of 0.02 N lithium methoxide. [Note—Restandardize the solution frequently.]

$$N = \frac{\text{mg benzoic acid}}{122.12 \times \text{mL lithium methoxide (corrected for the blank)}}$$

▲^{USP38}

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.] ▲^{USP40}

BRIEFING

Lithium Methoxide, Tenth-Normal (0.1 N) in Chlorobenzene, page 7208 of the *First Supplement* to *USP 38*. It is proposed to revise this volumetric solution to current *USP* style and include a note on standardization.

(HDQ: M. Marques.)

Correspondence Number—C165102

Comment deadline: January 31, 2016

Change to read:

Lithium Methoxide, Tenth-Normal (0.1 N) in Chlorobenzene

▲ 0.1 N Lithium Methoxide in Chlorobenzene VS ▲^{USP40}

CH₃OLi, **37.97**

3.798 g in 1000 mL

Dissolve 700 mg of freshly cut *lithium* metal in 150 mL of *methanol*, cooling the flask during addition of the metal. When the reaction is complete, add 850 mL of *chlorobenzene*. If cloudiness or precipitation occurs, add sufficient *methanol* to clarify the solution. Store preferably in the reservoir of an automatic delivery buret suitably protected from carbon dioxide and moisture. ~~Standardize the solution by titration against benzoic acid as described under Sodium Methoxide, Tenth Normal (0.1 N) in Toluene.~~

▲ **Standardization:** Accurately weigh about 400 mg of primary standard *benzoic acid*, and dissolve in 80 mL of *dimethylformamide* in a flask. Add 3 drops of a 1-in-100 solution of *thymol blue* in *dimethylformamide*, and titrate with the lithium methoxide solution to a blue endpoint. Correct for the volume of the lithium methoxide solution consumed by 80 mL of the *dimethylformamide*. Each 12.21 mg of benzoic acid is equivalent to 1 mL of 0.1 N lithium methoxide. ▲*USP40*

[Note—Restandardize the solution frequently.]

$$N = \frac{\text{mg benzoic acid}}{122.12 \times \text{mL lithium methoxide (corrected for the blank)}}$$

■ 1S (*USP38*)

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.] ▲*USP40*

BRIEFING

Lithium Methoxide, Tenth-Normal (0.1 N) in Methanol, page 7208 of the *First Supplement to USP 38*. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165103

Comment deadline: January 31, 2016

Change to read:

Lithium Methoxide, Tenth Normal (0.1 N) in Methanol

▲ **0.1 N Lithium Methoxide in Methanol VS** ▲*USP40*

CH₃OLi, **37.97**

3.798 g in 1000 mL

Dissolve 700 mg of freshly cut *lithium* metal in 150 mL of *methanol*, cooling the flask during addition of the metal. When the reaction is complete, add 850 mL of *methanol*. If cloudiness or precipitation occurs, add sufficient *methanol* to clarify the solution. Store preferably in the reservoir of an automatic delivery buret suitably protected from carbon dioxide and moisture. ~~Standardize the solution by titration against benzoic acid as described under Sodium Methoxide, Tenth Normal (0.1 N) in Toluene.~~

▲ **Standardization:** Accurately weigh about 400 mg of primary standard *benzoic acid*, and dissolve in 80 mL of *dimethylformamide* in a flask. Add 3 drops of a 1 in 100 solution of *thymol blue* in *dimethylformamide*, and titrate with the lithium methoxide solution to a blue endpoint. Correct for the volume of the lithium methoxide solution consumed by 80 mL of the

dimethylformamide. Each 12.21 mg of benzoic acid is equivalent to 1 mL of 0.1 N lithium methoxide. ▲*USP40*

[Note—Restandardize the solution frequently.]

$$N = \frac{\text{mg benzoic acid}}{122.12 \times \text{mL lithium methoxide (corrected for the blank)}}$$

■1S (*USP38*)

▲[Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Lithium Methoxide, Tenth-Normal (0.1 N) in Toluene, page 7208 of the *First Supplement* to *USP 38*. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165104

Comment deadline: January 31, 2016

Change to read:

~~**Lithium Methoxide, Tenth-Normal (0.1 N) in Toluene**~~

▲**0.1 N Lithium Methoxide in Toluene VS**▲*USP40*

CH₃OLi, **37.97**

3.798 g in 1000 mL

Dissolve 700 mg of freshly cut *lithium* metal in 150 mL of *methanol*, cooling the flask during addition of the metal. When reaction is complete, add 850 mL of *toluene*. If cloudiness or precipitation occurs, add sufficient *methanol* to clarify the solution. Store preferably in the reservoir of an automatic delivery buret suitably protected from carbon dioxide and moisture. ~~Standardize the solution by titration against *benzoic acid* as described under *Sodium Methoxide, Tenth-Normal (0.1 N) in Toluene*.~~

▲**Standardization:** Accurately weigh about 400 mg of primary standard *benzoic acid*, and dissolve in 80 mL of *dimethylformamide* in a flask. Add 3 drops of a 1-in-100 solution of *thymol blue* in *dimethylformamide*, and titrate with the lithium methoxide solution to a blue endpoint. Correct for the volume of the lithium methoxide solution consumed by 80 mL of the *dimethylformamide*. Each 12.21 mg of benzoic acid is equivalent to 1 mL of 0.1 N lithium methoxide. ▲*USP40*

[Note—Restandardize the solution frequently.]

$$N = \frac{\text{mg benzoic acid}}{122.12 \times \text{mL lithium methoxide (corrected for the blank)}}$$

■1S (*USP38*)

▲[Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Magnesium Chloride, 0.01 M, *USP 38* page 1897. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165105

Comment deadline: January 31, 2016

Change to read:

~~Magnesium Chloride, 0.01 M~~

▲ 0.01 M Magnesium Chloride VS▲*USP40*

MgCl₂·6H₂O, **203.30**

2.0330 g in 1000 mL

Dissolve about 2.04 g of *magnesium chloride* in 1000 mL of freshly boiled and cooled *water*. and standardize the solution as follows:

▲ Standardization: ▲*USP40*

Accurately measure 25 mL of the prepared magnesium chloride solution. Add 50 mL of *water*, 3 mL of *ammonia-ammonium chloride buffer TS* and 0.04 g of *eriochrome black T-sodium chloride* reagent. Titrate with 0.05 M *edetate disodium VS* until the red-purple color of the solution changes to blue-purple.

$$M = \frac{(\text{mL edetate disodium VS}) \times (\text{M edetate disodium})}{\text{mL magnesium chloride}}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Mercuric Nitrate, Tenth-Molar (0.1 M), *USP 38* page 1897. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165106

Comment deadline: January 31, 2016

Change to read:

~~Mercuric Nitrate, Tenth-Molar (0.1 M)~~

▲ 0.1 M Mercuric Nitrate VS▲*USP40*

Hg(NO₃)₂, **324.60**

32.46 g in 1000 mL

Dissolve about 35 g of *mercuric nitrate* in a mixture of 5 mL of *nitric acid* and 500 mL of *water*, and dilute with *water* to 1000 mL. Standardize the solution as follows:

▲ Standardization: ▲*USP40*

Transfer an accurately measured volume of about 20 mL of the solution to a conical flask, and add 2 mL of *nitric acid* and 2 mL of *ferric ammonium sulfate TS*. Cool to below 20°, and titrate with 0.1 N *ammonium thiocyanate VS* to the first appearance of a permanent brownish color.

$$M = \frac{\text{mL NH}_4\text{SCN} \times N \text{ NH}_4\text{SCN}}{\text{mL Hg(NO}_3)_2 \times 2}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Oxalic Acid, Tenth-Normal (0.1 N), *USP 38* page 1898. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C165108

Comment deadline: January 31, 2016

Change to read:

~~Oxalic Acid, Tenth-Normal (0.1 N)~~

▲ **0.1 N Oxalic Acid VS**▲*USP40*

H₂C₂O₄·2H₂O, **126.07**

6.303 g in 1000 mL

Dissolve 6.45 g of *oxalic acid* in *water* to make 1000 mL. ~~Standardize by titration against freshly standardized 0.1 N potassium permanganate VS as directed under Potassium Permanganate, Tenth-Normal (0.1 N).~~

▲ **Standardization:** Transfer 10 mL of oxalic acid solution to an appropriate flask and titrate with freshly standardized 0.1 N *potassium permanganate VS* until a pale pink color, which persists for 15 s, is produced.▲*USP40*

Preserve in glass-stoppered bottles, protected from light.

$$N = \frac{\text{mL KMnO}_4 \times N \text{ KMnO}_4}{\text{mL H}_2\text{C}_2\text{O}_4}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Perchloric Acid, Tenth-Normal (0.1 N) in Glacial Acetic Acid, *USP 38* page 1898. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C164482

Comment deadline: January 31, 2016

Change to read:**Perchloric Acid, Tenth-Normal (0.1 N) in Glacial Acetic Acid****▲ 0.1 N Perchloric Acid in Glacial Acetic Acid VS ▲*USP40***

HClO₄, **100.46**

10.05 g in 1000 mL

[Note—Where called for in the tests and assays, this volumetric solution is specified as “0.1 N perchloric acid.” Thus, where 0.1 N or other strength of this volumetric solution is specified, the solution in glacial acetic acid is to be used, unless the words “in dioxane” are stated. (See also *Perchloric Acid, Tenth-Normal (0.1 N) in Dioxane*

▲ 0.1 N Perchloric Acid in Dioxane VS. ▲*USP40*

)]

Mix 8.5 mL of *perchloric acid* with 500 mL of *glacial acetic acid* and 21 mL of *acetic anhydride*, cool, and add *glacial acetic acid* to make 1000 mL. Alternatively, the solution may be prepared as follows. Mix 11 mL of 60% *perchloric acid* with 500 mL of *glacial acetic acid* and 30 mL of *acetic anhydride*, cool, and add *glacial acetic acid* to make 1000 mL.

Allow the prepared solution to stand for 1 day for the excess acetic anhydride to be combined, and determine the water content by *Method I* (see *Water Determination* (921)), except to use a test specimen of about 5 g of the 0.1 N perchloric acid that is expected to contain approximately 1 mg of water and the *Reagent* (see *Reagent in Water Determination* (921), *Method 1a*) diluted such that 1 mL is equivalent to about 1–2 mg of water. If the water content exceeds 0.5%, add more acetic anhydride. If the solution contains no titratable water, add sufficient water to obtain a content of between 0.02% and 0.5% of water. Allow the solution to stand for 1 day, and again titrate the water content. The solution so obtained contains between 0.02% and 0.5% of water, indicating freedom from acetic anhydride.

~~Standardize the solution as follows.~~

▲ Standardization: ▲*USP40*

Accurately weigh about 700 mg of *potassium biphthalate*, previously crushed lightly and dried at 120° for 2 h, and dissolve it in 50 mL of *glacial acetic acid* in a 250-mL flask. Add 2 drops of *crystal violet TS*, and titrate with the perchloric acid solution until the violet color changes to blue-green. Deduct the volume of the perchloric acid consumed by 50 mL of the *glacial acetic acid*. Each 20.422 mg of potassium biphthalate is equivalent to 1 mL of 0.1 N perchloric acid.

$$N = \frac{\text{g KHC}_8\text{H}_4\text{O}_4}{0.20422 \times \text{mL HClO}_4 \text{ (corrected for the blank)}}$$

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.] ▲*USP40*

BRIEFING

0.05 N Silver Nitrate VS. It is proposed to add this new volumetric solution.

(HDQ: M. Marques.)

Correspondence Number—C165224

Comment deadline: January 31, 2016

Add the following:

▲ **0.05 N Silver Nitrate VS**

AgNO₃, **169.87**

8.49 in 1000 mL

Dissolve 8.75 g of *silver nitrate* in 1000 mL of *water*.

Standardization: Transfer about 50 mg, accurately weighed, of *sodium chloride*, previously dried at 110° for 2 h, to a 150-mL beaker. Dissolve in 5 mL of *water* and add 5 mL of *acetic acid*, 50 mL of *methanol*, and about 0.5 mL of *eosin Y TS*. Stir, preferably with a magnetic stirrer, and titrate with the silver nitrate solution.

$$N = \frac{\text{mg NaCl}}{\text{mL AgNO}_3 \times 58.44}$$

▲*USP40*

BRIEFING

0.1 N Sodium Hydroxide VS. It is proposed to add this new volumetric solution.

(HDQ: M. Marques.)

Correspondence Number—C163684

Comment deadline: January 31, 2016

Add the following:

▲ **0.1 N Sodium Hydroxide VS**

NaOH, **40.00**

4.0 g in 1000 mL

Transfer 100 mL of *1 N sodium hydroxide VS* to a 1000-mL volumetric flask. Dilute with *water*, *carbon dioxide-free* to volume.

Standardization: Dissolve about 200 mg of *benzoic acid* in 15 mL of *carbon dioxide-free water*. Add 2 drops of *phenolphthalein TS*, and titrate with *0.1 N sodium hydroxide VS* until a permanent pale pink color is produced.

$$N = \frac{\text{mg benzoic acid}}{122.1 \times \text{mL sodium hydroxide}}$$

[Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

Standard Dichlorophenol–Indophenol Solution, *USP 38* page 1895. It is proposed to revise this volumetric solution to current *USP* style and include a note about standardization.

(HDQ: M. Marques.)

Correspondence Number—C164717

Comment deadline: January 31, 2016

Change to read:

Standard Dichlorophenol–Indophenol Solution

To 50 mg of *2,6-dichlorophenol–indophenol sodium* that has been stored in a desiccator over *soda lime* add 50 mL of *water* containing 42 mg of *sodium bicarbonate*, shake vigorously, and when the dye is dissolved, add *water* to make 200 mL. Filter into an amber, glass-stoppered bottle. Use within 3 days and standardize immediately before use. ~~Standardize the solution as follows:~~

▲ **Standardization:** ▲*USP40*

Accurately weigh 50 mg of *USP Ascorbic Acid RS*, and transfer to a glass-stoppered, 50-mL volumetric flask with the aid of a sufficient volume of *metaphosphoric–acetic acids TS* to make 50 mL. Immediately transfer 2 mL of the ascorbic acid solution to a 50-mL conical flask containing 5 mL of the *metaphosphoric–acetic acids TS*, and titrate rapidly with the dichlorophenol–indophenol solution until a distinct rose-pink color persists for at least 5 s. Perform a blank titration by titrating 7 mL of the *metaphosphoric–acetic acids TS* plus a volume of *water* equal to the volume of the dichlorophenol solution used in titrating the ascorbic acid solution. Express the concentration of the standard solution in terms of its equivalent in mg of ascorbic acid.

▲ [Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

0.1 M Zinc Sulfate VS. It is proposed to add this new volumetric solution.

(HDQ: M. Marques.)

Correspondence Number—C164323

Comment deadline: January 31, 2016

Add the following:

▲ **0.1 M Zinc Sulfate VS**

ZnSO₄·7H₂O, **287.54**

28.8 g in 1000 mL

Dissolve 28.8 g of *zinc sulfate heptahydrate* in water to make 1 L.

Standardization: Accurately measure about 10 mL of 0.1 M *edetate disodium VS* into a 125-mL conical flask, and add, in the order given, 10 mL of *acetic acid–ammonium acetate buffer TS*, 50 mL of *alcohol*, and 2 mL of *dithizone TS*. Titrate with the zinc sulfate solution to a clear, rose-pink color.

$$M = \frac{\text{mL edetate disodium} \times M \text{ edetate disodium}}{\text{mL ZnSO}_4}$$

[Note—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]▲*USP40*

BRIEFING

L19, *USP 38* page 1902. It is proposed to increase the particle size range of this column.

Additionally, minor editorial changes have been made to update the column packing to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C163827

Comment deadline: January 31, 2016

Change to read:

L19—Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the calcium form, ~~about 9~~

▲5–15▲*USP40*

µm in diameter.

BRIEFING

L22, *USP 38* page 1902. It is proposed to increase the particle size range of this column.

Additionally, minor editorial changes have been made to update the column packing to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C163828

Comment deadline: January 31, 2016

Change to read:

L22—A cation-exchange resin made of porous polystyrene gel with sulfonic acid groups, ~~about ±0~~

▲5–15▲*USP40*

µm in size

▲diameter.▲*USP40*

BRIEFING

L40, *USP 38* page 1903. It is proposed to expand the particle size range of this column.

Additionally, minor editorial changes have been made to update the column packing to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C165113

Comment deadline: January 31, 2016

Change to read:

L40—Cellulose tris-3,5-dimethylphenylcarbamate-coated, porous silica particles, 5

▲3▲*USP40*

–20 µm in diameter.

BRIEFING

L45, *USP 38* page 1903. It is proposed to increase the particle size range of this column.

Additionally, minor editorial changes have been made to update the column packing to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C163829

Comment deadline: January 31, 2016

Change to read:

L45—Beta cyclodextrin, *R,S*-hydroxypropyl ether derivative, bonded to porous silica particles, 5
to 10

▲3–10▲*USP40*

µm in diameter.

BRIEFING

L51, *USP 38* page 1903. It is proposed to expand the particle size range of this column packing.

Additionally, minor editorial changes have been made to update the column packing to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C165114

Comment deadline: January 31, 2016

Change to read:

L51—Amylose tris-3,5-dimethylphenylcarbamate-coated, porous, spherical, silica particles, 5

▲3▲*USP40*

–10 µm in diameter. [Note—Available as Chiralpak AD from Chiral Technologies, Inc. (www.chiraltech.com).]

BRIEFING

L54, *USP 38* page 1903. It is proposed to increase the particle size range of this column.

Additionally, minor editorial changes have been made to update the column packing to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C163831

Comment deadline: January 31, 2016

Change to read:

L54—A size exclusion medium made of covalent bonding of dextran to highly cross-linked porous agarose beads, ~~about 13~~

▲ 5–15 ▲ *USP40*

µm in diameter.

[Note—Available as Superdex Peptide HR 10/30 from www.gelifesciences.com.]

BRIEFING

L80, *USP 38* page 1904. It is proposed to add a particle size range to this column.

Additionally, minor editorial changes have been made to update the column packing to current *USP* style.

(HDQ: M. Marques.)

Correspondence Number—C165223

Comment deadline: January 31, 2016

Change to read:

L80—Cellulose tris(4-methylbenzoate)-coated, porous, spherical, silica particles,

▲ 5–20 ▲ *USP40*

µm in diameter.

CONTAINERS FOR DISPENSING CAPSULES AND TABLETS

BRIEFING

Container Specifications for Capsules and Tablets, page 7697 of the *Second Supplement* to *USP 38*.

(HDQ.)

Correspondence Number—C141734; C143400; C143401; C143402; C143403; C162941; C162945

The following table is provided as a reminder for the pharmacist engaged in the typical dispensing situation who already is acquainted with the *Packaging and Storage* requirements set forth in the individual monographs. It lists the capsules and tablets that are official in the *United States Pharmacopeia* and indicates the relevant tight (T), well-closed (W), and light-resistant (LR) specifications applicable to containers in which the drug that is repackaged should be dispensed.

This table is not intended to replace, nor should it be interpreted as replacing, the definitive

requirements stated in the individual monographs.

Container Specifications for Capsules and Tablets

Monograph Title	Container Specification
Add the following: ▲ Acamprosate Delayed-Release Tablets	T, LR▲ <i>USP40</i>
Delete the following: ▲ Phenytoin Sodium Capsules, Prompt	‡▲ <i>USP40</i>
Delete the following: ▲ Secobarbital Sodium and Amobarbital Sodium Capsules	W▲ <i>USP40</i>
Add the following: ▲ Tienchi Ginseng Root and Rhizome Dry Extract Capsules	W, LR▲ <i>USP40</i>
Add the following: ▲ Tienchi Ginseng Root and Rhizome Dry Extract Tablets	W, LR▲ <i>USP40</i>
Add the following: ▲ Tienchi Ginseng Root and Rhizome Powder Capsules	W, LR▲ <i>USP40</i>
Add the following: ▲ Tienchi Ginseng Root and Rhizome Powder Tablets	W, LR▲ <i>USP40</i>

BRIEFING

Description and Relative Solubility of USP and NF Articles, page 7708 of the *Second Supplement to USP 38*.

(HDQ.)

Correspondence Number—C136074; C138696; C146273

Add the following:

▲ **Carglumic Acid:** White, crystalline powder. Soluble in boiling water; slightly soluble in cold water; practically insoluble in organic solvents.▲*USP40*

Add the following:

▲ **Pemetrexed Disodium:** White or almost white powder. Freely soluble in water; very slightly soluble in dehydrated alcohol; practically insoluble in methylene chloride.▲*USP40*

Add the following:

▲ **Pregabalin:** White to off-white, crystalline solid. Freely soluble in water and in both basic and acidic aqueous solutions.▲*USP40*

BRIEFING

Acamprosate Delayed-Release Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is being proposed.

1. The isocratic liquid chromatographic procedures in the *Assay* and *Dissolution* tests are based on analyses performed with the Luna Phenyl-Hexyl brand of L11 column. The retention times for acamprosate are about 9.5 and 5.7 min, respectively.
2. The isocratic liquid chromatographic procedure in the test for *Limit of Acamprosate Related Compound A* is based on analyses performed with the Discovery HS C18 brand of L1 column with a 3- μm particle size manufactured by Supelco. The Intersil ODS-3V brand of L1 column with a 5- μm particle size may also be suitable. The retention time for acamprosate related compound A is about 13.2 min.
3. The gradient liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with the Luna Phenyl-Hexyl brand of L11 column. The retention time for acamprosate is about 10.5 min.

All interested parties are encouraged to submit their U.S. FDA-approved specifications to USP if they are wider than those proposed.

(CHM4: H. Joyce.)

Correspondence Number—C141734

Comment deadline: January 31, 2016

Add the following:

▲ Acamprosate Delayed-Release Tablets

DEFINITION

Acamprosate Delayed-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acamprosate calcium ($\text{C}_{10}\text{H}_{20}\text{CaN}_2\text{O}_8\text{S}_2$).

IDENTIFICATION

- **A. Infrared Absorption** (197K)

Standard: USP Acamprosate Calcium RS

Sample: Remove the coating from 5 Tablets. Powder the remaining material, mix it well, and use a suitable portion of the mixture.

Analysis

Samples: *Standard* and *Sample*

Acceptance criteria: The *Sample* exhibits maxima only at the same wavelengths as the *Standard* over the range of 1750–600 cm^{-1} .

- **B.** The retention time of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: Add 1.0 mL of *triethylamine* per 1 L of water and adjust with *phosphoric acid* to a pH of 2.5.

Mobile phase: *Methanol* and *Buffer* (2:98)

Standard solution: 0.3 mg/mL of USP Acamprosate Calcium RS in *Mobile phase*. Sonication may be used to promote dissolution.

Sample stock solution: Nominally 3 mg/mL of acamprosate calcium from Tablets prepared as follows. Transfer a suitable portion of powdered Tablets (NLT 20) to an appropriate volumetric flask. Add 40% of the final flask volume of *Mobile phase* and sonicate for 30 min with intermittent shaking to avoid lump formation. Dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.3 mg/mL of acamprosate calcium from the *Sample stock solution* in *Mobile phase* passed through a suitable filter. Use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-μm packing *L11*

Column temperature: 30°

Flow rate: 0.7 mL/min

Injection volume: 10 μL

Run time: NLT 1.2 times the retention time of acamprosate

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acamprosate calcium (C₁₀H₂₀CaN₂O₈S₂) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Acamprosate Calcium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acamprosate calcium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Dissolution** (711)

Acid stage

Acid stage medium: 0.1 N hydrochloric acid; 1000 mL

Apparatus 1: 180 rpm

Time: 2 h

Buffer stage

Buffer stage medium: pH 6.8 citrate buffer. [After 2 h with *Acid stage medium*, replace the contents of the dissolution vessels with *pH 6.8 citrate buffer* (21 g/L of *citric acid* and 12 g/L of *sodium hydroxide* in water adjusted with 2 N *sodium hydroxide TS* or 420.3 g/L of *citric acid* in water to a pH of 6.8).]; 1000 mL

Apparatus 1: 180 rpm

Time: 3 h. The time in the *Buffer stage medium* does not include the time in the *Acid stage medium*.

Mobile phase: Add 0.4 mL of *trifluoroacetic acid* per 1 L of water.

Standard stock solution: 1.6 mg/mL of USP *Acamprosate Calcium RS* in *Mobile phase*

Acid stage standard solution: ($L/1000$) mg/mL of USP *Acamprosate Calcium RS* from the *Standard stock solution* in *Acid stage medium*, where L is the label claim of *acamprosate calcium* in mg/Tablet

Buffer stage standard solution: ($L/1000$) mg/mL of USP *Acamprosate Calcium RS* from the *Standard stock solution* in *Buffer stage medium*, where L is the label claim of *acamprosate calcium* in mg/Tablet

Acid stage sample solution: Pass a portion of solution under test through a suitable filter and use the filtrate.

Buffer stage sample solution: Pass a portion of solution under test through a suitable filter and use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25.0-cm; 5- μ m packing *L11*

Column temperature: 30°

Flow rate: 0.8 mL/min

Injection volume: 10 μ L

Run time: NLT 2 times the retention time of *acamprosate*

System suitability

Samples: *Acid stage standard solution* and *Buffer stage standard solution*

Suitability requirements

Tailing factor: NMT 2.0, *Acid stage standard solution* and *Buffer stage standard solution*

Relative standard deviation: NMT 2.0%, *Acid stage standard solution* and *Buffer stage standard solution*

Analysis

Samples: *Acid stage standard solution*, *Buffer stage standard solution*, *Acid stage sample solution*, and *Buffer stage sample solution*

Calculate the percentage of the labeled amount of acamprosate calcium ($C_{10}H_{20}CaN_2O_8S_2$) dissolved in the *Acid stage medium*:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of acamprosate from the *Acid stage sample solution*

r_S = peak response of acamprosate from the *Acid stage standard solution*

C_S = concentration of USP Acamprosate Calcium RS in the *Acid stage standard solution* (mg/mL)

V = volume of *Acid stage medium*, 1000 mL

L = label claim of acamprosate calcium (mg/Tablet)

Calculate the percentage of the labeled amount of acamprosate calcium ($C_{10}H_{20}CaN_2O_8S_2$) dissolved in the *Buffer stage medium*:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of acamprosate from the *Buffer stage sample solution*

r_S = peak response of acamprosate from the *Buffer stage standard solution*

C_S = concentration of USP Acamprosate Calcium RS in the *Buffer stage standard solution* (mg/mL)

V = volume of *Buffer stage medium*, 1000 mL

L = label claim of acamprosate calcium (mg/Tablet)

Tolerances: The requirements must be met for both the *Acid stage* and the *Buffer stage*.

Acid stage: NMT 2% of the labeled amount of acamprosate calcium ($C_{10}H_{20}CaN_2O_8S_2$) is dissolved.

Buffer stage: NLT 80% (Q) of the labeled amount of acamprosate calcium ($C_{10}H_{20}CaN_2O_8S_2$) is dissolved.

- **Uniformity of Dosage Units** (905): Meet the requirements

IMPURITIES

- **Limit of Acamprosate Related Compound A**

Solution A: 5 g/L of *fluorescamine* in acetonitrile. Use within 4 h.

Buffer: 13.6 g/L of *monobasic potassium phosphate* (0.1 M phosphate buffer) prepared as follows. Transfer a suitable amount of *monobasic potassium phosphate* to a volumetric flask. Dissolve in 95% of the final flask volume of water. Adjust with 1.8 N *potassium hydroxide TS* or 1 N *phosphoric acid TS* to a pH of 6.5. Dilute with water to volume.

Mobile phase: *Acetonitrile, methanol, and Buffer* (10:10:80)

Diluent: 6.2 g/L of *boric acid*, 7.5 g/L of *potassium chloride*, and 3.6 g/L of *sodium hydroxide* prepared as follows. Transfer suitable amounts of *boric acid, potassium chloride, and sodium hydroxide* to a suitable volumetric flask. Dissolve in 95% of the final flask volume of water. Adjust with 2.5 N *sodium hydroxide TS* to a pH of 10.4. Dilute with water to volume.

Standard stock solution A: 250 µg/mL of USP Acamprosate Related Compound A RS in water

Standard stock solution B: 1 µg/mL of USP Acamprosate Related Compound A RS from *Standard stock solution A* in *Diluent*

Standard solution: Transfer 3.0 mL of *Standard stock solution B* to an appropriate container. Add 0.15 mL of *Solution A* and shake vigorously for 30 s. Heat in a water bath at 50° for 30 min. Cool under a stream of cold water, centrifuge, and pass the supernatant through a suitable membrane filter.

Sample stock solution A: Nominally 20 mg/mL of acamprosate calcium from Tablets prepared as follows. Transfer a suitable amount of powdered Tablets (NLT 5) to an appropriate volumetric flask. Dissolve in 60% of the final flask volume of water and sonicate for NLT 30 min. Dilute with water to volume.

Sample stock solution B: Nominally 2 mg/mL of acamprosate calcium from *Sample stock solution A* in *Diluent*. Pass through a suitable filter and use the filtrate.

Sample solution: Transfer 3.0 mL of *Sample stock solution B* to an appropriate container. Add 0.15 mL of *Solution A* and shake for 30 s. Heat in a water bath at 50° for 30 min. Cool under a stream of cold water, centrifuge, and pass the supernatant through a suitable membrane filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 261 nm

Column: 4.6-mm × 15-cm; 3- or 5-µm packing *L1*

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 2 times the retention time of acamprosate related compound A

System suitability

Sample: *Standard solution*

[Note—The relative retention times for fluorescamine and acamprosate related compound A are 0.3 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between fluorescamine and acamprosate related compound A

Relative standard deviation: NMT 5.0% for acamprosate related compound A

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acamprosate related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Acamprosate Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acamprosate calcium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.10%

• **Organic Impurities**

Buffer: Add 1.0 mL of *triethylamine* per 1 L of water and adjust with *phosphoric acid* to a pH of 2.5.

Solution A: *Buffer*

Solution B: *Acetonitrile*

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	100	0
50	80	20
60	100	0
75	100	0

System suitability solution: 5 mg/mL of USP Acamprosate Calcium RS, 0.005 mg/mL of USP Acamprosate Related Compound B RS, and 0.005 mg/mL of *glacial acetic acid* in *Buffer*. Sonication may be used to aid in dissolution.

Standard solution: 0.005 mg/mL of USP Acamprosate Calcium RS in *Buffer*. Sonication may be used to aid in dissolution.

Sample solution: Nominally 5 mg/mL of acamprosate calcium from Tablets prepared as follows. Transfer a suitable portion of powdered Tablets (NLT 10) to an appropriate volumetric flask. Add 70% of the final flask volume of *Buffer* and sonicate for 30 min with intermittent shaking to avoid lump formation. Dilute with *Buffer* to volume and pass through a suitable filter. Use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5- μ m packing *L11*

Column temperature: 40°

Flow rate: 0.7 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between acetic acid and acamprosate related compound B; NLT 1.5 between acamprosate related compound B and acamprosate, *System suitability solution*

Relative standard deviation: NMT 5% for acamprosate, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of acamprosate from the *Standard solution*

C_S = concentration of USP Acamprosate Calcium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acamprosate calcium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Calcium ^a	0.6	—
Acetic acid ^b	0.7	—
Acamprosate related compound B ^b	0.8	—
Acamprosate	1.0	—
<i>N</i> -Methyl acamprosate ^c	2.7	—
Any individual, unspecified degradation products	—	0.1
Total degradation products ^d	—	1.0

^a Included for identification only. This peak is due to the calcium counterion and hence is not an impurity.

^b This is a process impurity that is included in the table for identification purposes. It is controlled in the drug substance and is not to be reported or included in the total degradation products for the drug product.

^c 3-(*N*-Methylacetamido)propane-1-sulfonate.

^d The sum of acamprosate related compound A from the *Limit of Acamprosate Related Compound A* and all degradation products from the test for *Organic Impurities*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP Reference Standards** <11>
 - USP Acamprosate Calcium RS
 - USP Acamprosate Related Compound A RS
 - 3-Aminopropane-1-sulfonic acid.
 - C₃H₉NO₃S 139.17
 - USP Acamprosate Related Compound B RS
 - Calcium 3-formamidopropane-1-sulfonate.

$C_8H_{16}CaN_2O_8S_2$ 372.42

▲USP40

BRIEFING

Acetaminophen and Codeine Phosphate Oral Solution, *USP 38* page 2031. As part of the USP monograph modernization initiative and based on correspondence from the FDA regarding modernization, the addition of an HPLC procedure for monitoring 4-aminophenol, based on general chapter *4-Aminophenol in Acetaminophen-Containing Drug Products* (227), is being proposed. This liquid chromatographic procedure is based on analyses performed with the Dionex Acclaim Mixed Mode WCX-1 brand of L85 column. The typical retention time for 4-aminophenol is about 4.2–5.3 min. The proposed limit of this nephrotoxin is 0.15% and is based on input provided by the FDA and the pharmaceutical industry to the USP Acetaminophen Expert Panel, which reports to the Chemical Medicines Monographs 2 Expert Committee.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM2: C. Anthony.)

Correspondence Number—C161675

Comment deadline: January 31, 2016

Acetaminophen and Codeine Phosphate Oral Solution**DEFINITION**

Acetaminophen and Codeine Phosphate Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amounts of acetaminophen ($C_8H_9NO_2$) and codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solutions* correspond to those of the *Standard solutions*, as obtained in the *Assays for Acetaminophen and Codeine Phosphate*.

- **B. Thin-Layer Chromatography**

Standard solution: 12 mg/mL each of USP Acetaminophen RS and USP Codeine Phosphate RS in methanol

Sample solution: Transfer a volume of Oral Solution, equivalent to 12 mg of codeine phosphate, to a separator. Add 1 mL of ammonium hydroxide and 5 mL of methylene chloride. Shake for 1 min, and allow the layers to separate. Use the clear lower layer.

Developing solvent system: Methanol and ammonium hydroxide (49:1)

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Locate the spots on the plate by examination under short-wavelength UV light.

Acceptance criteria: The R_F values of the two principal spots of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY

Change to read:

• Acetaminophen

Mobile phase: Methanol and water (3:7)

Standard solution: 0.48 mg/mL of USP Acetaminophen RS in *Mobile phase*

Sample solution: Nominally 0.48 mg/mL of acetaminophen in *Mobile phase*, prepared by adding a volume of Oral Solution, equivalent to 120 mg of acetaminophen, to a 250-mL volumetric flask. Dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: ~~NLT 1000 theoretical plates~~

▲▲USP40

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*

r_S = peak response of acetaminophen from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

● **Codeine Phosphate**

Mobile phase: Dissolve 4.44 g of docusate sodium in 1000 mL of a mixture of methanol, tetrahydrofuran, phosphoric acid, and water (600:40:1:360) with stirring, and pass through a membrane filter of 0.45- μ m or finer pore size.

Diluent: Methanol and water (3:7)

Standard solution: 0.12 mg/mL of USP Codeine Phosphate RS in *Diluent*

Sample solution: Nominally 0.12 mg/mL of codeine phosphate hemihydrate in *Diluent*, prepared by adding a volume of Oral Solution, equivalent to 12 mg of codeine phosphate hemihydrate, to a 100-mL volumetric flask. Dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: ~~NLT 1500 theoretical plates~~

▲▲USP40

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of codeine phosphate from the *Sample solution*

r_S = peak response of codeine phosphate from the *Standard solution*

C_S = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of codeine phosphate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of codeine phosphate hemihydrate, 406.37

M_{r2} = molecular weight of anhydrous codeine phosphate, 397.37

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Uniformity of Dosage Units** (905)

For single-unit containers

Acceptance criteria: Meets the requirements

- **Deliverable Volume** (698)

For multiple-unit containers

Acceptance criteria: Meets the requirements

IMPURITIES

Add the following:

- ▲ ● **4-Aminophenol in Acetaminophen-Containing Drug Products** (227): Meets the requirements ▲*USP40*

SPECIFIC TESTS

- **pH** (791): 4.0–6.1
- **Alcohol Determination** (611), *Method II*(if present): 90.0%–120.0% of the labeled quantity of alcohol (C₂H₅OH), acetone being used as the internal standard

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP Reference Standards** (11)
 - USP Acetaminophen RS
 - USP Codeine Phosphate RS

BRIEFING

Acetaminophen and Codeine Phosphate Oral Suspension, *USP 38* page 2032. As part of the USP monograph modernization initiative and based on correspondence from the FDA regarding modernization, the addition of an HPLC procedure for monitoring 4-aminophenol, based on general chapter *4-Aminophenol in Acetaminophen-Containing Drug Products* (227), is proposed. This liquid chromatographic procedure is based on analyses performed with the Dionex Acclaim Mixed Mode WCX-1 brand of L85 column. The typical retention time for 4-aminophenol is about 4.2–5.3 min. The proposed limit of this nephrotoxin is 0.15% and is based on input provided by the FDA and the pharmaceutical industry to the USP Acetaminophen Expert Panel, which reports to the Chemical Medicines Monographs 2 Expert Committee.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM2: C. Anthony.)

Correspondence Number—C161676

Comment deadline: January 31, 2016

Acetaminophen and Codeine Phosphate Oral Suspension

DEFINITION

Acetaminophen and Codeine Phosphate Oral Suspension is a suspension of Acetaminophen and Codeine Phosphate in a suitable aqueous vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of acetaminophen ($C_8H_9NO_2$) and codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

- **B. Thin-Layer Chromatography**

Standard solution: 12 mg/mL each of USP Acetaminophen RS and USP Codeine Phosphate RS in methanol

Sample solution: Transfer a volume of Oral Suspension, equivalent to 12 mg of codeine phosphate, to a separator. Add 1 mL of ammonium hydroxide and 5 mL of methylene chloride, shake for 1 min, and allow the layers to separate. Use the clear lower layer.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Developing solvent system: Methanol and ammonium hydroxide (49:1)

Application volume: 10 μ L

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Locate the spots on the plate by examination under short-wavelength UV light.

Acceptance criteria: The R_f values of the two principal spots of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY

Change to read:

- **Procedure**

Diluent: Methanol and 0.01 N sodium hydroxide (30:70)

Mobile phase: Dissolve 4.9 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 3.9, and add 216 mg of sodium 1-octanesulfonate. Add 100 mL of acetonitrile, and filter.

Codeine phosphate standard stock solution: 0.5 mg/mL of USP Codeine Phosphate RS in *Diluent*

Standard stock solution: Transfer a quantity of 5J mg of USP Acetaminophen RS (J being

the ratio of the labeled amount, in mg, of acetaminophen to the labeled amount, in mg, of codeine phosphate hemihydrate) and 10.0 mL of *Codeine phosphate standard stock solution* to a 100-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume.

System suitability stock solution: 0.02 mg/mL of sodium benzoate and 0.03 mg/mL of methylparaben in *Diluent*

System suitability solution: Transfer 10.0 mL of the *System suitability stock solution* to a 50-mL volumetric flask, add 10.0 mL of *Standard stock solution*, and dilute with *Mobile phase* to volume.

Standard solution: 0.01 mg/mL of USP Codeine Phosphate RS and 0.01J mg/mL of USP Acetaminophen RS in *Mobile phase*. Prepare by diluting 10.0 mL of the *Standard stock solution* with *Mobile phase* to 50 mL in a volumetric flask.

Sample stock solution: Nominally 0.5 mg/mL of acetaminophen ▲ and 0.5J mg/mL of codeine phosphate hemihydrate ▲^{USP40} in *Diluent* prepared as follows. Transfer a measured volume of well-mixed Oral Suspension, equivalent to 50 mg of acetaminophen, to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and mix by mechanical means for 30 min. Dilute with *Diluent* to volume. Foaming may be minimized by adding a few drops of acetonitrile before diluting with *Diluent* to volume. Centrifuge a portion of this mixture.

Sample solution: Dilute 10.0 mL of the clear supernatant from the *Sample stock solution* with *Mobile phase* to 50 mL in a volumetric flask.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-μm packing L11

Flow rate: 2 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for acetaminophen, benzoate, codeine, and methylparaben are about 0.25, 0.5, 1.0, and 1.3, respectively.]

Suitability requirements

Resolution: NLT 2 between each pair of adjacent peaks, *System suitability solution*

Tailing factor: NMT 2 for each analyte peak, *Standard solution*

Column efficiency: ~~NLT 500 theoretical plates, *Standard solution*~~

▲ ▲^{USP40}

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen (C₈H₉NO₂) in the

portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*

r_S = peak response of acetaminophen from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

Calculate the percentage of the labeled amount of codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of codeine from the *Sample solution*

r_S = peak response of codeine from the *Standard solution*

C_S = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of codeine phosphate hemihydrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of codeine phosphate hemihydrate, 406.37

M_{r2} = molecular weight of anhydrous codeine phosphate, 397.37

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Uniformity of Dosage Units** (905)

For single-unit containers

Acceptance criteria: Meets the requirements

- **Deliverable Volume** (698)

For multiple-unit containers

Acceptance criteria: Meets the requirements

IMPURITIES

Add the following:

- ▲ ● **4-Aminophenol in Acetaminophen-Containing Drug Products** (227): Meets the requirements ▲*USP40*

SPECIFIC TESTS

- **pH** (791): 4.0–6.1

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

- **USP Reference Standards** (11)

USP Acetaminophen RS

USP Codeine Phosphate RS

BRIEFING

Albumin Human, *USP 38* page 2067. On the basis of comments received, the following changes are proposed:

1. Revise the *Definition* to remove aspects of processing.
2. Revise the *Definition* to add appropriate reference to the Code of Federal Regulations.
3. Add *Identification* methods and zone electrophoresis.
4. Add the *Assay* based on protein determination.
5. Add the *Impurities* test *Prekallikrein Activator* (165).
6. Add the *Other Components* test for *Content of Sodium Caprylate* based on ion chromatography using the Microsorb C18 brand of 5- μ m guard column and the Nucleosil C18 brand of 5- μ m analytical column.
7. Add the *Other Components* test for *Content of N-Acetyl-dl-tryptophan* based on HPLC using the TSK G3000SW brand of guard column and the TSK G3000SW brand of analytical column.
8. Add *Specific Tests* for *Bacterial Endotoxins Test* (85), *Biological Reactivity Tests* (88), *In Vivo*, *Sterility Tests* (71), and *pH* (791).
9. Add to *Specific Tests* an HPLC method for *Molecular Weight Distribution*, using the TSK G3000SW brand of guard column and the TSK G3000SW brand of analytical column.
10. Add to *Specific Tests* a method for *Heat Stability*.
11. Add to *Specific Tests* atomic adsorption methods for the *Content of Sodium* and *Limit of Potassium*.
12. Revise the *Labeling* section to align with the Code of Federal Regulations.
13. Add USP Albumin Human RS and USP Endotoxin RS to the *USP Reference Standards* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BIO3: K. Carrick.)

Correspondence Number—C162107

Comment deadline: January 31, 2016

Albumin Human

DEFINITION

Change to read:

~~Albumin Human conforms to the regulations of the federal Food and Drug Administration concerning biologics (640.80 to 640.86) (see *Biologics* (1041)). It is a sterile, nonpyrogenic preparation of serum albumin obtained by fractionating material (source blood, plasma, serum, or placentas) from healthy human donors, the source material being tested for the absence of~~

hepatitis B surface antigen. It is made by a process that yields a product that is safe for intravenous use.

▲ Albumin Human is a sterile, nonpyrogenic preparation of serum albumin. The source material of Albumin Human shall be plasma recovered from Whole Blood prepared as prescribed in the Code of Federal Regulations (CFR) Title 21 sections 640.1 through 640.5, or Source Plasma prepared as prescribed in sections 640.60 through 640.76. Albumin Human is manufactured by a process that yields a product that is safe for intravenous use. Heating of the final containers of Albumin Human begins within 24 h after completion of filling. Heat treatment is conducted so that the solution is heated continuously for NLT 10 and NMT 11 h, at an attained temperature of $60 \pm 0.5^\circ$. All final containers of Albumin Human are incubated at 20° – 35° for at least 14 days following the heat treatment. ▲*USP40*

NLT 96% of its total protein is albumin. ~~It is a solution containing, in each 100 mL, either 25 g of serum albumin osmotically equivalent to 500 mL of normal human plasma, or 20 g equivalent to 400 mL, or 5 g equivalent to 100 mL, or 4 g equivalent to 80 mL, and contains NLT 93.75% and NMT 106.25% of the labeled amount in the case of the solution containing 4 g in each 100 mL, and NLT 94.0% and NMT 106.0% of the labeled amount in the other cases.~~

▲ The final product shall conform to one of the following concentrations: 40 ± 2.5 ; 50 ± 3.0 ; 200 ± 12 ; and 250 ± 15 g/L solution of protein. ▲*USP40*

It contains no added antimicrobial agent, but may contain sodium acetyltryptophanate with or without sodium caprylate as a stabilizing agent. ~~It has a heme content such that the absorbance of a solution, diluted to contain 1% of protein, in a 1-cm holding cell, measured at a wavelength of 403-nm, is NMT 0.25. It meets the requirements of the test for heat stability and for pH.~~

▲ Albumin Human conforms to the regulations of the federal Food and Drug Administration concerning biologics (sections 640.80–640.84). ▲*USP40*

IDENTIFICATION

Add the following:

▲ ● A. Immunodiffusion

Buffer A: Dissolve 1.98 g of barbituric acid, 1.03 g of sodium barbital, and 8.7 g of sodium chloride in 900 mL of water. Adjust with a sodium hydroxide solution to a pH of 8.0, dilute with water to 1 L, and mix.

Agar-gel immunodiffusion plates: Prepare agar-gel immunodiffusion plates each containing a central well and 5 or 6 outer wells concentric with the central well using 1.9 g of agar in 150 mL of *Buffer A*.

Standard solution: 5 μ L of USP Albumin Human RS

Sample solution: 5 μ L of Albumin Human

System suitability

Sample: *Standard solution*

Suitability requirements: The plate containing the *Standard solution* shows an arc between the central well and the well containing antiserum.

Analysis

Samples: *Standard solution* and *Sample solution*

Place *Sample solution* in the central well of one plate and *Standard solution* in the central well of another plate. Add 5 μL of an antiserum specific to human plasma proteins from a suitable commercial source in one of the appropriately marked outer wells of each plate. Place 5 μL each of antisera specific to plasma proteins of animals commonly used in the preparation of materials of biological origin in other appropriately marked outer wells of each plate. [Note—At the minimum, antisera against plasma proteins of bovine, porcine, and ovine should be used.] Incubate the plates at 2°–8° for NLT 24 h.

Acceptance criteria: The plates containing *Sample solution* observed in a bright white light shows a sharp white arc between the central well and the well containing antiserum specific to human plasma proteins, and no arc between the central well and the wells containing antisera specific to plasma proteins of other animals. ▲*USP40*

Add the following:

▲ ● B. Protein Composition by Zone Electrophoresis

Solution A: 0.9% (w/v) Sodium chloride in water

Solution B: 5% (v/v) Acetic acid in water

Running buffer: 1.38 g of barbital, 8.76 g of barbital sodium, and 0.38 g of calcium lactate. Dilute with water to 1000.0 mL.

Fixative dye solution: A 5-g/L solution of amido black 10B in a mixture of methanol and glacial acetic acid (90:10)

Destaining solution: Methanol and glacial acetic acid (90:10)

Clearing solution: Methanol and glacial acetic acid (81:19)

Support media: Use strips of suitable cellulose acetate gel or agarose gel. [Note—Method may be adapted to an automated system.]

Standard solution: USP Albumin Human RS diluted to 50 g/L of protein in *Running buffer*

Sample solution: Albumin Human diluted to 50 g/L of protein in *Running buffer*

Electrophoretic system

Run buffer: *Running buffer*

Voltage: Apply suitable voltage such that the most rapid band migrates at least 30 mm.

Loading volume: Apply 2.5 μL of the *Sample solution* as a 10 mm band or apply 0.25 $\mu\text{L}/\text{mL}$ if a narrower strip is used.

Staining: Immerse the strips in *Fixative dye solution* for 5 min.

Destaining: Immerse in 10 volumes of *Destaining solution* until just free of color. Develop with *Clearing solution*.

System suitability

Sample: *Standard solution*

Suitability requirements: In the electropherogram obtained with the *Standard solution*, the proportion of protein in the principal band is within the limits stated in the certificate for USP Albumin Human RS.

Analysis

Sample: *Sample solution*

Scan membranes measuring the absorbance at 600 nm. Integrate the dye density and report the relative percent areas of any peaks.

Calculate the percentage of albumin human in the portion of Albumin Human taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of albumin from the *Sample solution*

r_T = sum of all the peak responses present from the *Sample solution*

Acceptance criteria: NLT 96% for Albumin Human ▲*USP40*

ASSAY

Add the following:

▲ ● Procedure

Biuret reagent: Dissolve 3.46 g of cupric sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 10 mL of hot water. Dissolve 34.6 g of sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) and 20.0 g of sodium carbonate (Na_2CO_3) in 80 mL of hot water. When the solutions cool, mix together, and dilute to a final volume with water to 200 mL.

Solution A: 6% Sodium hydroxide

Solution B: 0.9% Sodium chloride

Standard solutions: 0.5, 4.75, and 10 g/L of USP Albumin Human RS in *Solution B*

Sample solution: Albumin Human diluted to a protein concentration in the range of the *Standard solutions* with *Solution B*

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: Vis

Wavelength: 545 nm

Blank: *Solution B*

System suitability

Samples: *Standard solutions* and *Sample solution*

Suitability requirements: The r^2 value of the standard curve must be at NLT 0.99. The absorbance of the *Sample solution* must fall within the range of the absorbance of the *Standard solutions*.

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

To 1 volume of the *Blank*, *Standard solutions*, and *Sample solution*, add 1 volume of *Solution A*, and mix. Immediately add 0.4 volumes of *Biuret reagent*, and mix. Between 30 and 60 min, zero the suitable spectrophotometer with the *Blank* and record the absorbance of the *Standard solutions* and *Sample solution*. Generate a standard curve using linear regression by the least squares method for the peak areas of the *Standard solutions* versus the *Standard solutions* concentration. From the standard curve, calculate the protein concentration of the *Sample solution* and correct for the dilution.

Acceptance criteria: The final product shall conform to one of the following

concentrations: 40 ± 2.5 ; 50 ± 3.0 ; 200 ± 12 ; and 250 ± 15 g/L solution of protein. ▲USP40

OTHER COMPONENTS

Add the following:

▲ ● Content of Sodium Caprylate

Solution A: 90% Acetonitrile

Solution B: 0.1 N hydrochloric acid

Solution C: 0.1 N hydrochloric acid in *Solution A*

Solution D: 0.005 N hydrochloric acid

Solution E: 0.0025 N potassium hydroxide

Mobile phase: Acetonitrile, methanol, hydrochloric acid, and water (36: 27: 0.0004: 37)

Standard solutions: 10, 55, and 120 mM sodium caprylate in *Solution C*

Sample solution: Pipet 1 mL of Albumin Human into a 10-mL beaker. Rinse the pipet.

Collect this rinse in the same 10 mL beaker and record for calculating dilution. Using

Solution B, adjust the pH of the sample to between 3.8 and 4.0. Transfer 1 mL of the pH-adjusted Albumin Human to a 50-mL volumetric flask. Fill with *Solution A* to volume and mix. Transfer a portion to a centrifuge tube and centrifuge at 16,000 g for 10 min. Use the supernatant as *Sample solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Conductivity

Suppressor: AMMS-ICE

Columns

Guard: 4.6-mm × 1.5-cm; 5- μ m packing L1

Analytical: 4.0-mm × 25.0-cm; 5- μ m packing L1

Flow rate: 0.7 mL/min

Injection volume: 20 μ L

Regenerant: *Solution E*

System suitability

Samples: *Standard solutions* and *Sample solution*

Suitability requirements: The r^2 value of the standard curve must be at least 0.98. The *Sample solution* peak area must fall within the range of the highest and lowest standards.

Analysis

Samples: *Standard solutions* and *Sample solution*

Generate the standard curve by the linear regression by the least squares method of the peak areas of the *Standard solutions* versus the caprylate concentration. Determine the *Sample solution* concentration by comparison to the standard curve and correct for dilution.

Acceptance criteria: 0.08 ± 0.016 mM sodium caprylate ▲*USP40*

Add the following:

▲ ● **Content of *N*-Acetyl-dl-tryptophan**

[Note—Perform if *N*-acetyl-dl-tryptophan is used in the product.]

Mobile phase: 0.10 M dibasic sodium phosphate anhydrous, 0.10 M monobasic sodium phosphate monohydrate, 0.15 M sodium chloride, and 0.05% sodium azide. Adjust with 10 N sodium hydroxide to a pH of 6.8.

Standard solution: 0.004 M *N*-acetyl-dl-tryptophan

Sample solution: 50 g/L of Albumin Human, diluted in *Mobile phase* if necessary

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Columns

Guard: 7.5-mm × 7.5-cm; packing L59

Analytical: 7.5-mm × 60-cm; packing L59

Flow rate: 1.0 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2500 theoretical plates

Tailing factor: 0.9–1.2

Analysis

Samples: *Standard solution* and *Sample solution*

Perform duplicate injections using the average peak area.

Calculate the concentration of *N*-acetyl-dl-tryptophan in the portion of Albumin Human taken:

$$\text{Result} = (r_U/r_S) \times C_S \times D$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of *N*-acetyl-dl-tryptophan in the *Standard solution*

D = dilution factor

Acceptance criteria: 0.08 ± 0.016 mM sodium acetyltryptophanate ▲*USP40*

IMPURITIES

Add the following:

▲ ● **Prekallikrein Activator** (165): NMT 35 IU/mL ▲*USP40*

SPECIFIC TESTS

Add the following:

▲ ● **Bacterial Endotoxins Test** (85): It contains NMT 0.5 USP Endotoxin Units/mL. ▲*USP40*

Add the following:

▲ ● **Biological Reactivity tests** (88), *In Vivo, Safety Tests—Biologicals*: Meets the requirements ▲*USP40*

Add the following:

▲ ● **Sterility Tests** (71): Meets the requirements ▲*USP40*

Add the following:

▲ ● **pH** (791)

Sample solution: Albumin Human diluted to 10 g/L in 0.15 M sodium chloride

Acceptance criteria: 6.4–7.4 ▲*USP40*

Add the following:

▲ ● **Molecular Weight Distribution**

Mobile phase: Prepare a solution containing 7.337 g/L of dibasic sodium phosphate heptahydrate, 1.741 g/L of monobasic sodium phosphate monohydrate, and 11.688 g/L of sodium chloride in water, degas, and filter.

Standard solution: Dilute a solution of USP Human Albumin RS in *Mobile phase* to obtain a solution with a concentration of about 10 mg/mL of protein.

Sample solution: Dilute a solution of Albumin Human in *Mobile phase* to obtain a solution with about the same concentration of protein as the *Standard solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV-Vis

Wavelength: 280 nm

Columns

Guard: 7.5-mm × 7.5-cm; L59

Analytical: 7.5-mm × 60-cm; L59

Flow rate: 0.5 mL/min

Injection volume: 25 μL

System suitability

Sample: *Standard solution*

Suitability requirements: The test is not valid unless the value obtained for USP Human Albumin RS is within the value stated in the USP Human Albumin RS certificate.

Analysis

Sample: *Sample solution*

Measure the areas of the major peak of all other peaks, excluding the solvent peaks. Calculate the percentage of the major peak in the portion of Albumin Human taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: The area of the major peak is NLT 85% of the total area in the chromatogram. ▲*USP40*

Add the following:

- ▲ ● **Heat Stability:** A final container sample of Albumin Human shall remain unchanged, as determined by visual inspection, after heating at 57° for 50 h, when compared to its control consisting of a sample, from the same lot, which has not undergone the heat treatment. ▲*USP40*

Add the following:**▲ ● Content of Sodium**

Standard solutions: 1.0 mEq/L of sodium with 25 mEq/L of potassium and 0.6 mEq/L of sodium with 25 mEq/L of potassium

Sample solution: Dilute Albumin Human with water such that the sodium concentration is within the range of the standards.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: Atomic absorption spectrophotometry

Lamp: Sodium

Analytical wavelength: 589 nm

System suitability

Sample: *Sample solution*

Suitability requirements: Duplicate absorbance readings must be within 3.0 mEq/L.

Analysis

Samples: *Standard solutions* and *Sample solution*

Establish a stable baseline with water. Read the absorption of the *Standard solutions* and *Sample solution*.

Calculate the sodium concentration of the *Sample solution* from the response line of the *Standard solutions* and correct for the *Sample solution* dilution.

Acceptance criteria: 130–160 mEq/L ▲*USP40*

Add the following:**▲ ● Limit of Potassium**

Standard solution: 0.06 mEq/L of potassium and 40 mEq/L of sodium

Sample solution: Dilute Albumin Human such that the potassium concentration is within the range of the standard.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: Atomic absorption spectrometry

Lamp: Potassium

Analytical wavelength: 766 nm

System suitability

Samples: *Standard solution* and *Sample solution*

Suitability requirements: Duplicate absorbances readings must be within ± 0.005 AU

Analysis

Samples: *Standard solution* and *Sample solution*

Establish a stable baseline with water. Read the absorption of the *Standard solution* and *Sample solution*.

Calculate the sodium concentration of the *Sample solution* from the response line of the *Standard solution*, and correct for dilution.

Acceptance criteria: NMT 2 mEq/L ▲*USP40*

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at the temperature recommended by the manufacturer or indicated on the label.
- **Expiration Date:** The expiration date is not later than 5 years after issue from manufacturer's cold storage (5°, 3 years) if labeling recommends storage between 2° and 10°; not later than 3 years after issue from manufacturer's cold storage (5°, 3 years) if labeling recommends storage at temperatures not higher than 37°; and not later than 10 years after date of manufacture if in a hermetically sealed metal container and labeling recommends storage between 2° and 10°.

Change to read:

- **Labeling:** ~~Label it to state that it is not to be used if it is turbid and that it is to be used within 4 h after the container is entered. Label it also to state the osmotic equivalent in terms of plasma, the sodium content, and the type of source material (venous plasma, placental plasma, or both) from which it was prepared. Label it also to indicate that additional fluids are needed when the 20-g/100-mL or 25-g/100-mL product is administered to a markedly dehydrated patient.~~

▲ Label it to state: the osmotic equivalent in terms of plasma and the sodium concentration in terms of a value or a range in mEq/L; the cautionary statement placed in a prominent position on the label, "Do Not Use if Turbid. Do Not Begin Administration More Than 4 Hours After the Container Has Been Entered." The need for additional fluids when 20% or 25% albumin is administered to a patient with marked. The protein concentration, expressed as a 4%, 5%, 20%, or 25% solution. Label it to indicate the volume of the preparation, storage conditions, the expiration date, and the name and concentration of

any added substance (for example, stabilizer). ▲*USP40*

Add the following:

▲ ● **USP Reference Standards** (11)

USP Albumin Human RS

USP Endotoxin RS

▲*USP40*

BRIEFING

Alprazolam Tablets, *USP 38* page 2099. As part of *USP* modernization efforts, it is proposed to revise the monograph as follows:

1. A new validated test for *Organic Impurities* is proposed. This new liquid chromatographic procedure is also proposed to replace the existing *Assay* and is based on analyses conducted with the Luna C8(2) brand of L7 column. The typical retention time for alprazolam is about 5 min. The limits listed in the proposed test for *Organic Impurities* are based on the currently official monograph for *Alprazolam*. Stakeholders are encouraged to submit their approved specifications to *USP* if they are different from those proposed in this revision.
2. The *Identification* section is revised to replace *Identification* test *A* with a test based on the retention time agreement based on the proposed *Assay* and to add *Identification* test *B* based on UV spectral agreement based on the proposed *Assay*. This approach eliminates the use of chloroform, which is a safety hazard.
3. The cross-references to the existing *Assay* in the test for *Uniformity of Dosage Units* are expanded to include the procedure details.
4. *USP* Alprazolam Related Compound A RS and *USP* Clordiazepoxide Related Compound A RS are added to the *USP Reference Standards* section to support the proposed revisions to the *Assay* and test for *Organic Impurities*.

Interested parties are encouraged to submit validated dissolution procedures, which test individual units, and validated uniformity of dosage form procedures, which avoid the use of internal standards and chloroform, along with supporting data for each to further improve this monograph.

Interested parties are also encouraged to submit validated identification tests to add to the *Alprazolam Orally Disintegrating Tablets* monograph.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM4: H. Joyce.)

Correspondence Number—C120149

Comment deadline: January 31, 2016

Alprazolam Tablets

DEFINITION

Alprazolam Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam

(C₁₇H₁₃ClN₄).

IDENTIFICATION

Delete the following:

▲ ● A. Infrared Absorption

Sample: An amount of finely powdered Tablets, equivalent to 15 mg of alprazolam, prepared as follows. Dissolve the *Sample* in 10 mL of 10 mg/mL of sodium carbonate solution. Add 15 mL of chloroform, and shake vigorously for 30 min. Centrifuge, withdraw the aqueous layer, and transfer the chloroform to a clean container. Add 200 mg of potassium bromide. Evaporate the chloroform from this mixture to dryness, and dry the dispersion in vacuum at 60° for 24 h. Grind this dispersion into a fine powder. Prepare a suitable pellet for testing by placing 100 mg of dried potassium bromide into a die. Sprinkle 20 mg of the finely ground alprazolam-potassium bromide dispersion onto the dried potassium bromide layer, and cover with another specimen of 100 mg of dried potassium bromide.

Acceptance criteria: The IR absorption spectrum of the potassium bromide dispersion so obtained exhibits maxima characteristic of alprazolam, as compared to that of a similar preparation of USP Alprazolam RS, at the following wavenumbers: at 1609, 1578, 1566, 1539, 1487, and 1379 wavenumbers in the region of 1650–1300 cm⁻¹; at 932, 891, 826, 779, 746, 696, and 658 wavenumbers in the region of 975–600 cm⁻¹. ▲USP40

Add the following:

▲ ● **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP40

Add the following:

▲ ● **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP40

ASSAY

Change to read:

● **Procedure**

Mobile phase: Acetonitrile, chloroform, butyl alcohol, glacial acetic acid, and water (850: 80: 50: 0.5: 20)

Internal standard solution: 0.25 mg/mL of triazolam in acetonitrile

Standard stock solution: 0.25 mg/mL of USP Alprazolam RS in *Internal standard solution*

Standard solution: 25 µg/mL of USP Alprazolam RS from *Standard stock solution* in acetonitrile

Sample solution: Nominally 25 µg/mL of alprazolam from finely powdered Tablets (NLT 20) prepared as follows. Transfer a suitable amount of the powdered tablets to a suitable volumetric flask. Add 1% of the flask volume of water. Transfer 10% of the flask volume of *Internal standard solution*, shake vigorously for 10 min, and dilute with acetonitrile to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6 mm x 30 cm, packing L3

Flow rate: 2 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between triazolam and alprazolam

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area response ratio of the alprazolam peak relative to the internal standard peak from the *Sample solution*

R_S = peak area response ratio of the alprazolam peak relative to the internal standard peak from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of alprazolam in the *Sample solution* (µg/mL)

▲ Solution A: 0.77 g/L of ammonium acetate prepared as follows. Dissolve 0.77 g of ammonium acetate in each L of water and adjust with acetic acid to a pH of 4.7.

Solution B: *Chromatographic acetonitrile*

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
2.5	60	40
9.0	5	95
9.1	60	40
11.0	60	40

Diluent: *Chromatographic acetonitrile* and *water* (40:60)

System suitability solution: 50 µg/mL of USP Alprazolam RS, 1 µg/mL of USP Alprazolam Related Compound A RS, and 1 µg/mL of USP Chlordiazepoxide Related Compound A RS in

Diluent

Standard solution: 50 µg/mL of USP Alprazolam RS in *Diluent*

Sample solution: Nominally 50 µg/mL of alprazolam from Tablets prepared as follows.

Transfer a suitable portion of powder from NLT 10 Tablets to an appropriate volumetric flask. Add 80% of the total flask volume of *Diluent*. Sonicate for NLT 10 min. Dilute with *Diluent* to volume. Centrifuge a portion and use the clear supernatant. [Note—The use of a centrifuge speed of 3500 rpm for 10 min may be suitable.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 260 nm. For *Identification* test B, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 3-µm packing L7

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between chlordiazepoxide related compound A and alprazolam related compound A, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam (C₁₇H₁₃ClN₄) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of alprazolam in the *Sample solution* (µg/mL)

▲USP40

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Dissolution** (711), *Procedure, Apparatus 1 and Apparatus 2, Procedure for a Pooled Sample*

Buffer stock solution: Dissolve 80 g of *monobasic potassium phosphate* and 20 g of *dibasic potassium phosphate* in 1 L of *water*. Add, with mixing, *phosphoric acid* or

potassium hydroxide solution (45 in 100), as necessary to adjust the solution, such that the resulting solution has a pH of 6.0 ± 0.1 .

Buffer: Prepare a 1-in-10 dilution of the *Buffer stock solution* to obtain a solution that has a pH of 6.0 ± 0.1 .

Medium: *Buffer*; 500 mL

Apparatus 1: 100 rpm

Time: 30 min

Mobile phase: *Acetonitrile, tetrahydrofuran, and Buffer* (35:5:60)

Standard stock solution: 0.05 mg/mL of USP Alprazolam RS in *methanol*

Standard solution: Add 50 mL of *Buffer stock solution* and 250 mL of *water* to a 500-mL flask. Add to the flask 5.0 mL of *Standard stock solution* for every 0.25 mg of alprazolam contained in the Tablet being assayed. Dilute with *water* to volume.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 10-cm; packing L7

Flow rate: 1 mL/min

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 500 theoretical plates

Relative standard deviation: NMT 3.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved.

Tolerances: NLT 80% (Q) of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) is dissolved.

Change to read:

- **Uniformity of Dosage Units** (905)

~~**Mobile phase, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.~~

▲ **Mobile phase:** *Acetonitrile, chloroform, butyl alcohol, glacial acetic acid, and water* (850: 80: 50: 0.5: 20) ▲*USP40*

Internal standard solution: 0.032 mg/mL of triazolam in *acetonitrile*

Standard solution: 0.025 mg/mL of USP Alprazolam RS in *Internal standard solution*

Sample solution: Transfer 1 Tablet to a container. Add 0.4 mL of *water* directly onto the Tablet, allow the Tablet to stand for 2 min, and then swirl the container to disperse the Tablet. For every 0.25 mg of alprazolam contained in the Tablet, add 10.0 mL of *Internal*

standard solution to the container. Shake, and centrifuge if necessary.

▲ **Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 30-cm; packing L3

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between triazolam and alprazolam

Relative standard deviation: NMT 2.0% for replicate injections

▲ **USP40**

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam (C₁₇H₁₃ClN₄) in the Tablet taken:

$$\text{Result} = (R_U/R_S) \times C \times V \times (100/L)$$

R_U = peak area response ratio of alprazolam relative to the internal standard from the *Sample solution*

R_S = peak area response ratio of alprazolam relative to the internal standard from the *Standard solution*

C = concentration of USP Alprazolam RS in the *Standard solution* (mg/ml)

V = volume of the *Internal standard solution* used to prepare the *Sample solution* (mL)

L = label claim (mg/Tablet)

Acceptance criteria: Meet the requirements

IMPURITIES

Add the following:

▲ ● **Organic Impurities**

Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.05 µg/mL of USP Alprazolam RS in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between chlordiazepoxide related compound A and alprazolam related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of alprazolam from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of alprazolam in the *Sample solution* ($\mu\text{g/mL}$)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Chlordiazepoxide related compound A ^a	0.7	1.1	(0.15)
Alprazolam related compound A ^a	0.8	1.7	(0.15)
Alprazolam	1.0	—	—
Nordazepam ^{a,b}	1.2	1.3	(0.15)
Alprazolam quinoline derivative ^{a,c}	1.4	1.1	(0.15)
2-Amino-5-chlorobenzophenone ^a	1.6	1.4	(0.15)
Any individual unspecified degradation product	—	1.0	0.15
Total degradation products	—	—	1.0

^a If present, it is controlled as an unspecified degradation product.
^b 7-Chloro-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one.
^c 7-Chloro-1-methyl-5-phenyl[1,2,4]triazolo[4,3-*a*]quinolin-4-amine.

▲USP40

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

Change to read:

- **USP Reference Standards** <11>

USP Alprazolam RS

▲ USP Alprazolam Related Compound A RS

2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3*H*-1,4-benzodiazepine.

$C_{17}H_{15}ClN_4O$ 326.78

USP Chlordiazepoxide Related Compound A RS

7-Chloro-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepine 4-oxide;

Also known as 7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.

$C_{15}H_{11}ClN_2O_2$ 286.71

▲USP40

BRIEFING

Amiloxate, USP 38 page 2174. On the basis of comments received, it is proposed to make the following changes:

1. Delete the absorptivities under *Identification* test B and add *Identification* test C that uses the retention time agreement based on the *Assay*.
2. Revise the system suitability requirement for *Relative standard deviation* in the *Assay* from NMT 1.0% to NMT 0.73% to be consistent with the repeatability requirements in *Chromatography* (621), *System Suitability*.
3. Add the volumetric solution (VS) for 0.1 N sodium hydroxide in the test for *Acidity*.

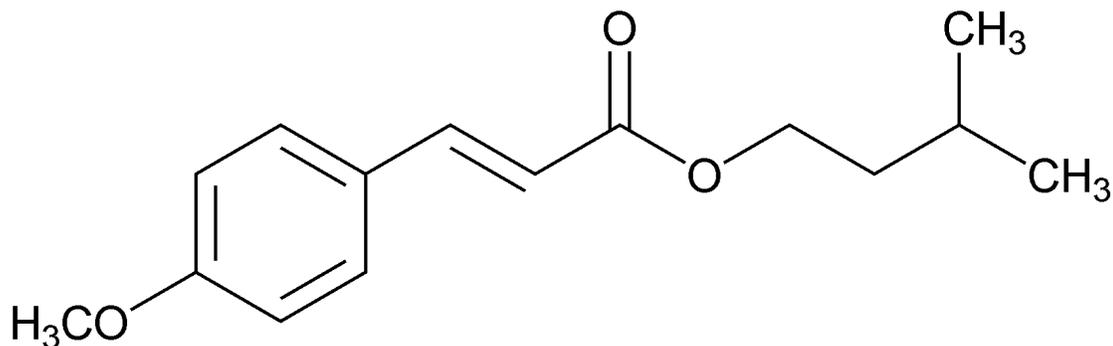
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(CHM6: F. Mao.)

Correspondence Number—C134498

Comment deadline: January 31, 2016

Amiloxate



$C_{15}H_{20}O_3$ 248.32

4-Methoxycinnamic acid, isoamyl ester;

3-Methylbutyl 3-(4-methoxyphenyl)-(E)-2-propenoate [71617-10-2].

DEFINITION

Amiloxate contains NLT 98.0% and NMT 102.0% of amiloxate ($C_{15}H_{20}O_3$).

IDENTIFICATION

- **A. Infrared Absorption** (197F)

Change to read:

- **B. Ultraviolet Absorption** (197U)

Sample solution: 5.0 µg/mL in alcohol

Acceptance criteria: Absorptivities, calculated on the as-is basis, do not differ by more than 3.0%.

▲▲USP40

Add the following:

- ▲● **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲USP40

ASSAY

Change to read:

- **Procedure**

Standard solution: 20 mg/mL of USP Amiloxate RS in *tert-butyl methyl ether*

Sample solution: 20 mg/mL of Amiloxate in *tert-butyl methyl ether*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 25-m; coated with a 0.1-µm film of phase *G1*

Temperatures

Injection port: 240°

Detector: 260°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	8	240	10

Carrier gas: Helium

Flow rate: 6 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT $\pm 0.0\%$

▲ 0.73% ▲ USP40

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of amiloxate ($C_{15}H_{20}O_3$) in the portion of Amiloxate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Amiloxate RS in the *Standard solution* (mg/mL)

C_U = concentration of Amiloxate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• Organic Impurities

Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Amiloxate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses, excluding the solvent peak, from the *Sample solution*

Acceptance criteria

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **Specific Gravity** (841): 1.037–1.041
- **Refractive Index** (831): 1.556–1.560 at 20°

Change to read:

• Acidity

Sample solution: Transfer 50 mL of *alcohol* to a suitable container. Add 1 mL of phenolphthalein TS and sufficient 0.1 N sodium hydroxide to obtain a persistent pink color. Transfer 50 mL of this solution to a suitable container, and add 5.0 mL of Amiloxate.

Analysis: Titrate with 0.1 N sodium hydroxide

▲ VS. ▲ USP40

Acceptance criteria: NMT 0.2 mL of titrant per mL of Amiloxate is required for neutralization.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** <11>

USP Amiloxate RS

BRIEFING

Ascorbic Acid Injection, *USP 38* page 2286. In preparation for the omission of the flame tests from *Identification Tests—General* <191>, proposed in *PF 41(2)* [Mar.–Apr. 2015], the reference to <191> in *Identification test C* is deleted and the acceptance criteria for the flame test is included.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(NBDS: N. Davydova.)

Correspondence Number—C164836

Comment deadline: January 31, 2016

Ascorbic Acid Injection

DEFINITION

Ascorbic Acid Injection is a sterile solution, in Water for Injection, of Ascorbic Acid prepared with the aid of Sodium Hydroxide, Sodium Carbonate, or Sodium Bicarbonate. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ascorbic acid (C₆H₈O₆).

IDENTIFICATION

- **A.**

Analysis: To a volume of Injection, equivalent to 40 mg of ascorbic acid, add 4 mL of 0.1 N hydrochloric acid, then add 4 drops of methylene blue TS, and warm to 40°.

Acceptance criteria: The deep blue color becomes appreciably lighter or is completely discharged within 3 min.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the *Assay*.

Delete the following:

▲ ● ~~**C. Identification Tests—General, Sodium**~~ <191>: Meets the requirements ▲*USP40*

Add the following:

▲ ● **C.** Ascorbic Acid Injection imparts an intense yellow color to a nonluminous flame. ▲*USP40*

ASSAY

- **Procedure**

Mobile phase: Dissolve 15.6 g of *dibasic sodium phosphate* and 12.2 g of *monobasic potassium phosphate* in 2000 mL of water, and adjust with *phosphoric acid* to a pH of 2.5 ± 0.05.

Standard solution: 0.5 mg/mL of USP Ascorbic Acid RS in *Mobile phase*. [Note—Refrigerate and store protected from light until use. The solution is stable for at least 24 h. Inject within 3 h after removal from the refrigerator.]

Sample solution: Dilute the Injection, if necessary, with *Mobile phase* to obtain a solution with a concentration of about 0.5 mg/mL. [Note—Refrigerate and store protected from light until use. The solution is stable for at least 24 h. Inject within 3 h after removal from the refrigerator.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 150-cm × 6-mm; packing L39

Flow rate: 0.6 mL/min

Injection volume: 4 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3500 theoretical plates

Tailing factor: NMT 1.6

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ascorbic acid (C₆H₈O₆) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ascorbic Acid RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ascorbic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• Limit of Oxalate

Analysis: Dilute a volume of Injection, equivalent to 50 mg of ascorbic acid, with water to 5 mL. Add 0.2 mL of acetic acid and 0.5 mL of *calcium chloride TS*.

Acceptance criteria: No turbidity is produced in 1 min.

SPECIFIC TESTS

- **pH** (791): 5.5–7.0
- **Other Requirements:** It meets the requirements in *Injections* (1).
- **Bacterial Endotoxins Test** (85): It contains NMT 1.2 USP Endotoxin Units/mg of ascorbic acid.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in light-resistant, single-dose containers, preferably of Type I or Type II glass.
- **Labeling:** In addition to meeting the requirements in *Injections* (1), *Labels and Labeling*, *Labeling*, fused-seal containers of the Injection in concentrations of 250 mg/mL and greater are labeled to indicate that since pressure may develop on long storage, precautions should be taken to wrap the container in a protective covering while it is being opened.
- **USP Reference Standards** (11)
USP Ascorbic Acid RS
USP Endotoxin RS

BRIEFING

Aspirin, *USP 38* page 2289. On the basis of comments received, it is proposed to make the following changes.

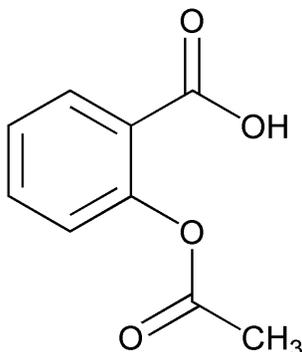
1. Replace the nonspecific *Identification* test *A* based on an outdated color test with a retention time agreement using the procedure in the proposed *Assay*.
2. Revise *Identification* test *B* to allow for both potassium bromide dispersion and attenuated total reflectance methods.
3. Replace the current titrimetric *Assay* procedure with an HPLC method. The HPLC procedure was validated using the Merck KGaA Lichrosorb RP-18 brand of L1 column in which aspirin elutes at about 2.5 min.
4. Change the *Acceptance criteria* in the *Assay* from 99.5%–100.5% to 98.0%–102.0% because this is consistent with the precision of typical HPLC assay procedures compared to typical titrations.
5. Replace the limit test for salicylic acid in the *Organic Impurities* section with a chromatographic procedure based on the *European Pharmacopoeia V.8.5* monograph for *Acetylsalicylic Acid*. The procedure was verified using the Phenomenex Kinetix XB-C18 brand of L1 column in which aspirin and salicylic acid elutes at about 3.8 and 5.1 min, respectively.
6. Remove the *Readily Carbonizable Substances Test* (271) and the *Substances Insoluble in Sodium Carbonate TS* test as they are no longer required with the introduction of the proposed chromatographic procedure in the test for *Organic Impurities*.
7. Add USP Salicylic Acid RS to the *USP Reference Standards* section to support the proposed *Assay* and *Organic Impurities* tests.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM6: A. Potts.)

Correspondence Number—C135549

Comment deadline: January 31, 2016

AspirinC₉H₈O₄ 180.16

Benzoic acid, 2-(acetyloxy)-;
 Salicylic acid acetate;
 2-Acetoxybenzoic acid [50-78-2].

DEFINITION**Change to read:**

Aspirin contains ~~NLT 99.5% and NMT 100.5%~~
~~▲ NLT 98.0% and NMT 102.0%▲USP40~~
 of aspirin (C₉H₈O₄), calculated on the dried basis.

IDENTIFICATION**Delete the following:**▲ ● ~~A.~~

~~**Sample solution:** Prepare a solution of Aspirin in water.~~

~~**Analysis:** Heat *Sample solution* for several min, cool, and add 1 or 2 drops of *ferric chloride TS*.~~

~~**Acceptance criteria:** A violet-red color is produced.▲USP40~~

Add the following:

▲ ● **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲USP40

Change to read:

- **B. Infrared Absorption** (197K)
 ▲ or (197A)▲USP40

ASSAY**Change to read:**

- **Procedure**

Sample: 1.5 g of Aspirin

Titrimetric system:

See *Titrimetry* (541)

Mode: Residual titration

Titrant: 0.5N sodium hydroxide VS

Back-Titrant: 0.5N sulfuric acid VS

Endpoint detection: Visual

Analysis: Transfer the Sample to a suitable flask. Add 50.0 mL of *Titrant* and boil the mixture gently for 10 min. Add phenolphthalein TS, and titrate the excess sodium hydroxide with *Back-Titrant*. Perform a blank determination. Each mL of *Back-Titrant* is equivalent to 45.04 mg of aspirin ($C_9H_8O_4$).

Acceptance criteria: 99.5%–100.5% on the dried basis

▲ **Mobile phase:** Acetonitrile, phosphoric acid, and water (400:2:600, v/v/v)

Standard solution: 0.5 mg/mL of USP Aspirin RS in acetonitrile

Sample solution: 0.5 mg/mL of Aspirin in acetonitrile

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 25-cm; 5- μ m packing L1

Flow rate: 2 mL/min

Injection volume: 10 μ L

Run time: 2 times the retention time of the aspirin peak

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of aspirin ($C_9H_8O_4$) in the portion of Aspirin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aspirin from the *Sample solution*

r_S = peak response of aspirin from the *Standard solution*

C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)

C_U = concentration of Aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ▲*USP40*

IMPURITIES

- **Residue on Ignition** (281): NMT 0.05%
- **Chloride and Sulfate** (221), *Chloride*

Standard solution: Transfer 0.10 mL of 0.020 N hydrochloric acid to a suitable container. Dilute with water to 25 mL.

Sample solution: Transfer 1.5 g of Aspirin to a suitable container. Dilute with water to 75 mL. Boil for 5 min, cool, add sufficient water to restore the original volume, and filter.

Analysis: To the *Standard solution* and the *Sample solution*, separately add 1 mL of *silver nitrate TS*.

Acceptance criteria: A 25-mL portion of filtrate of the *Sample solution* shows no more chloride than that of the *Standard solution* (0.014%).

- **Sulfate**

Sample: Transfer 6.0 g of Aspirin to a suitable container. Dissolve in 37 mL of *acetone* and add 3 mL of water.

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.02 M lead perchlorate, prepared by dissolving 9.20 g of *lead perchlorate* in water to make 1000 mL of solution

Endpoint detection: Potentiometric

Analysis: Titrate with *Titrant* using a pH meter capable of a minimum reproducibility of ± 0.1 mV (see *pH* (791)) and equipped with an electrode system consisting of a lead-specific electrode and a silver–silver chloride reference glass-sleeved electrode containing a solution of *tetraethylammonium perchlorate* in *glacial acetic acid* (1 in 44).

[Note—After use, rinse the lead-specific electrode with water, drain the reference electrode, flush with water, rinse with methanol, and allow to dry.]

Acceptance criteria: NMT 1.25 mL of 0.02 M lead perchlorate is consumed (0.04%).

Delete the following:

- **Heavy Metals**

Sample: 2-g

Analysis: Dissolve *Sample* in 25 mL of *acetone*, and add 1 mL of water. Add 1.2 mL of *thioacetamide glycerin base TS* and 2 mL of *pH 3.5 Acetate Buffer* (see *Heavy Metals* (231)), and allow to stand for 5 min.

Acceptance criteria: Any color produced is not darker than that of a control made with 25 mL of *acetone* and 2 mL of *Standard Lead Solution* (see *Heavy Metals* (231)), treated in the same manner (NMT 10 ppm). • (Official 1-Jan-2018)

Change to read:

- **Organic Impurities**

Limit of Free Salicylic Acid

Standard solution: 0.10 mg/mL of salicylic acid in water

Sample solution: Transfer 2.5 g of Aspirin to a 25.0 mL volumetric flask and dilute to volume in alcohol.

Diluted ferric ammonium sulfate solution: Add 1 mL of 1 N hydrochloric acid and 2 mL of ferric ammonium sulfate TS to a 100 mL volumetric flask, and dilute with water.

Analysis: To each of two matched color comparison tubes add 48 mL of water and 1 mL of a freshly prepared *Diluted ferric ammonium sulfate solution*. Into one tube pipet 1 mL of a *Standard solution*. Into the second tube pipet 1 mL of the *Sample solution*. Mix the contents of each tube.

Acceptance criteria: After 30 s, the color in the tube containing the *Standard solution* is not more intense than that in the tube containing the *Sample solution* (0.1%).

▲ [Note—Use freshly prepared samples for analysis.]

Mobile phase: Acetonitrile, phosphoric acid, water (400:2:600, v/v/v)

System suitability solution: 0.02 mg/mL of USP Aspirin RS and 0.01 mg/mL of USP Salicylic Acid RS in *Mobile phase*

Standard solution: 0.01 mg/mL of USP Salicylic Acid RS in *Mobile phase*

Sample solution: 10 mg/mL of Aspirin in *acetonitrile*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 237 nm

Column: 4.6-mm × 25-cm; 5-µm packing *L1*

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 8 times the retention time of the aspirin peak

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for relative retention times for aspirin and salicylic acid.]

Suitability requirements

Resolution: NLT 6.0 between aspirin and salicylic acid, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual specified or unspecified impurity in the portion of Aspirin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual specified or unspecified impurity from the *Sample solution*

r_S = peak response of salicylic acid from the *Standard solution*

C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.03%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid related compound A ^a	0.7	0.15
Salicylic acid related compound B ^b	0.8	0.15
Aspirin	1.0	—
Salicylic acid	1.3	0.15
Acetylsalicylate ^c	2.3	0.15
Salsalate ^d	3.2	0.15
Aspirin anhydride ^e	6.0	0.15
Individual unspecified impurity	—	0.05
Total impurities	—	0.25

a 4-Hydroxybenzoic acid.
 b 4-Hydroxyisophthalic acid.
 c 2-[(2-Acetoxybenzoyl)oxy]benzoic acid.
 d 2-[(2-Hydroxybenzoyl)oxy]benzoic acid.
 e 2-Acetoxybenzoic anhydride.

▲USP40

SPECIFIC TESTS

- **Loss on Drying** (731)

Analysis: Dry over silica gel for 5 h.

Acceptance criteria: NMT 0.5%

Delete the following:

- ▲ ● **Readily Carbonizable Substances Test** (271)

Sample solution: 100 mg/mL in sulfuric acid

Acceptance criteria: The solution has no more color than *Matching Fluid Q*. ▲USP40

Delete the following:

- ▲ ● **Substances Insoluble in Sodium Carbonate TS**

Sample solution: A solution of 500 mg of Aspirin dissolved in 10 mL of warm *sodium carbonate TS*.

Acceptance criteria: The *Sample solution* is clear. ▲USP40

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

Change to read:

- **USP Reference Standards** <11>

USP Aspirin RS

▲ USP Salicylic Acid RS ▲*USP40*

BRIEFING

Azathioprine Sodium for Injection, *USP 38* page 2337. As part of the USP monograph modernization effort, it is proposed to make the following changes:

1. The polarographic *Assay* is replaced with a validated stability-indicating HPLC procedure. The liquid chromatographic procedure in the *Assay* is based on analyses performed with the μ Bondapak C18 brand of L1 column. The typical retention time for azathioprine is 4.3 min.
2. *Identification* test *B*, by retention time agreement based on the *Assay*, is added.
3. The redundant test for *Completeness of Solution* is deleted, as the monograph already contains *Injections* <1>.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM3: F. Mao.)

Correspondence Number—C139064

Comment deadline: January 31, 2016

Azathioprine Sodium for Injection**DEFINITION**

Azathioprine Sodium for Injection is a sterile solid prepared by the freeze-drying of an aqueous solution of Azathioprine and Sodium Hydroxide. It contains NLT 93.0% and NMT 107.0% of the labeled amount of azathioprine ($C_9H_7N_7O_2S$).

IDENTIFICATION

- **A.** The principal spot from the *Sample solution* shows the same R_F value as that obtained from *Standard solution A* in the test for *Limit of Mercaptopurine*.

Add the following:

- ▲ ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP40*

ASSAY**Change to read:**

- **Procedure**

~~**Standard solution:** 0.1 mg/mL of USP Azathioprine RS prepared as follows. Transfer USP Azathioprine RS into a suitable volumetric flask, dissolve in 0.1 N sodium hydroxide equivalent to 5% of the final volume, and dilute with water to volume. Transfer 10.0 mL~~

of this solution into a 50-mL volumetric flask, and dilute with 0.1 N sulfuric acid to volume.

Sample solution: Transfer the contents of 1 vial of Azathioprine Sodium for Injection with the aid of water into a 100-mL volumetric flask, and dilute with water to volume. Transfer 10 mL of this solution into another 100-mL volumetric flask, and dilute with 0.1 N sulfuric acid to volume.

Analysis: Transfer 20 mL each of the *Standard solution* and the *Sample solution*, separately, to polarographic cells, and deaerate for 10 min with nitrogen that previously has been saturated with 0.1 N sulfuric acid. Blanket the solution with saturated nitrogen, insert the dropping mercury electrode of a suitable polarograph, and record the polarogram from -0.60 to -1.00 V, using a saturated calomel electrode as the reference electrode. Determine the height of the diffusion current as the difference between the residual current and diffusion current plateau.

Calculate the percentage of the labeled amount of azathioprine ($C_9H_7N_7O_2S$) in the portion of Azathioprine Sodium for Injection taken:

$$\text{Result} = [(i_d)_U / (i_d)_S] \times (C_S / C_U) \times 100$$

$(i_d)_U$ = diffusion currents of the *Sample solution*

$(i_d)_S$ = diffusion currents of the *Standard solution*

C_S = concentration of USP Azathioprine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of azathioprine in the *Sample solution* (mg/mL)

▲ Solution A: 1.6 g/L of sodium 1-heptanesulfonate in water

Mobile phase: Methanol and Solution A (30:70). Adjust with 1 N hydrochloric acid to a pH of 3.5 ± 0.1 .

Standard stock solution: 0.5 mg/mL of USP Azathioprine RS prepared as follows. Transfer USP Azathioprine RS to a suitable volumetric flask. Add 25% of the flask volume of methanol and 1% of ammonium hydroxide to the flask, swirl, and sonicate for 2 min or until dissolved. Dilute with methanol to volume.

Standard solution: 0.1 mg/mL of USP Azathioprine RS in water from the *Standard stock solution*

Sample solution: Nominally equivalent to 0.1 mg/mL of azathioprine in water from Azathioprine Sodium for Injection

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10- μ m packing L1

Flow rate: 1.8 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements**Tailing factor:** NMT 2**Relative standard deviation:** NMT 1.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of azathioprine ($C_9H_7N_7O_2S$) in the portion of Azathioprine Sodium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of azathioprine from the *Sample solution* r_S = peak response of azathioprine from the *Standard solution* C_S = concentration of USP Azathioprine RS in the *Standard solution* (mg/mL) C_U = nominal concentration of azathioprine in the *Sample solution* (mg/mL)

▲USP40

Acceptance criteria: 93.0%–107.0%**PERFORMANCE TESTS**

- **Uniformity of Dosage Units** (905): Meets the requirements

IMPURITIES

- **Limit of Mercaptopurine**

Standard solution A: 10 mg/mL of USP Azathioprine RS in *dimethylformamide***Standard solution B:** 100 µg/mL of USP Mercaptopurine RS in *dimethylformamide***Sample solution:** 10 mg/mL of Azathioprine Sodium for Injection in *dimethylformamide***Chromatographic system**(See *Chromatography* (621), *Thin-Layer Chromatography*.)**Mode:** TLC**Adsorbent:** 0.25-mm layer of microcrystalline cellulose**Application volume:** 5 µL for *Standard solution A* and the *Sample solution*, and 15 µL for *Standard solution B***Developing solvent system:** *Butyl alcohol* saturated with 5 N *ammonium hydroxide***Analysis****Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Proceed as directed in the chapter. Apply the *Samples* at points 2 cm from the bottom edge of a TLC plate. Allow the spots to dry, and develop the chromatogram in a suitable chamber until the solvent front has moved 15 cm from the point of application. Remove the plate, air-dry, and locate the spots by viewing under short- and long-wavelength UV light.

Acceptance criteria: 3.0%; any spot from the *Sample solution*, other than the principal spot, is not more intense than the spot from *Standard solution B*.

SPECIFIC TESTS**Delete the following:****▲ ● Completeness of Solution** (641)

Sample: 1 container of Azathioprine Sodium for Injection

Acceptance criteria: The contents of the *Sample* are soluble in 10 mL of water to give a clear, bright yellow solution, essentially free from foreign matter. ▲USP40

● **pH** (791)

Sample solution: The contents of 1 container dissolved in 10 mL of *water*

Acceptance criteria: 9.8–11.0

- **Bacterial Endotoxins Test** (85): It contains NMT 1.0 USP Endotoxin Unit/mg of azathioprine.
- **Water Determination** (921), *Method I*: NMT 7.0%, when the *Sample solution* is prepared as directed for a hygroscopic specimen
- **Other Requirements:** It meets the requirements in *Injections* (1).

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as directed in *Containers for Sterile Solids* as described in *Injections* (1), at controlled room temperature.
- **USP Reference Standards** (11)
 - USP Azathioprine RS
 - USP Endotoxin RS
 - USP Mercaptopurine RS

BRIEFING

Buprenorphine Hydrochloride, *USP 38* page 2486. On the basis of comments received, it is proposed to make the following revisions:

1. Replace the wet chemistry procedure for *Identification* test *B* with an HPLC retention time agreement based on the proposed chromatographic procedure for the *Assay*.
2. Revise *Identification* test *C* based on the corresponding procedure in the current edition of the *European Pharmacopoeia* to improve sample solubility and accuracy of the test.
3. Replace the titration procedure for the *Assay* with an HPLC procedure. The proposed liquid chromatographic procedure is based on analyses performed with the Sunfire C18 brand of L1 column. The typical retention time for buprenorphine is about 10.8 min.
4. Revise the *Definition* and the *Acceptance criteria* in the *Assay* from "98.5%–101.0%" to "98.0%–102.0%," which is typical for a chromatographic assay.
5. Replace the test for *Organic Impurities* with an HPLC procedure, based on the corresponding procedure in the current edition of the *European Pharmacopoeia*, to improve the resolution and accuracy of the test. The limits for organic impurities are proposed based on the specifications approved by FDA. The proposed liquid chromatographic procedure is based on analyses performed with the Sunfire C18 brand of L1 column. The typical retention time for buprenorphine is about 8.5 min.
6. Replace the test for *pH* with the test for *Acidity or Alkalinity* to improve sample solubility and accuracy of the test, based on the corresponding procedure in the current edition of the *European Pharmacopoeia*.

7. Add the chemical information of buprenorphine related compound A and USP Buprenorphine System Suitability Mixture RS to the *USP Reference Standards* section.

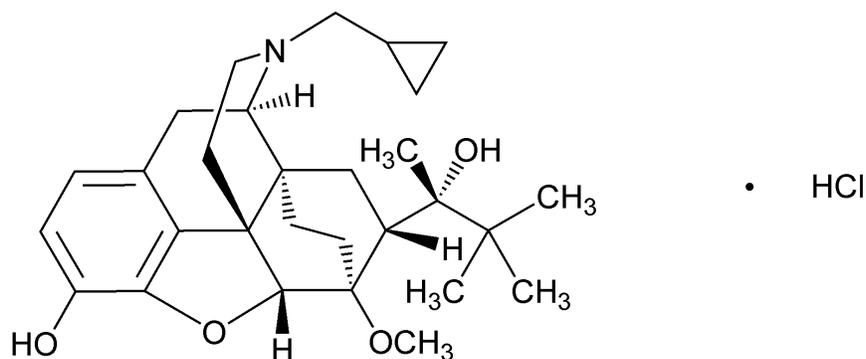
Additionally, minor editorial changes have been made to update the monograph to the current *USP* style.

(CHM2: H. Cai.)

Correspondence Number—C100874

Comment deadline: January 31, 2016

Buprenorphine Hydrochloride



$C_{29}H_{41}NO_4 \cdot HCl$ 504.11

6,14-Ethenomorphinan-7-methanol, 17-(cyclopropylmethyl)- α -(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy- α -methyl-, hydrochloride, [5 α ,7 α (S)]-; 21-Cyclopropyl-7 α -[(S)-1-hydroxy-1,2,2-trimethylpropyl]-6,14-*endo*-ethano-6,7,8,14-tetrahydrooripavine hydrochloride [53152-21-9].

DEFINITION

Change to read:

Buprenorphine Hydrochloride contains ~~NLT 98.5% and NMT 101.0%~~

~~▲ NLT 98.0% and NMT 102.0%~~▲*USP40*

of buprenorphine hydrochloride ($C_{29}H_{41}NO_4 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)

Delete the following:

- ▲ ● **B.**

Sample solution: ~~50 mg/mL of Buprenorphine Hydrochloride in methanol~~

Analysis: ~~To 0.5 mL of the Sample solution add 0.2 mL of a freshly prepared 100 mg/mL potassium ferricyanide TS solution and 0.5 mL of ferric chloride TS.~~

Acceptance criteria: ~~A blue color appears immediately.~~▲*USP40*

Add the following:

- ▲ ● **B.** The retention time of the buprenorphine peak of the *Sample solution* corresponds to

that of the *Standard solution*, as obtained in the *Assay*.▲*USP40*

Change to read:

• **C. Identification Tests—General** (191), *Chloride*

~~**Sample solution:** 10 mg/mL~~

~~**Acceptance criteria:** Meet the requirements~~

▲ **Sample stock solution:** 50 mg/mL of Buprenorphine Hydrochloride in *methanol*

Sample solution: 10 mg/mL of Buprenorphine Hydrochloride in carbon dioxide-free water from the *Sample stock solution*

Analysis: Use 3 mL of the *Sample solution*.

Acceptance criteria: Meets the requirements▲*USP40*

ASSAY

Change to read:

• **Procedure**

~~**Sample:** 0.8 g~~

~~**Titrimetric system**~~

~~**Mode:** Direct titration~~

~~**Titrant:** 0.1 N perchloric acid VS~~

~~**Endpoint detection:** Visual~~

~~**Analysis:** Dissolve the *Sample* in 50 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS and 2 drops of crystal violet TS, and titrate with *Titrant* to a green endpoint. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of *Titrant* is equivalent to 50.41 mg of buprenorphine hydrochloride ($C_{29}H_{41}NO_4 \cdot HCl$).~~

~~**Acceptance criteria:** 98.5%–101.0% on the anhydrous basis~~

▲ **Buffer:** 5.44 g/L of *monobasic potassium phosphate* prepared as follows. Initially dissolve in 90% volume of water, and adjust with 5% (v/v) of *phosphoric acid* to a pH of 4.5. Dilute with water to volume.

Solution A: *Acetonitrile* and *Buffer* (10:90)

Solution B: *Acetonitrile*

Mobile phase: See *Table 1*. Return to the original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	89	11
2	89	11
12	64	36
15	41	59
20	39	61

Diluent: *Methanol* and water (80:20)

Standard solution: 2.0 mg/mL of USP Buprenorphine Hydrochloride RS in *Diluent*

Sample solution: 2.0 mg/mL of Buprenorphine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 10-cm; 3.5-μm packing *L1*

Column temperature: 30°

Flow rate: 1.3 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of buprenorphine hydrochloride ($C_{29}H_{41}NO_4 \cdot HCl$) in the portion of Buprenorphine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of buprenorphine from the *Sample solution*

r_S = peak response of buprenorphine from the *Standard solution*

C_S = concentration of USP Buprenorphine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Buprenorphine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis ▲*USP40*

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

Change to read:

- **Organic Impurities**

Mobile phase: ~~Methanol, 1% solution of ammonium acetate, and glacial acetic acid (60:10:0.01)~~

Standard solution: ~~12.5 μg/mL each of USP Buprenorphine Hydrochloride RS and USP Buprenorphine Related Compound A RS in *Mobile phase*~~

Sample solution: ~~5 mg/mL of buprenorphine hydrochloride in *Mobile phase*~~

~~Chromatographic system~~

~~(See Chromatography (621), System Suitability.)~~

~~**Mode:** LC~~

~~**Detector:** UV 288 nm~~

~~**Column:** 4.6 mm x 25 cm, packing L1~~

~~**Column temperature:** 40°~~

~~**Flow rate:** 1 mL/min~~

~~**Injection volume:** 20 µL~~

~~**System suitability**~~

~~**Sample:** Standard solution~~

~~**Suitability requirements**~~

~~**Resolution:** NLT 3.0 between buprenorphine hydrochloride and buprenorphine-related compound A~~

~~**Column efficiency:** NLT 6500 theoretical plates~~

~~**Relative standard deviation:** NMT 2.0%~~

~~**Analysis**~~

~~**Samples:** Standard solution and Sample solution~~

~~Allow the Sample solution to elute for NLT two times the retention time of buprenorphine hydrochloride.~~

~~Calculate the percentage of each impurity in the portion of Buprenorphine Hydrochloride taken:~~

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

~~r_U = peak response for each impurity from the Sample solution~~

~~r_S = peak response of buprenorphine hydrochloride from the Standard solution~~

~~C_S = concentration of USP Buprenorphine Hydrochloride RS in the Standard solution (mg/mL)~~

~~C_U = concentration of Buprenorphine Hydrochloride in the Sample solution (mg/mL)~~

~~**Acceptance criteria**~~

~~**Individual impurity:** NMT 0.25%~~

~~**Total impurities:** NMT 0.65%~~

▲ Buffer, Solution A, Solution B, and Mobile phase: Prepare as directed in the Assay.

System suitability solution: 5.0 mg/mL of USP Buprenorphine System Suitability Mixture RS in *methanol*

Standard solution: 0.005 mg/mL of USP Buprenorphine Hydrochloride RS and 0.01 mg/mL of USP Buprenorphine Related Compound A RS in *methanol*

Sample solution: 5.0 mg/mL of Buprenorphine Hydrochloride in *methanol*

Chromatographic system: Proceed as directed in the Assay except for the *Column*.

Column: 4.6-mm × 5.0-cm; 3.5-μm packing L1

System suitability

Samples: System suitability solution and Standard solution

Suitability requirements

Resolution: NLT 1.5 between buprenorphine and ethenobuprenorphine, System suitability solution

Relative standard deviation: NMT 5% for buprenorphine and buprenorphine related compound A, Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of buprenorphine related compound A in the portion of Buprenorphine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of buprenorphine related compound A from the Sample solution

r_S = peak response of buprenorphine related compound A from the Standard solution

C_S = concentration of USP Buprenorphine Related Compound A RS in the Standard solution (mg/mL)

C_U = concentration of Buprenorphine Hydrochloride in the Sample solution (mg/mL)

Calculate the percentage of each other individual impurity in the portion of Buprenorphine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each other individual impurity from the Sample solution

r_S = peak response of buprenorphine from the Standard solution

C_S = concentration of USP Buprenorphine Hydrochloride RS in the Standard solution (mg/mL)

C_U = concentration of Buprenorphine Hydrochloride in the Sample solution (mg/mL)

F = relative response factor (see Table 2)

Acceptance criteria: See Table 2. Disregard any peaks below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Buprenorphine	1.0	—	—
Ethenobuprenorphine ^a	1.1	1.0	0.10
Buprenorphine related compound A	1.4	—	0.20
Buprenorphine 2,2'-dimer ^b	1.8	3.3	0.10
Any other individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.65
^a (S)-2-[17-(Cyclopropylmethyl)- 4,5a-epoxy-3-hydroxy-6-methoxy-6a,14-ethenomorphinan-7a-yl]- 3,3-dimethylbutan-2-ol. ^b 2,2'-Bi{17-(cyclopropylmethyl)- 4,5a-epoxy-3-hydroxy-7a-[(S)-2-hydroxy-3,3-dimethylbutan-2-yl]-6-methoxy-6a, 14-ethanomorphinan}.			

▲USP40

SPECIFIC TESTS

- **Optical Rotation** (781S), *Procedures, Specific Rotation*

Sample solution: 20 mg/mL in methanol

Acceptance criteria: -92° to -98°

Delete the following:

- ▲ ● **pH** (791)

Sample: 10 mg/mL solution

Acceptance criteria: 4.0–6.0 ▲USP40

Add the following:

- ▲ ● **Acidity or Alkalinity**

Sample stock solution: 50 mg/mL of Buprenorphine Hydrochloride in *methanol*

Sample solution: 10 mg/mL of Buprenorphine Hydrochloride in carbon dioxide-free water from *Sample stock solution*

Analysis: Add 0.05 mL of *methyl red TS 2* to 10 mL of *Sample solution* and titrate with 0.02 N sodium hydroxide or 0.02 N hydrochloric acid

Acceptance criteria: NMT 0.2 mL of 0.02 N sodium hydroxide or 0.02 N hydrochloric acid is required to change the color of the indicator. ▲USP40

- **Water Determination** (921), *Method I*: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

- **USP Reference Standards** (11)

USP Buprenorphine Hydrochloride RS

USP Buprenorphine Related Compound A RS

▲ (S)-2-[17-(But-3-en-1-yl)-4,5a-epoxy-3-hydroxy-6-methoxy-6a,14-ethanomorphinan-7a-yl]-3,3-dimethylbutan-2-ol.

C₂₉H₄₁NO₄ 467.65

USP Buprenorphine System Suitability Mixture RS

It contains buprenorphine hydrochloride and about 0.5% of ethenobuprenorphine as follows:

(S)-2-[17-(Cyclopropylmethyl)-4,5a-epoxy-3-hydroxy-6-methoxy-6a,14-ethenomorphinan-7a-yl]-3,3-dimethylbutan-2-ol.

C₂₉H₃₉NO₄ 465.63

▲USP40

BRIEFING

Carboplatin, USP 38 page 2602. On the basis of comments received, it is proposed to make the following changes:

1. The *Definition* is revised to calculate the *Assay* based on the dried basis to be consistent with the monograph in the *European Pharmacopoeia*. The test for *Water Determination* is replaced with the test for *Loss on Drying*.
2. *Identification* test A is revised to include *Infrared Absorption* (197A).
3. *Identification* test B, based on the retention time agreement in the *Assay*, is added.
4. The system suitability requirements for *Capacity factor* and *Column efficiency* are deleted from the *Assay* because the remaining criteria are adequate to evaluate the system suitability.
5. The system suitability requirement for *Column efficiency* is deleted from the test for *Limit of 1,1-Cyclobutanedicarboxylic Acid* because the remaining criteria are adequate to evaluate the system suitability.
6. The column size in the *Assay* is changed to be consistent with the procedure for *Related substances* in the *European Pharmacopoeia* monograph. The typical retention time for carboplatin is about 7 min.
7. The *Organic Impurities* test is revised to include the specified impurity of cisplatin and the run time.
8. The test for *Platinum Content* is revised to avoid the use of the toxic reagent, hydrazine hydrate.
9. The storage temperature is added to the *Packaging and Storage* section.

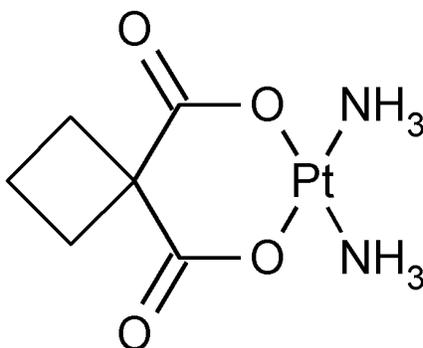
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(CHM3: F. Mao.)

Correspondence Number—C150436

Comment deadline: January 31, 2016

Carboplatin



$C_6H_{12}N_2O_4Pt$ 371.25

Platinum, diammine[1,1-cyclobutanedicarboxylato(2-)-O,O]-, (SP-4-2);
cis-Diammine(1,1-cyclobutanedicarboxylato)platinum [41575-94-4].

DEFINITION

Change to read:

Carboplatin contains NLT 98.0% and NMT 102.0% of carboplatin ($C_6H_{12}N_2O_4Pt$), calculated on the anhydrous

▲ dried ▲*USP40*

basis.

[**Caution**—Great care should be taken in handling Carboplatin because it is a suspected carcinogen.]

IDENTIFICATION

Change to read:

● **A. Infrared Absorption** ~~(197K)~~

▲ **Infrared Absorption** (197)

[Note—Methods described in (197K) or (197A) may be used.] ▲*USP40*

Add the following:

▲ ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP40*

ASSAY

Change to read:

● Procedure

Mobile phase: *Acetonitrile* and *water* (87:13)

Standard solution: 1 mg/mL of USP Carboplatin RS in *water*. Use it within 2 h.

Sample solution: 1 mg/mL of Carboplatin in *water*. Use it within 2 h.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: ~~4.0-mm × 30-cm; packing L8~~

▲ 4.6-mm × 25-cm; 5-µm packing L8▲USP40

Flow rate: 2.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor, k' : ~~NLT 3.0~~

Column efficiency: ~~NLT 2500 theoretical plates~~

▲▲USP40

Tailing factor: NMT 2.5

Relative standard deviation: NMT 1.2%

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of carboplatin ($C_6H_{12}N_2O_4Pt$) in the portion of Carboplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Carboplatin RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the *anhydrous*

▲ dried▲USP40

basis

OTHER COMPONENTS

Change to read:

● **Platinum Content**

~~Thoroughly cleanse all glassware with nitric acid, and rinse with water to prevent “mirroring” of platinum precipitate.~~

Sample solution: ~~Transfer 0.25 g of Carboplatin to a 600-mL beaker. Add 400 mL of water, and slowly dissolve by heating almost to the boiling point, stirring frequently with a glass rod.~~

Analysis: ~~When the *Sample solution* is complete, remove the insulating pad, and boil for about 10 min. Remove the beaker from the hot plate, allow to cool for 1 min without stirring, and pass through quantitative, fine porosity, smooth, dense, ashless filter paper, collecting the filtrate in a 600-mL beaker, and completing the transfer to the filter with~~

hot water. Wash the filter with hot water. Place the beaker containing the combined filtrate and washings on a hot plate, and evaporate to a volume of about 300 mL. Place a glass stirring rod in the beaker, and heat the solution to boiling. Slowly add to the center of the beaker, by dropwise additions, 10.0 mL of hydrazine hydrate, 85%. [**Caution**—Hydrazine is toxic.] Add 2 drops of 10 N sodium hydroxide, boil for 10 min to coagulate the precipitate for ease of filtration, cool, and pass through quantitative, medium-porosity, smooth, ashless filter paper. Rinse the beaker with hot water, and pour the rinsings onto the filter. Wipe the beaker and the stirring rod with small pieces of the same kind of paper used for this filtration, and place these and the filter containing the precipitate in a No. 1 porcelain crucible, previously ignited to constant weight. Dry on a hot plate covered with an insulating pad, slowly increase the heat to char, and ignite for 1 h at 800°. Cool in a desiccator, and weigh again.

Acceptance criteria: The weight of the platinum so obtained is between 52.0% and 53.0% of the carboplatin taken on the anhydrous basis.

▲ **Sample:** 0.2 g of Carboplatin, from *Loss on Drying*

Analysis: Ignite the *Sample* to constant weight at $800 \pm 50^\circ$, and weigh the residue. The residue is platinum.

Calculate the platinum content in the portion of Carboplatin taken:

$$\text{Result} = (W_U/W_S) \times 100$$

W_U = weight of platinum

W_S = weight of *Sample*

Acceptance criteria: 52.0%–53.0% on the dried basis ▲*USP40*

IMPURITIES

Change to read:

- **Limit of 1,1-Cyclobutanedicarboxylic Acid**

Solution A: Dissolve 8.5 g of *tetrabutylammonium hydrogen sulfate* in 80 mL of *water*. Add 3.4 mL of *phosphoric acid*, and adjust with 10 N *sodium hydroxide* to a pH of 7.55.

Mobile phase: *Acetonitrile*, *Solution A*, and *water* (100:20:880)

Standard solution: 5 µg/mL of 1,1-cyclobutanedicarboxylic acid in *Mobile phase*

System suitability solution: 2.5 µg/mL of 1,1-cyclobutanedicarboxylic acid and 0.5 mg/mL of Carboplatin in *Mobile phase* prepared as follows. Mix 1.0 mL of *Standard solution* with 1.0 mL of *Standard solution* in the *Assay*.

Sample solution: 1 mg/mL of Carboplatin in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.0-mm × 30-cm; packing *L1*

Flow rate: 2 mL/min

Injection volume: 100 μ L

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for carboplatin and 1,1-cyclobutanedicarboxylic acid are 0.65 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between the carboplatin and 1,1-cyclobutanedicarboxylic acid peaks

Column efficiency: ~~NLT 1500 theoretical plates, cyclobutanedicarboxylic acid peak~~

▲▲USP40

Relative standard deviation: NMT 10%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 1,1-cyclobutanedicarboxylic acid in the portion of Carboplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 1,1-cyclobutanedicarboxylic acid from the *Sample solution*

r_S = peak response of 1,1-cyclobutanedicarboxylic acid from the *Standard solution*

C_S = concentration of 1,1-cyclobutanedicarboxylic acid in the *Standard solution* (mg/mL)

C_U = concentration of Carboplatin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5%

Change to read:

- **Organic Impurities**

Mobile phase, Sample solution, Chromatographic system, and System suitability:

~~Proceed as directed in the Assay.~~

Standard solution: ~~2.5 μ g/mL of USP Carboplatin RS in water, from the Standard solution in the Assay~~

Analysis

Samples: ~~Sample solution and Standard solution~~

Record the chromatograms, and measure the peak responses.

Acceptance criteria

Individual impurities: ~~NMT 0.25%; no single peak response is greater than that of the carboplatin peak from the Standard solution.~~

Total impurities: ~~NMT 0.5%; the sum of the peak responses, excluding the carboplatin and 1,1-cyclobutanedicarboxylic acid responses, from the Sample solution, is NMT 2 times the carboplatin response from the Standard solution.~~

▲ Mobile phase, Standard solution, Sample solution, and System suitability: Proceed

as directed in the *Assay*.

Diluted standard solution: 2.5 µg/mL of USP Carboplatin RS in *water*, from the *Standard solution*

Chromatographic system: Proceed as directed in the *Assay*, and the run time is at least 2.5 times the retention time of the carboplatin peak.

Analysis

Samples: *Sample solution* and *Diluted standard solution*

Calculate the percentage of each impurity in the portion of Carboplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of carboplatin from the *Diluted standard solution*

C_S = concentration of USP Carboplatin RS in the *Diluted standard solution* (mg/mL)

C_U = concentration of Carboplatin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cisplatin ^a	0.3	0.25
Carboplatin	1.0	—
Any individual unspecified impurity	—	0.25
Total impurities	—	0.5
^a <i>cis</i> -Diamminedichloroplatinum(II).		

▲USP40

SPECIFIC TESTS

- **Crystallinity** (695): Meets the requirements
- **pH** (791)

Sample solution: 10 mg/mL in water

Acceptance criteria: 5.0–7.0

Delete the following:

- ▲ ● **Water Determination, Method I** (921): NMT 0.5%, using anhydrous formamide as the solvent ▲USP40

Add the following:

- ▲ ● **Loss on Drying** (731)

Sample: 1 g

Analysis: Dry the *Sample* at 105° to constant weight.

Acceptance criteria: NMT 0.5% ▲USP40

- **Transmittance**

Sample solution: 10 mg/mL of Carboplatin in *water*

Analysis: Determine the percent transmittance in 1-cm cells at a wavelength of 440 nm, using *water* as the blank.

Acceptance criteria: NLT 97%

- **Water-Insoluble Matter**

Sample: 1 g

Analysis: Transfer the *Sample* to a 150-mL beaker. Add 100 mL of *water*, and dissolve by stirring with a stirring bar for 30 min. With the aid of suction, pass through a tared filtering crucible. Rinse the beaker with *water*, and transfer the rinsings to the crucible. Dry the crucible at $130 \pm 10^\circ$ to constant weight.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers, protected from light.

▲ Store at room temperature. ▲*USP40*

- **USP Reference Standards** (11)

USP Carboplatin RS

BRIEFING

Carboplatin for Injection, *USP 38* page 2603. On the basis of comments received, it is proposed to make the following changes:

1. *Identification* test *B*, based on the retention time agreement in the *Assay*, is added.
2. The column size in the *Assay* is changed to be consistent with the *Assay* procedure in the *Carboplatin* monograph. The typical retention time for carboplatin is about 7 min.
3. The system suitability requirements for *Capacity factor* and *Column efficiency* are deleted from the *Assay* because the remaining criteria are adequate to evaluate the system suitability.
4. The system suitability requirement for *Column efficiency* is deleted from the test for *Limit of 1,1-Cyclobutanedicarboxylic Acid* because the remaining criteria are adequate to evaluate the system suitability.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM3: F. Mao.)

Correspondence Number—C158807

Comment deadline: January 31, 2016

Carboplatin for Injection

DEFINITION

Carboplatin for Injection is a sterile, lyophilized mixture of Carboplatin and Mannitol. It contains NLT 90.0% and NMT 110.0% of the labeled amount of carboplatin ($C_6H_{12}N_2O_4Pt$).

[**Caution**—Great care should be taken in handling Carboplatin because it is a suspected carcinogen.]

IDENTIFICATION

• A. Thin-Layer Chromatography

Standard solution: 10 mg/mL of USP Carboplatin RS in *water*

Sample solution: Nominally equivalent to 10 mg/mL of carboplatin in *water* from the contents of 1 container

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 μ L

Developing solvent system: Acetone and water (80:20)

Spray reagent: Add 5.6 g of *stannous chloride* to 10 mL of *hydrochloric acid*, and stir for 5 min. [Note—It is not necessary that all of the solids dissolve.] Add 90 mL of *water* and 1 g of *potassium iodide*, and stir. Prepare this solution fresh daily.

Analysis

Samples: *Standard solution* and *Sample solution*

Place the plate in a chromatographic chamber lined with filter paper and equilibrated for 2 h in *Developing solvent system*. Develop the chromatogram until the solvent front has moved 10 cm from the origin. Remove the plate from the chamber, and air-dry at room temperature for 2 h. Spray with the *Spray reagent*, and heat at 110° for 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in appearance and R_F value to that of the *Standard solution*.

Add the following:

- ▲ • B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP40

ASSAY

Change to read:

• Procedure

Mobile phase: *Acetonitrile* and *water* (87:13)

Standard solution: 1 mg/mL of USP Carboplatin RS. Use this solution within 2 h.

Sample solution: Nominally equivalent to 1 mg/mL of carboplatin from the contents of 1 container diluted with *water*. Complete chromatographic analysis of this solution within 2 h.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.0-mm × 30-cm; packing L8

▲ 4.6-mm × 25-cm; 5-μm packing L8▲USP40

Flow rate: 2.0 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor, k' : NLT 3.0

Column efficiency: NLT 2500 theoretical plates

▲▲USP40

Tailing factor: NMT 2.5

Relative standard deviation: NMT 1.2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carboplatin ($C_6H_{12}N_2O_4Pt$) in the portion of Carboplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Carboplatin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of carboplatin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Uniformity of Dosage Units** (905): Meets the requirements

IMPURITIES

Change to read:

- **Limit of 1,1-Cyclobutanedicarboxylic Acid**

Solution A: Dissolve 8.5 g of *tetrabutylammonium hydrogen sulfate* in 80 mL of water. Add 3.4 mL of *phosphoric acid*, and adjust with 10 N *sodium hydroxide* to a pH of 7.55.

Mobile phase: *Acetonitrile*, *Solution A*, and *water* (100:20:880)

Standard solution A: 0.01 mg/mL of 1,1-cyclobutanedicarboxylic acid in *Mobile phase*

Standard solution B: 5 µg/mL of 1,1-cyclobutanedicarboxylic acid in *Mobile phase*

System suitability solution: 2.5 µg/mL of 1,1-cyclobutanedicarboxylic acid and 0.5 mg/mL of carboplatin prepared as follows. Mix 1.0 mL of *Standard solution B* with 1.0 mL of *Standard solution* in the Assay.

Sample solution: Nominally equivalent to 1 mg/mL of carboplatin from the contents of 1 container diluted with *Mobile phase*. Complete the chromatographic analysis of the solution within 2 h.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.0-mm × 30-cm; packing *L1*

Flow rate: 2 mL/min

Injection volume: 100 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for carboplatin and 1,1-cyclobutanedicarboxylic acid are 0.65 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between the carboplatin and 1,1-cyclobutanedicarboxylic acid peaks

Column efficiency: ~~NLT 1500 theoretical plates, cyclobutanedicarboxylic acid peak~~



Relative standard deviation: NMT 10%

Analysis

Samples: *Standard solution A* and *Sample solution*

Calculate the percentage of 1,1-cyclobutanedicarboxylic acid in the portion of Carboplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 1,1-cyclobutanedicarboxylic acid from the *Sample solution*

r_S = peak response of 1,1-cyclobutanedicarboxylic acid from *Standard solution A*

C_S = concentration of 1,1-cyclobutanedicarboxylic acid in *Standard solution A* (mg/mL)

C_U = nominal concentration of carboplatin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

- **Bacterial Endotoxins Test** <85>: NMT 0.54 USP Endotoxin Units/mg of carboplatin

- **Sterility Tests** (71), *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **Constituted Solution**: At the time of use, it meets the requirements in *Injections* (1), *Constituted Solutions*.
- **pH** (791)
Sample solution: Use Sterile Water for Injection, and constitute as directed in the labeling.
Acceptance criteria: 5.0–7.0
- **Water Determination** (921), *Method I*
Analysis: Use *anhydrous formamide* as the extraction solvent. Introduce 50 mL of *anhydrous formamide* into the titration vessel, and titrate with the *Reagent* to the electrometric endpoint. Use the formamide thus dried to rinse a suitable glass syringe equipped with an 8-cm long, 22-gauge needle. Add the rinse back to the titration vessel, and titrate the vessel contents again, if necessary. Via the syringe, withdraw 5 mL of the formamide thus titrated and, through the closure of the container, expel the contents into the container. Shake the container to obtain a solution. With the same syringe, withdraw all of the contents of the container, and transfer to the titration vessel. Titrate to the endpoint, adjusting the feeding speed control to the lowest setting to avoid overtitration.
Acceptance criteria: NMT 3.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage**: Preserve as described in *Injections* (1), *Packaging, Containers for Sterile Solids*, and protect from light.
- **USP Reference Standards** (11)
 USP Carboplatin RS
 USP Endotoxin RS

BRIEFING

Carglumic Acid. Because there is no existing *USP* monograph for this drug substance, a new monograph based on validated methods of analysis is being proposed. The stability-indicating liquid chromatographic procedure in the *Assay* and the test for *Organic Impurities* is based on analysis performed using the Nucleodur C18 Pyramid brand of L1 column. The typical retention time for carglumic acid is about 3.5 min based on conditions specified. The liquid chromatographic procedure in the test for *Limit of d-Carglumic Acid* is based on analysis performed using the 5- μ m Astec Chirobiotic TAG brand of L63 column. The typical retention times for carglumic acid and d-carglumic acid are about 3.4 and 7.5 min, respectively.

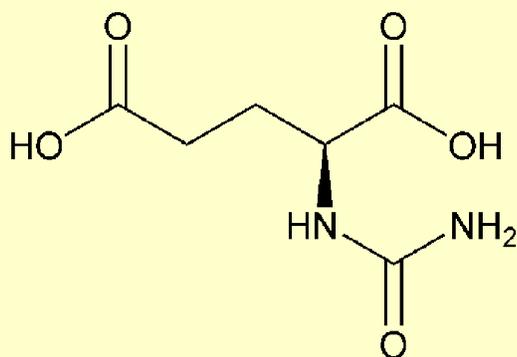
(CHM2: S. Ramakrishna.)

Correspondence Number—C120395

Comment deadline: January 31, 2016

Add the following:

▲ Carglumic Acid



$C_6H_{10}N_2O_5$ 190.15

L-Glutamic acid, *N*-(aminocarbonyl)-;
N-Carbamoyl-L-glutamic acid [1188-38-1].

DEFINITION

Carglumic Acid contains NLT 98.0% and NMT 102.0% of carglumic acid ($C_6H_{10}N_2O_5$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197): Meets the requirements for (197A) or (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Limit of d-Carglumic Acid*.

ASSAY

• Procedure

Solution A: 0.135 g/L of *monobasic potassium phosphate* in water. Adjust with *phosphoric acid* to a pH of 2.5.

Solution B: *Methanol*

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
6	100	0
10	70	30
15	70	30
17	100	0

System suitability solution: 0.2 mg/mL each of USP Carglumic Acid RS, USP L-Glutamic Acid RS, and USP Pyroglutamic Acid RS in water. Sonication may be necessary for complete dissolution.

Standard solution: 1 mg/mL of USP Carglumic Acid RS in water

Sample solution: 1 mg/mL of Carglumic Acid in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3-μm packing L1

Column temperature: 20°

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between l-glutamic acid and carglumic acid; NLT 4.0 between carglumic acid and pyroglutamic acid, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of carglumic acid (C₆H₁₀N₂O₅) in the portion of Carglumic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carglumic acid from the *Sample solution*

r_S = peak response of carglumic acid from the *Standard solution*

C_S = concentration of USP Carglumic Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Carglumic Acid in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

- **Organic Impurities**

All samples containing Carglumic Acid are prepared fresh at the time of analysis.

Solution A, Solution B, and System suitability solution: Proceed as directed in the *Assay*.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solvent A (%)	Solvent B (%)
0	100	0
8	100	0
14	90	10
28	90	10
30	100	0
40	100	0

Standard solution: 5 µg/mL each of USP Carglumic Acid RS, USP Carglumic Acid Related Compound A RS, USP Carglumic Acid Related Compound B RS, USP Carglumic Acid Related Compound C RS, USP L-Glutamic Acid RS, and USP Pyroglutamic Acid RS in water

Sample solution: 10 mg/mL of Carglumic Acid in water

Chromatographic system: Proceed as directed in the *Assay*, except for the following.

Injection volume: 100 µL

Run time: NLT 9 times the retention time of the carglumic acid peak

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between L-glutamic acid and carglumic acid; NLT 3.0 between carglumic acid and pyroglutamic acid, *System suitability solution*

Relative standard deviation: NMT 5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of carglumic acid related compound A, carglumic acid related compound B, carglumic acid related compound C, L-glutamic acid, and pyroglutamic acid in the portion of Carglumic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carglumic acid related compound A, carglumic acid related compound B, carglumic acid related compound C, L-glutamic acid, or pyroglutamic acid from the *Sample solution*

r_S = peak response of carglumic acid related compound A, carglumic acid related compound B, carglumic acid related compound C, L-glutamic acid, or pyroglutamic acid from the *Standard solution*

C_S = concentration of USP Carglumic Acid Related Compound A RS, USP Carglumic Acid Related Compound B RS, USP Carglumic Acid Related Compound C RS, USP L-Glutamic Acid RS, and USP Pyroglutamic Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Carglumic Acid in the *Sample solution* (mg/mL)

Calculate the percentage of dicarbamoyl L-glutamic acid, 1-carglumic dimer, and any unspecified impurity in the portion of Carglumic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dicarbamoyl l-glutamic acid, 1-carglumic dimer, and any unspecified impurity from the *Sample solution*

r_S = peak response of carglumic acid from the *Standard solution*

C_S = concentration of the corresponding USP Carglumic Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Carglumic Acid in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
l-Glutamic acid ^a	0.4	0.05
Carglumic acid	1.0	0.05
Pyroglutamic acid	1.4	0.05
Carglumic acid related compound A	2.0	0.05
Carglumic acid related compound C	2.8	0.05
Carglumic acid related compound B	2.9	0.05
Dicarbamoyl l-glutamic acid ^b	4.1	0.05
1-Carglumic dimer ^c	5.6	0.05
Any unspecified impurity	—	0.05
Total impurities	—	0.2

^a 2-Amino-pentanedioic acid.
^b (S)-2-(3-Carbamoylureido)pentanedioic acid.
^c (S)-2-{3-[(S)-4-Carboxy-2-ureidobutanoyl]ureido}pentanedioic acid.

Limit of d-Carglumic acid

Buffer: 1.74 g/L of *dibasic potassium phosphate* in water. Adjust with 10% phosphoric acid solution to a pH of 8.0.

Mobile phase: *Acetonitrile* and *Buffer* (800:200)

System suitability solution: 25 µg/mL each of USP d-Carglumic Acid RS and USP Carglumic Acid RS in *Mobile phase*

Standard solution: 5 µg/mL of USP d-Carglumic Acid RS in *Mobile phase*

Sample solution: 5 mg/mL of Carglumic Acid in *Mobile phase*

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 25-cm; 5-µm packing L63

Column temperature: 20°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 3 times the retention time of carglumic acid

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 6.0 between l-carglumic acid and d-carglumic acid

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of d-carglumic acid in the portion of Carglumic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of d-carglumic acid from the *Sample solution*

r_S = peak response of d-carglumic acid from the *Standard solution*

C_S = concentration of USP d-Carglumic Acid RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of Carglumic Acid in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carglumic acid	0.45	—
d-Carglumic acid	1.00	0.1

SPECIFIC TESTS

- **Loss on Drying** (731)

Sample: 1.0 g

Analysis: Dry the *Sample* at 105° to constant weight.

Acceptance criteria: NMT 0.5%

- **Optical Rotation** (781), *Procedures, Specific Rotation*

Sample solution: 10 mg/mL in water

Temperature: 20°

Acceptance criteria: -6.5° to -5.5°

- **pH** (791)

Sample solution: 5 mg/mL in water

Acceptance criteria: 2.2–3.2

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store at controlled room temperature in tight containers in a dry place.

• USP Reference Standards <11>

USP Carglumic Acid RS

USP d-Carglumic Acid RS

d-Glutamic acid, N-(aminocarbonyl)-.

 $C_6H_{10}N_2O_5$ 190.15

USP Carglumic Acid Related Compound A RS

(S)-3-(2,5-Dioxoimidazolidin-4-yl)propanoic acid.

 $C_6H_8N_2O_4$ 172.14

USP Carglumic Acid Related Compound B RS

(S)-2,7-Dioxo-1,3-diazepane-4-carboxylic acid.

 $C_6H_8N_2O_4$ 172.14

USP Carglumic Acid Related Compound C RS

(S)-2-[(S)-4-Carboxy-2-ureidobutanamido]pentanedioic acid.

 $C_{11}H_{17}N_3O_8$ 319.27

USP L-Glutamic Acid RS

USP Pyroglutamic Acid RS

(S)-5-Oxopyrrolidine-2-carboxylic acid.

 $C_5H_7NO_3$ 129.11

▲USP40

BRIEFING

Cisplatin, USP 38 page 2832. On the basis of comments received, it is proposed to make the following changes:

1. *Identification* test B is revised to include *Infrared Absorption* <197A>.
2. *Identification* test C is deleted because the remaining requirements adequately identify this drug substance.
3. The requirement for *Relative standard deviation* in the *Assay* is changed from NMT 2.0% to NMT 0.73% to be consistent with what is stated in *Chromatography* <621>, *System Suitability*.
4. The time allowed for the use of the *Sample solution* is added in the *Assay* for clarification.
5. The pH adjustment for the *Mobile phase* is added in the test for *Limit of Trichloroamineplatin* for clarification.
6. The use of low-actinic glassware for preparation is replaced with protection from light to give flexibility in the test for *Limit of Trichloroamineplatin*.
7. The relative retention times are revised in the test for *Limit of Trichloroamineplatin* to be consistent with the current USP style.
8. The system suitability requirements for *Column efficiency* is deleted from the test for *Limit of Transplatin* because the remaining criteria are adequate to evaluate the system suitability.
9. The word "derivatized" is added in the test for *Limit of Transplatin* for clarification.
10. The peaks to determine the resolution are added for clarification in the test for *Limit of Transplatin*.
11. The derivatized transplatin peak is added to determine the relative standard deviation for clarification in the test for *Limit of Transplatin*.

12. The test for *Platinum Content* is revised to avoid the use of the toxic reagent hydrazine hydrate.
13. The storage temperature is added to the *Packaging and Storage* section.

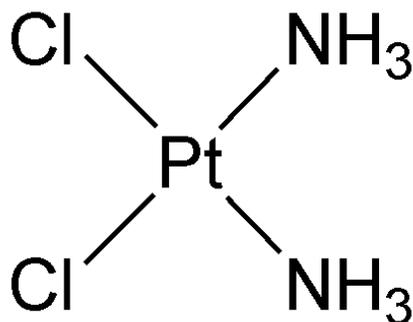
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM3: F. Mao.)

Correspondence Number—C162684; C164315

Comment deadline: January 31, 2016

Cisplatin



$\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$ 300.05

Platinum, diamminedichloro-, (*SP*-4-2)-;

cis-Diamminedichloroplatinum [15663-27-1].

DEFINITION

Cisplatin contains NLT 98.0% and NMT 102.0% of cisplatin ($\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$), calculated on the anhydrous basis.

[**Caution**—Cisplatin is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B. Infrared Absorption** ~~(197K)~~

▲ **Infrared Absorption** (197)

[Note—Methods described in (197K) or (197A) may be used.] ▲*USP40*

Delete the following:

- ▲ • **C. Thin-Layer Chromatography**

Standard solution: 1 mg/mL of USP Cisplatin RS in *dimethylformamide*

Sample solution: 1 mg/mL of Cisplatin in *dimethylformamide*

Solution A: ~~5.6 g of stannous chloride in 10 mL of hydrochloric acid. Stir for 5 min.~~

~~[Note—It is not necessary that all of the solids dissolve.]~~

Solution B: ~~0.2 g of potassium iodide in 90 mL of water~~

Chromatographic system

~~(See Chromatography (621), Thin-Layer Chromatography.)~~

Mode: ~~TLC~~

Adsorbent: ~~0.25-mm layer of chromatographic silica gel mixture~~

Application volume: ~~5 μ L~~

Developing solvent system: ~~Acetone and 1 N nitric acid (9:1)~~

Spray reagent A: ~~Mix Solution A and Solution B together. Disregard any precipitate that is formed. Store in the dark.~~

~~[Note—The solution is usable for at least 1 week.]~~

Spray reagent B: ~~20 mg/mL of potassium iodide in water~~

Analysis

Samples: ~~Standard solution and Sample solution~~

~~Develop with Developing solvent system, in a suitable chromatographic chamber containing a filter paper lining and equilibrated for 30 min with the Developing solvent system, for a distance of about 8 cm from the origin, followed by air drying. Complete the drying by heating in a forced air oven at about 100° for 1 min. Spray with Spray reagent A, heat in an oven at about 100° for 5 min, cool, and spray with Spray reagent B, to bring out the full color of the spots.~~

Acceptance criteria: ~~The principal spot from the Sample solution corresponds in appearance and R_f value to that produced by the Standard solution. ▲USP40~~

ASSAY

Change to read:

● Procedure

Mobile phase: *Ethyl acetate, methanol, dimethylformamide, and degassed water* (25:16:5:5)

Standard solution: 1 mg/mL of USP Cisplatin RS in *dimethylformamide*. Use within 1 h.

Sample solution: 1 mg/mL of Cisplatin in *dimethylformamide*.

▲ Use within 1 h. ▲USP40

Chromatographic system

(See *Chromatography (621), System Suitability.*)

Mode: LC

Detector: UV 310 nm

Column: 4.0-mm \times 30-cm; packing L8

Flow rate: 2.0 mL/min

Injection volume: 40 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

▲ 0.73% ▲ USP40

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cisplatin ($\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$) in the portion of Cisplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Cisplatin RS in the *Standard solution* (mg/mL)

C_U = concentration of Cisplatin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Change to read:

• Limit of Trichloroamineplatinatate

Mobile phase: 0.4 g/L of ammonium sulfate in water. ~~The pH of this solution is 5.9 ± 0.1.~~
[~~Note—Make adjustments to the ionic strength of the Mobile phase, if necessary, to meet the system suitability requirements.~~]

▲ Adjust with 6 N ammonium hydroxide to a pH of 5.9. ▲ USP40

Standard solution: 6 µg/mL of USP Potassium Trichloroamineplatinatate RS in saline TS.

~~Use low-actinic glassware for preparation~~

▲ Protect solution from light. ▲ USP40

Use the solution within 4 h.

Sample solution: 0.5 mg/mL of Cisplatin in saline TS. Completely dissolve by stirring by mechanical means for 30 min. ~~Use low-actinic glassware for preparation~~

▲ Protect solution from light. ▲ USP40

Use the solution within 4 h.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 209 nm

Column: 4.6-mm × 25-cm; packing L14

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[~~Note—The relative retention times for cisplatin (in the void volume) and~~

~~trichloroamineplatinatate are about 1.0 and 5.0, respectively.~~

▲ The relative retention times for saline and trichloroamineplatinatate are about 0.4 and 1.0, respectively. ▲ USP40

]

Suitability requirements

Resolution: NLT 2.0 between the saline and trichloroamineplatinatate peaks

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of trichloroamineplatinatate in the portion of Cisplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of trichloroamineplatinatate from the *Sample solution*

r_S = peak area of trichloroamineplatinatate from the *Standard solution*

C_S = concentration of USP Potassium Trichloroamineplatinatate RS in the *Standard solution* (mg/mL)

C_U = concentration of Cisplatin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of trichloroamineplatinatate, 318.48

M_{r2} = molecular weight of potassium trichloroamineplatinatate, 357.58

Acceptance criteria: NMT 1.0%

Change to read:

• Limit of Transplatin

Mobile phase: 0.18 M *monobasic potassium phosphate* in *water*. Adjust with *phosphoric acid* to a pH of 3.2.

Standard stock solution A: 0.05 mg/mL of USP Transplatin RS in *saline TS*. Dissolve by stirring by mechanical means for 30 min.

Standard stock solution B: Transfer 5 mL of *Standard stock solution A* to a 25-mL volumetric flask containing 12 mg of USP Cisplatin RS. Dilute with *saline TS* to volume, and stir by mechanical means for 30 min to dissolve.

System suitability stock solution: 0.05 mg/mL of USP Cisplatin RS in *saline TS*. Dissolve by stirring by mechanical means for 30 min.

System suitability solution: Transfer 10 mL each of *System suitability stock solution* and *Standard stock solution A* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of *thiourea* (prepared fresh daily) and 5.0 mL of *1 N hydrochloric acid*, and dilute with *saline TS* to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytef-lined closure, and heat in a heating block at $60 \pm 0.5^\circ$ for 60 min. Remove, and cool to room temperature.

Standard solution: Transfer 10 mL of *Standard stock solution B* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of *thiourea* (prepared fresh daily) and 5.0 mL of *1 N hydrochloric acid*, and dilute with *saline TS* to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytef-lined closure, and heat in a heating block at $60 \pm$

0.5° for 60 min. Remove, and cool to room temperature.

Sample stock solution: 0.5 mg/mL of Cisplatin in *saline TS*. Dissolve by stirring by mechanical means for 30 min.

Sample solution: Transfer 10 mL of *Sample stock solution* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of *thiourea* (prepared fresh daily) and 5.0 mL of 1 N *hydrochloric acid*, and dilute with *saline TS* to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytef-lined closure, and heat in a heating block at 60 ± 0.5° for 60 min. Remove, and cool to room temperature.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L9

Column temperature: 45°

Flow rate: 2.0 mL/min

Injection volume: 20 µL

[Note—Condition the *Column* by pumping *Mobile phase* at a flow rate of 2.0 mL/min for 30 min, then at 0.5 mL/min for 30 min, and then again at 2.0 mL/min for 30 min.]

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The retention time for derivatized transplatin is between 5.0 and 9.0 min; or, if it is not, modify the *Mobile phase* as necessary, and recondition the *Column*. The relative retention times for

▲ derivatized▲*USP40*

cisplatin and

▲ derivatized▲*USP40*

transplatin are about 1.0 and 1.3, respectively.]

Suitability requirements

Column efficiency: ~~NLT 2500 theoretical plates, *Standard solution*~~

▲▲*USP40*

Resolution: NLT 1.7

▲ between the derivatized cisplatin and derivatized transplatin peaks,▲*USP40*
System suitability solution

Relative standard deviation: NMT 4.0%

▲ for the derivatized transplatin peak,▲*USP40*

Standard solution

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of transplatin in the portion of Cisplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of
 ▲ derivatized ▲^{USP40}
 transplatin from the *Sample solution*

r_S = peak area of
 ▲ derivatized ▲^{USP40}
 transplatin from the *Standard solution*

C_S = concentration of USP Transplatin RS in the *Standard solution* (mg/mL)

C_U = concentration of Cisplatin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.0%

● **UV Purity Ratio**

Cleanse all glassware with a mixture of *hydrochloric acid* and *nitric acid* (3:1), rinse thoroughly with *water*, and dry before use. Do not use dichromate for cleaning. Do not use *acetone* or pressurized air for drying. Protect the *Sample solution* from light, and use within 1 h of preparation.

Sample: 98.5 ± 0.5 mg of ground Cisplatin

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: Maxima near 301 nm and minima near 246 nm

Cell: 2 cm

Blank: 0.1 N *hydrochloric acid*

Analysis

Transfer the *Sample* to a 100-mL volumetric flask, and add 0.1 N *hydrochloric acid* to volume. Using a clean magnetic stir bar, alternately stir at a high speed for 5 min and sonicate for 10 s until complete solution is effected, inverting the flask frequently to remove particles that may cling to the neck. Obtain the absorption spectrum. Calculate the absorbance ratio as follows:

$$\text{Result} = A_{301}/A_{246}$$

A_{301} = absorbance at near 301 nm

A_{246} = absorbance at near 246 nm

Acceptance criteria: NLT 4.5

SPECIFIC TESTS

Change to read:

● **Platinum Content**

~~[Note—Thoroughly cleanse all glassware with nitric acid, and rinse with Purified Water, to prevent “mirroring” of the platinum precipitate.]~~

~~**Analysis:** Transfer 0.5 g of Cisplatin to a 600-mL beaker. Add 300 mL of 0.1 N hydrochloric acid, and slowly dissolve by heating nearly to boiling on a hot plate covered with an insulating pad, and stirring frequently with a glass stirring rod. When solution is complete, remove the insulating pad, and boil for about 10 min. Remove the beaker from~~

the hot plate, allow to cool for 1 min without stirring, and pass through quantitative, fine porosity, smooth, dense, ashless filter paper, collecting the filtrate in a 600-mL beaker, completing the transfer to the filter with hot water. Wash the filter with hot water. Place the beaker containing the combined filtrate and washings on a hot plate, and evaporate to a volume of 300 mL. Place a glass stirring rod in the beaker, and heat the solution to boiling. Slowly add to the center of the beaker, by dropwise additions, 10.0 mL of hydrazine hydrate, 85%. [**Caution**—Hydrazine is toxic.] Add 2 drops of 10-N sodium hydroxide, boil for 10 min to coagulate the precipitate for ease of filtration, cool, and pass through quantitative, medium porosity, smooth, ashless filter paper. Rinse the beaker with hot water, and pour the rinsings onto the filter. Wipe the beaker and the stirring rod with small pieces of the same kind of paper used for this filtration, and place these and the filter containing the precipitate in a No. 1 porcelain crucible, previously ignited to constant weight. Dry on a hot plate covered with an insulating pad, slowly increase the heat to char, and ignite for 1 h at 800°. Cool in a desiccator, and again weigh.

Acceptance criteria: The weight of the platinum so obtained is between 64.42% and 65.22% of the weight of Cisplatin taken, on the anhydrous basis.

▲ **Sample:** 0.5 g of Cisplatin

Analysis: Ignite the *Sample* to constant weight at $800 \pm 50^\circ$, and weigh the residue. The residue is platinum.

Calculate the platinum content in the portion of Cisplatin taken:

$$\text{Result} = (W_U/W_S) \times 100$$

W_U = weight of platinum

W_S = weight of the *Sample*

Acceptance criteria: 64.42%–65.22% on the anhydrous basis ▲*USP40*

- **Crystallinity** (695): Meets the requirements
- **Water Determination** (921), *Method I*: NMT 1.0%

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers. Protect from light.
 - ▲ Store at room temperature. ▲*USP40*
- **USP Reference Standards** (11)
 - USP Cisplatin RS
 - USP Potassium Trichloroammineplatinate RS
 - Cl₃H₃KNPt 357.58
 - USP Transplatin RS

BRIEFING

Cisplatin for Injection, *USP 38* page 2833. On the basis of comments received, it is proposed to make the following changes:

1. *Identification* test *B* using the retention time agreement with the *Assay* is added.
2. The time allowed for the use of the *Sample solution* is added in the *Assay* and the test for *Limit of Trichloroamineplatin* for clarification.
3. The pH adjustment for the *Mobile phase* is added in the test for *Limit of Trichloroamineplatin* for clarification.
4. The use of low-actinic glassware for preparation is replaced with protection from light to give flexibility in the test for *Limit of Trichloroamineplatin*.
5. The relative retention times are revised in the test for *Limit of Trichloroamineplatin* to be consistent with the current *USP* style.
6. The word "derivatized" is added in the test for *Limit of Transplatin* for clarification.
7. The system suitability requirement for *Column efficiency* is deleted from the test for *Limit of Transplatin* because the remaining criteria are adequate to evaluate the system suitability.
8. The peaks to determine the resolution are added for clarification in the test for *Limit of Transplatin*.
9. The transplatin peak is added to determine the relative standard deviation for clarification in the test for *Limit of Transplatin*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM3: F. Mao.)

Correspondence Number—C163050

Comment deadline: January 31, 2016

Cisplatin for Injection

DEFINITION

Cisplatin for Injection is a sterile, lyophilized mixture of Cisplatin, Mannitol, and Sodium Chloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cisplatin ($\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$).

[**Caution**—Cisplatin is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.]

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Solution A: 5.6 g of *stannous chloride* in 10 mL of *hydrochloric acid*. Stir for 5 min. [Note —It is not necessary that all of the solids dissolve.]

Solution B: 0.2 g of *potassium iodide* in 90 mL of *water*

Standard solution: 1.0 mg/mL of USP Cisplatin RS, 9 mg/mL of *sodium chloride*, and 10 mg/mL of d-mannitol in *water*

Sample solution: Nominally equivalent to 1.0 mg/mL of cisplatin by dissolving the contents of 1 container of Cisplatin for Injection in *water*, based on the label claim

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

Developing solvent system: *Acetone* and 1 N *nitric acid* (9:1)

Spray reagent A: Mix *Solution A* and *Solution B* together. Disregard any precipitate that is formed. Store in the dark. [Note—The solution is usable for at least 1 week.]

Spray reagent B: 20 mg/mL of *potassium iodide* in water

Analysis

Samples: *Standard solution* and *Sample solution*

Develop with *Developing solvent system*, in a suitable chromatographic chamber containing a filter paper lining and equilibrated for 30 min with the *Developing solvent system*, for a distance of about 8 cm from the origin, followed by air drying. Complete the drying by heating in a forced-air oven at about 100° for 1 min. Spray with *Spray reagent A*, heat in an oven at about 100° for 5 min, cool, and spray with *Spray reagent B* to bring out the full color of the spots.

Acceptance criteria: The principal spot from the *Sample solution* corresponds in appearance and R_F value to that produced by the *Standard solution*.

Add the following:

- ▲ ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP40

ASSAY

Change to read:

- **Procedure**

Mobile phase: *Ethyl acetate*, *methanol*, *dimethylformamide*, and degassed water (25:16:5:5)

Standard solution: 1 mg/mL of USP Cisplatin RS in *dimethylformamide*. Use within 1 h.

Sample solution: Nominally equivalent to 1.0 mg/mL of cisplatin in *dimethylformamide* prepared as follows. Quantitatively dissolve 1 container of Cisplatin for Injection with *dimethylformamide* by sonicating for 5 min. Pass 5 mL of this solution through a suitable filter and collect the filtrate after discarding the first mL of the filtrate.

▲ Use within 1 h. ▲USP40

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 310 nm

Column: 4.0-mm \times 30-cm; packing L8

Flow rate: 2.0 mL/min

Injection volume: 40 μ L

System suitability**Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cisplatin ($\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$) in the portion of Cisplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of the USP Cisplatin RS in the *Standard solution* (mg/mL) C_U = nominal concentration of cisplatin in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**

- **Uniformity of Dosage Units** (905): Meets the requirements

IMPURITIES**Change to read:**

- **Limit of Trichloroammineplatinate**

Mobile phase: 0.4 g/L of *ammonium sulfate* in water. The pH of this solution is 5.9 ± 0.1 .

[~~Note—Make adjustments to the ionic strength of the *Mobile phase*, if necessary, to meet the system suitability requirements.~~]

▲ Adjust with 6 N *ammonium hydroxide* to a pH of 5.9. ▲*USP40*

Standard solution: 6 µg/mL of USP Potassium Trichloroammineplatinate RS in *saline TS*.

~~Use low-actinic glassware for preparation~~

▲ Protect solution from light. ▲*USP40*

Use the solution within 4 h.

Sample solution: Nominally equivalent to 0.5 mg/mL of cisplatin by quantitatively dissolving the contents of 1 container of Cisplatin for Injection with *water*. ~~Use low-actinic glassware for preparation~~

▲ Protect solution from light. Use the solution within 4 h. ▲*USP40*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC**Detector:** UV 209 nm**Column:** 4.6-mm × 25-cm; packing *L14***Flow rate:** 2 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[Note—The relative retention times for cisplatin (in the void volume) and trichloroamineplatin are about 1.0 and 5.0, respectively.

▲ The relative retention times for saline and trichloroamineplatin are about 0.4 and 1.0, respectively. ▲*USP40*

]

Suitability requirements

Resolution: NLT 2.0 between the saline and trichloroamineplatin peaks

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of trichloroamineplatin in the portion of Cisplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of trichloroamineplatin from the *Sample solution*

r_S = peak area of trichloroamineplatin from the *Standard solution*

C_S = concentration of USP Potassium Trichloroamineplatin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cisplatin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of trichloroamineplatin, 318.48

M_{r2} = molecular weight of potassium trichloroamineplatin, 357.58

Acceptance criteria: NMT 1.0%

Change to read:

• Limit of Transplatin

Mobile phase: 0.18 M *monobasic potassium phosphate* in water. Adjust with *phosphoric acid* to a pH of 3.2.

Standard stock solution A: 0.05 mg/mL of USP Transplatin RS in *saline TS*. Dissolve by stirring by mechanical means for 30 min.

Standard stock solution B: Transfer 5 mL of *Standard stock solution A* to a 25-mL volumetric flask containing 12 mg of USP Cisplatin RS. Dilute with *saline TS* to volume, and stir by mechanical means for 30 min to dissolve.

System suitability stock solution: 0.05 mg/mL of USP Cisplatin RS in *saline TS*. Dissolve by stirring by mechanical means for 30 min.

System suitability solution: Transfer 10 mL each of *System suitability stock solution* and *Standard stock solution A* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of *thiourea* (prepared fresh daily) and 5.0 mL of 1 N *hydrochloric acid*, and dilute with *saline TS* to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytef-lined closure, and heat in a heating block at $60 \pm 0.5^\circ$ for 60 min. Remove, and

cool to room temperature.

Standard solution: Transfer 10 mL of *Standard stock solution B* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of *thiourea* (prepared fresh daily) and 5.0 mL of *1 N hydrochloric acid*, and dilute with *saline TS* to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytef-lined closure, and heat in a heating block at $60 \pm 0.5^\circ$ for 60 min. Remove, and cool to room temperature.

Sample stock solution: Nominally equivalent to 0.5 mg/mL of cisplatin by dissolving the contents of 1 container of Cisplatin for Injection with *water*.

Sample solution: Transfer 10 mL of *Sample stock solution* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of *thiourea* (prepared fresh daily) and 5.0 mL of *1 N hydrochloric acid*, and dilute with *saline TS* to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytef-lined closure, and heat in a heating block at $60 \pm 0.5^\circ$ for 60 min. Remove, and cool to room temperature.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; packing *L9*

Column temperature: 45°

Flow rate: 2.0 mL/min

Injection volume: 20 μ L

[Note—Condition the *Column* by pumping the *Mobile phase* at a flow rate of 2.0 mL/min for 30 min, then at 0.5 mL/min for 30 min, and then again at 2.0 mL/min for 30 min.]

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The retention time for derivatized transplatin is between 5.0 and 9.0 min; or, if it is not, modify the *Mobile phase* as necessary, and recondition the *Column*. The relative retention times for

▲ derivatized ▲*USP40*

cisplatin and

▲ derivatized ▲*USP40*

transplatin are about 1.0 and 1.3, respectively.]

Suitability requirements

~~**Column efficiency:** NLT 2500 theoretical plates, *Standard solution*~~

▲ ▲*USP40*

Resolution: NLT 1.7

▲ between the derivatized cisplatin and derivatized transplatin peaks, ▲*USP40*
System suitability solution

Relative standard deviation: NMT 4.0%

▲ for the derivatized transplatin peak, ▲*USP40*
Standard solution

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of transplatin in the portion of Cisplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of
 ▲ derivatized ▲ USP40
 transplatin from the *Sample solution*

r_S = peak area of
 ▲ derivatized ▲ USP40
 transplatin from the *Standard solution*

C_S = concentration of USP Transplatin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cisplatin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.0%

SPECIFIC TESTS

- **pH** <791>:

Sample: Constituted as directed in the labeling, using Sterile Water for Injection

Acceptance criteria: 3.5–6.2

- **Bacterial Endotoxins Test** <85>: It contains NMT 2.0 USP Endotoxin Units/mg of cisplatin.
- **Sterility Tests** <71>, *Test for Sterility of the Product to be Examined, Membrane Filtration*: Meets the requirements
- **Water Determination** <921>, *Method I*

Sample: One container of Cisplatin for Injection

Analysis: Use *anhydrous formamide* as the extraction solvent, and use the following procedure. Introduce 50 mL of *anhydrous formamide* into the titration vessel, and titrate with the *Reagent* to the electrometric endpoint. Use the formamide thus dried to rinse a suitable glass syringe equipped with a 22-gauge needle, about 8 cm long. Add the rinse back to the titration vessel, and again titrate the vessel contents, if necessary. Via the syringe, withdraw 5 mL of the formamide thus titrated, and, through the closure of the container, expel the contents into the container. Shake the container to obtain a solution. With the same syringe, withdraw all of the contents of the container, and transfer to the titration vessel. Titrate to the endpoint, adjusting the feeding speed control to the lowest setting, to avoid over-titration.

Acceptance criteria: NMT 2.0%

- **Constituted Solutions:** At the time of use, it meets the requirements for *Injections* <1>, *Constituted Solutions*.
- **Other Requirements:** It meets the requirements for *Injections* <1>, *Labels and Labeling*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in *Containers for Sterile Solids* as described in *Injections* <1>. Protect from light.

- **USP Reference Standards** <11>

USP Cisplatin RS

USP Endotoxin RS

USP Potassium Trichloroammineplatinate RS

Cl₃H₃KNPt 357.58

USP Transplatin RS

BRIEFING

Desogestrel. Because there is no existing *USP* monograph for this drug substance, a new monograph based on the *European Pharmacopoeia* is being proposed. The liquid chromatographic procedures in the *Assay* and in the *Organic Impurities* tests are based on analyses performed with the Zorbax SB-C18 brand of *L1* column. The typical retention times for desogestrel in the *Assay* and the *Organic Impurities* tests are about 26 min. The Phenomenex Luna C18(2) brand of *L1* column may also be suitable.

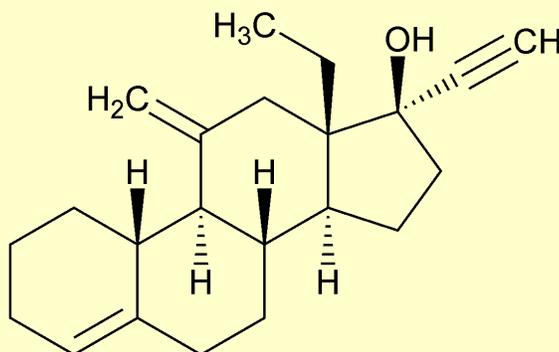
(CHM5: R.-H. Yeh, D. Vicchio.)

Correspondence Number—C157584

Comment deadline: January 31, 2016

Add the following:

▲ Desogestrel



C₂₂H₃₀O 310.47

18,19-Dinorpregn-4-en-20-yn-17-ol, 13-ethyl-11-methylene-, (17α)-;
13-Ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol [54024-22-5].

DEFINITION

Desogestrel contains NLT 98.0% and NMT 102.0% of desogestrel (C₂₂H₃₀O), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** <197>: [Note—Methods described in <197K> or <197A> may be used.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• Procedure**

Mobile phase: *Acetonitrile* and water (73:27)

System suitability solution: 400 µg/mL of USP Desogestrel RS and 0.4 µg/mL each of USP Desogestrel Related Compound A RS and USP Desogestrel Related Compound D RS prepared as follows. Dissolve the material in *acetonitrile* equivalent to 50% of the volume of a suitable volumetric flask and dilute with water to volume.

Standard solution: 400 µg/mL of USP Desogestrel RS prepared as follows. Dissolve the material in *acetonitrile* equivalent to 50% of the volume of a suitable volumetric flask and dilute with water to volume.

Sample solution: 400 µg/mL of Desogestrel prepared as directed in the *Standard solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 25-cm; 5-µm packing *L1*

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 15 µL

Run time: 1.5 times the retention time of desogestrel

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 1.3 between desogestrel and desogestrel related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Peak-to-valley ratio: NLT 2.0 between desogestrel and desogestrel related compound D, *System suitability solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of desogestrel (C₂₂H₃₀O) in the portion of Desogestrel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desogestrel from the *Sample solution*

r_S = peak response of desogestrel from the *Standard solution*

C_S = concentration of USP Desogestrel RS in the *Standard solution* (µg/mL)

C_U = concentration of Desogestrel in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%
- **Organic Impurities**

Mobile phase, System suitability solution, Chromatographic system, and Sample solution: Proceed as directed in the *Assay*.

Diluent: *Acetonitrile* and water (50:50)

Standard stock solution: 0.4 mg/mL each of USP Desogestrel RS, USP Desogestrel Related Compound B RS, and USP Desogestrel Related Compound C RS, and 0.8 mg/mL each of USP Desogestrel Related Compound A RS and USP Desogestrel Related Compound D RS prepared as follows. Dissolve the materials in *acetonitrile* equivalent to 50% of the volume of a suitable volumetric flask and dilute with water to volume.

Standard solution: 0.4 µg/mL each of USP Desogestrel RS, USP Desogestrel Related Compound B RS, and USP Desogestrel Related Compound C RS, and 0.8 µg/mL each of USP Desogestrel Related Compound A RS and USP Desogestrel Related Compound D RS in *Diluent*, from *Standard stock solution*

System suitability

Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 1.3 between desogestrel and desogestrel related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0% for each corresponding peak present in the *Standard solution*

Peak-to-valley ratio: NLT 2.0 between desogestrel and desogestrel related compound D, *System suitability solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of desogestrel related compound A, desogestrel related compound B, desogestrel related compound C, or desogestrel related compound D in the portion of Desogestrel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desogestrel related compound A, desogestrel related compound B, desogestrel related compound C, or desogestrel related compound D from the *Sample solution*

r_S = peak response of the corresponding Reference Standard from the *Standard solution*

C_S = concentration of USP Desogestrel Related Compound A RS, USP Desogestrel Related Compound B RS, USP Desogestrel Related Compound C RS, or USP Desogestrel Related Compound D RS in the *Standard solution* (µg/mL)

C_U = concentration of Desogestrel in the *Sample solution* (µg/mL)

Calculate the percentage of 11-methylene lynestrenol and any other individual unspecified impurity in the portion of Desogestrel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 11-methylene lynestrenol or any other individual unspecified impurity from the *Sample solution*

r_S = peak response of desogestrel from the *Standard solution*

C_S = concentration of USP Desogestrel RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of Desogestrel in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desogestrel related compound B	0.16	0.10
Desogestrel related compound C	0.19	0.1
11-Methylene lynestrenol ^a	0.71	0.2
Desogestrel related compound A	0.96	0.2
Desogestrel	1.0	—
Desogestrel related compound D	1.06	0.2
Any other individual unspecified impurity	—	0.10
Total impurities	—	0.5
^a 11-Methylene-19-nor-17 α -pregn-4-en-20-yn-17-ol.		

SPECIFIC TESTS

- **Optical Rotation** (781S), *Procedure, Specific Rotation*

Sample solution: 10 mg/mL of Desogestrel in *absolute alcohol*

Acceptance criteria: +53° to +57° (dried substance)

- **Loss on Drying** (731)

Analysis: Dry under vacuum at a pressure not exceeding 15 mm of mercury at room temperature to constant weight.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP Reference Standards** (11)

USP Desogestrel RS

USP Desogestrel Related Compound A RS

13-Ethyl-11-methylene-18,19-dinor-5 α ,17 α -pregn-3-en-20-yn-17-ol;

Desogestrel Δ 3-isomer.

$C_{22}H_{30}O$ 310.47

USP Desogestrel Related Compound B RS

13-Ethyl-3-hydroxy-11-methylene-18,19-dinor-17 α -pregn-4-en-20-yn-17-ol.

$C_{22}H_{30}O_2$ 326.48

USP Desogestrel Related Compound C RS

13-Ethyl-11-methylene-18,19-dinor-17 α -pregn-4-en-20-yn-17-ol-3-one.

$C_{22}H_{28}O_2$ 324.46

USP Desogestrel Related Compound D RS

13-Ethyl-11-methylenegon-4-en-17-one.

$C_{20}H_{28}O$ 284.44

▲USP40

BRIEFING

Fenbendazole, USP 38 page 3453. The following revisions are proposed:

1. *Identification* test A is revised to provide multiple analysis options to allow flexibility to the user.
2. Comments were received indicating that the *Organic Impurities* calculation uses an incorrect factor that could potentially lead to under-reporting of impurities. The proposed calculation format eliminates the use of the factor. In addition, *Table 2* is added to list the relative retention times and acceptance criteria.

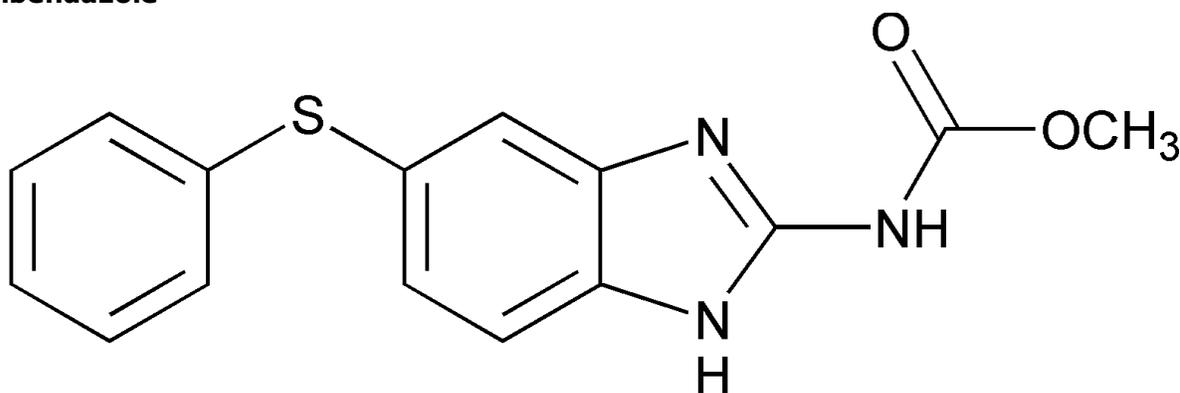
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(CHM3: M. Puderbaugh.)

Correspondence Number—C165049

Comment deadline: January 31, 2016

Fenbendazole



$C_{15}H_{13}N_3O_2S$ 299.35

Carbamic acid, [5-(phenylthio)-1*H*-benzimidazol-2-yl]-, methyl ester;
Methyl 5-(phenylthio)-2-benzimidazolecarbamate [43210-67-9].

DEFINITION

Fenbendazole contains NLT 98.0% and NMT 101.0% of fenbendazole ($C_{15}H_{13}N_3O_2S$), calculated on the dried basis.

IDENTIFICATION

Delete the following:

- ~~▲ ● A. Infrared Absorption (197K) ▲^{USP40}~~

Add the following:

- ▲ ● A. Infrared Absorption (197): [Note—Methods described in (197K), (197M), or (197A) may be used.]▲^{USP40}

ASSAY

● Procedure

Sample: 200 mg

Analysis: Dissolve the *Sample* in 30 mL of *glacial acetic acid*, warming if necessary to effect solution. Allow to cool, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 29.94 mg of fenbendazole ($C_{15}H_{13}N_3O_2S$).

Acceptance criteria: NLT 98.0%–101.0% on the dried basis

IMPURITIES

- Residue on Ignition (281): NMT 0.3%

Delete the following:

- ~~● ● Heavy Metals, Method II (231): NMT 10 ppm ● (Official 1-Jan-2018)~~

Change to read:

● Organic Impurities

Solution A: *Methanol*, *acetic acid*, and water (30:1:70)

Solution B: *Methanol*, *acetic acid*, and water (70:1:30)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	0	100
40	0	100
50	100	0

Diluent: *Methanol* and *hydrochloric acid* (99:1)

System suitability stock solution: 0.1 mg/mL each of USP Fenbendazole RS and USP Mebendazole RS in *methanol*

System suitability solution: 0.01 mg/mL each of USP Fenbendazole RS and USP Mebendazole RS from the *System suitability stock solution* in *Diluent*

Standard stock solution A: 0.025 mg/mL of USP Fenbendazole RS prepared as follows. Dissolve a weighed quantity of USP Fenbendazole RS in *Diluent* to obtain a solution with a known concentration of 5 mg/mL. Dilute 1.0 mL of this solution with *methanol* to 200.0 mL.

Standard solution A: 0.0125 mg/mL of USP Fenbendazole RS from *Standard stock solution A* in *Diluent*

Standard stock solution B: 0.1 mg/mL of USP Fenbendazole Related Compound A RS in *methanol*

Standard solution B: 0.01 mg/mL of USP Fenbendazole Related Compound A RS from *Standard stock solution B* in *Diluent*

Standard stock solution C: 0.1 mg/mL of USP Fenbendazole Related Compound B RS in *methanol*

Standard solution C: 0.01 mg/mL of USP Fenbendazole Related Compound B RS from *Standard stock solution C* in *Diluent*

Sample solution: Dissolve 5.0 mg/mL of Fenbendazole in *Diluent*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: *System suitability solution*, ~~*Standard solution B*~~, and ~~*Standard solution C*~~

[~~Note—The retention time for fenbendazole is about 19 min; and the relative retention times are about 0.85 for mebendazole and 1.0 for fenbendazole (*System suitability solution*); the relative retention time is about 0.25 for fenbendazole related compound A (*Standard solution B*); the relative retention time is about 0.65 for fenbendazole related compound B (*Standard solution C*).]~~

▲ [Note—The relative retention times for mebendazole and fenbendazole are about 0.85 and 1.0, respectively.] ▲ *USP40*

Suitability requirements

Resolution: NLT 1.5 between mebendazole and fenbendazole, ~~*System suitability solution*~~

▲ *USP40*

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Calculate the percentages of fenbendazole related compound A and fenbendazole related compound B in the portion of Fenbendazole taken:

$$\text{Result} = 0.1 \times (C/W) \times (r_i/r_{St})$$

- C = concentration of USP Fenbendazole Related Compound A RS or USP Fenbendazole Related Compound B RS in *Standard solution B* or *Standard solution C* (mg/mL)
 W = weight of Fenbendazole taken to prepare the *Sample solution* (mg/mL)
 r_i = peak area of the relevant related compound from the *Sample solution*
 r_{Si} = peak area of the relevant related compound from the *Standard solution B* or *Standard solution C*, as appropriate

[~~Note—Disregard any impurity peak with a response that is less than one-fifth that of r_{Si} .~~]

Acceptance criteria 1: ~~NMT 0.5% of related compound A or related compound B~~

Calculate the percentage of any other impurity in the portion of Fenbendazole taken:

$$\text{Result} = 0.1 \times (C/W) \times (r_i/r_{Si})$$

- C = concentration of USP Fenbendazole RS in *Standard solution A* (mg/mL)
 W = weight of Fenbendazole taken to prepare the *Sample solution* (mg/mL)
 r_i = peak area of any other impurity from the *Sample solution*
 r_{Si} = peak area of the main peak from *Standard solution A*

[~~Note—Disregard any impurity peak with a response that is less than one-fifth that of r_{Si} .~~]

Acceptance criteria 2-

Any other impurity: ~~NMT 0.5%~~

Total impurities: ~~The sum of all impurities found, including fenbendazole related compound A and fenbendazole related compound B, is NMT 1%.~~

- ▲ Calculate the percentages of fenbendazole related compound A and fenbendazole related compound B in the portion of Fenbendazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of each related compound from the *Sample solution*
 r_S = peak response of the corresponding related compound from *Standard solution B* or *Standard solution C*
 C_S = concentration of the corresponding related compound in *Standard solution B* or *Standard solution C* (mg/mL)
 C_U = concentration of Fenbendazole in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Fenbendazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of any impurity from the *Sample solution*
 r_S = peak response of USP Fenbendazole RS from *Standard solution A*
 C_S = concentration of USP Fenbendazole RS in *Standard solution A* (mg/mL)
 C_U = concentration of Fenbendazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting level for impurities is 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fenbendazole related compound A	0.25	0.5
Fenbendazole related compound B	0.65	0.5
Fenbendazole	1.0	—
Any individual impurity	—	0.5
Total impurities	—	1

▲USP40

SPECIFIC TESTS● **Loss on Drying** (731)**Analysis:** Dry at 100°–105° for 3 h.**Acceptance criteria:** NMT 1.0%**ADDITIONAL REQUIREMENTS****Change to read:**

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers, and store at 25°, with excursions permitted between 15° and 30°

▲ room temperature. ▲USP40

- **Labeling:** Label to indicate that it is for veterinary use only.

● **USP Reference Standards** (11)USP Fenbendazole RS USP Fenbendazole Related Compound A RS Methyl (1*H*-benzimidazole-2-yl)carbamate.C₉H₉N₃O₂ 191.19USP Fenbendazole Related Compound B RS

Methyl [5(6)-chlorobenzimidazole-2-yl]carbamate.

C₉H₈ClN₃O₂ 225.63USP Mebendazole RS **BRIEFING**

Fluticasone Propionate Nasal Spray, USP 38 page 3590. As part of the USP monograph modernization initiative, it is proposed to make the following changes.

1. Replace the titration procedure for the *Content of Benzalkonium Chloride* test, which uses chloroform, with an HPLC method using the Kinetex C18 brand of L1 column manufactured by Phenomenex, in which the C₁₂, C₁₄, and C₁₆ homologs of benzalkonium elute at about 1.5, 2.0, and 2.4 min, respectively.
2. Remove the stringent constraints for pH adjustment for buffer preparation in both the *Assay* and *Organic Impurities* procedures.
3. Replace the USP Fluticasone Propionate Nasal Spray Resolution Mixture RS used in the *Assay* and *Delivered Dose Uniformity* tests with the individual components, USP Fluticasone Propionate RS and USP Fluticasone Propionate Related Compound D RS.

4. Replace the USP Fluticasone Propionate Related Compounds Mixture RS used in the *Organic Impurities* test with two of the three individual components, USP Fluticasone Propionate RS and USP Fluticasone Propionate Related Compound D RS.
5. Remove the *Foreign Particulates* test from the monograph as it is non-value added for a public standard and can remain a part of the private standard.
6. Remove the *Droplet Size Distribution* test from the monograph as it does not have a requirement. This is dependent on the spray valve, making it a manufacturer specific parameter. Thus, it is not appropriate to include in the public standard.
7. Remove the *Spray Pattern* test from the monograph as it does not have a requirement. This is dependent on the spray valve, making it a manufacturer specific parameter. Thus, it is not appropriate to include in the public standard.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM4: P. Pabba, R. Ravichandran.)
Correspondence Number—C153461

Comment deadline: January 31, 2016

Fluticasone Propionate Nasal Spray

DEFINITION

Fluticasone Propionate Nasal Spray is a white, opaque suspension of Fluticasone Propionate in water. It is supplied in a form suitable for nasal administration. It contains NLT 95.0% and NMT 115.0% of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$).

IDENTIFICATION

- **A. Infrared Absorption** (197M)

Sample: Transfer 30 g of Nasal Spray equally into two 50-mL centrifuge tubes. Add 10 mL of water to each tube, insert the stopper, and shake vigorously for 2 min. Centrifuge at 3500 rpm for 10 min, and discard the supernatant. Add 10 mL of water to each tube, insert the stopper, and shake vigorously for 2 min. Centrifuge at 3500 rpm for 10 min, and discard the supernatant. Add 10 mL of water to each tube, insert the stopper, and shake vigorously for 2 min. Centrifuge at 3500 rpm for 10 min, and discard the supernatant. To one tube add 10 mL of methanol. Shake to disperse the residue, and transfer to the other tube. Shake the other tube for 1 min. Centrifuge at 3500 rpm for 10 min. Decant the supernatant into an agate mortar. Evaporate the methanol either by carefully blowing dry with compressed air or nitrogen, or by allowing the methanol to evaporate naturally. If using an air or nitrogen line, use a suitable in-line filter to avoid contamination. Allow the residue to dry overnight in a desiccator over silica gel.

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:● **Procedure**

Diluent: *Acetonitrile* and 0.001 M hydrochloric acid (60:40)

Buffer: 1.2 g/L of *monobasic ammonium phosphate*. Adjust with *phosphoric acid* to a pH of 3.5. ~~±0.05.~~

▲▲*USP40*

Mobile phase: *Methanol*, *acetonitrile*, and *Buffer* (50:15:35)

System suitability solution: 50 µg/mL of USP Phenylethyl Alcohol RS, 10 µg/mL of ~~USP Fluticasone Propionate Nasal Spray Resolution Mixture RS~~

▲ USP Fluticasone Propionate RS, and 1 µg/mL of USP Fluticasone Propionate Related Compound D RS ▲*USP40*
in *Diluent*

Standard solution: 10 µg/mL of USP Fluticasone Propionate RS in *Diluent*

Sample solution: Nominally 10 µg/mL of fluticasone propionate prepared as follows.

Transfer an amount of the Nasal Spray containing 0.5 mg of fluticasone propionate to a 50-mL volumetric flask. Add about 40 mL of *Diluent*, and sonicate the flask for 10 min.

Dilute with *Diluent* to volume, and shake. Allow to stand for 10 min until the supernatant is a clear solution. Inject the clear supernatant into the chromatograph.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 and 239 nm

Column: 4.6-mm × 25-cm; 5-µm packing *L1*

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Record the chromatogram at 210 nm for 5 min and then change the wavelength to 239 nm.

[Note—The relative retention times for phenylethyl alcohol, fluticasone propionate, and fluticasone propionate related compound D are about 0.42, 1.0, and 1.10, respectively.]

Suitability requirements

Resolution: NLT 1.5 between fluticasone propionate and fluticasone propionate related compound D, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluticasone propionate (C₂₅H₃₁F₃O₅S) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of fluticasone propionate in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 95.0%–115.0%

OTHER COMPONENTS

• Content of Phenylethyl Alcohol

Diluent, Mobile phase, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Standard solution: 0.05 mg/mL of USP Phenylethyl Alcohol RS in *Diluent*

Sample solution: Transfer 1.0 g of the Nasal Spray to a 50-mL volumetric flask. Add about 40 mL of *Diluent*, and sonicate for 10 min until supernatant is clear. Use the clear supernatant for analysis.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in mg/g, of phenylethyl alcohol in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/W)$$

r_U = peak response of phenylethyl alcohol from the *Sample solution*

r_S = peak response of phenylethyl alcohol from the *Standard solution*

C_S = concentration of USP Phenylethyl Alcohol RS in the *Standard solution* (mg/mL)

V = volume of the *Sample solution*, 50 mL

W = weight of the Nasal Spray in the *Sample solution* (g)

Acceptance criteria

For 50 sprays: 1.75–2.63 mg/g

For 120 sprays: 1.88–2.63 mg/g

Change to read:

• Content of Benzalkonium Chloride

~~**Buffer:** 250 mg/mL of *citric acid*. Adjust the solution with 2-N *sodium hydroxide TS* to a pH of 3.5 ± 0.05 .~~

~~**Standard solution:** 200 $\mu\text{g/mL}$ [0.02% (w/w)] of USP Benzalkonium Chloride RS in water~~

~~**Docosate sodium titrant:** Dissolve 0.22 g of USP Docosate Sodium RS in 100 mL of warm water. Dilute with water to make 1000 mL.~~

~~**Eosin Y indicator:** Dissolve 25 mg of eosin Y in 50 mL of acetone. Add 450 mL of chloroform and 5.0 ± 0.5 g of citric acid. Shake thoroughly until no discoloration occurs. Filter the mixture to remove any undissolved citric acid. Store in an amber bottle.~~

Titer value of docusate sodium: Pipet 10 mL of the *Standard solution* into a 250 mL glass stoppered flask containing 40 mL of water, 5 mL of *Eosin Y indicator*, and 2 mL of *Buffer*. Insert the stopper into the flask, and shake, releasing any build-up of pressure. Titrate with *Docusate sodium titrant* with vigorous shaking to a point when pink coloration is discharged from the chloroform layer. Perform a blank determination, substituting 10 mL of water for the *Standard solution*, and make any necessary correction (see *Titrimetry* (541)).

Calculate the titer value (T) of the *Docusate sodium titrant*, in $\mu\text{g/mL}$, of benzalkonium chloride:

$$\text{Result} = C_S \times (V_S/V_D)$$

C_S = concentration of USP Benzalkonium Chloride RS in the *Standard solution* ($\mu\text{g/mL}$)

V_S = volume of the *Standard solution*, 10 mL

V_D = volume of the *Docusate sodium titrant* used in the titration of the *Standard solution* (mL)

Analysis-

Sample: 10 g of Nasal Spray

Transfer the *Sample* into a 250 mL glass stoppered flask containing 40 mL of water, 5 mL of *Eosin Y indicator*, and 2 mL of *Buffer*. Repeat the procedure as given above for the *Standard solution*. To clarify the endpoint, place the flask in an ultrasonic bath for 1–2 min to separate the chloroform layer from the aqueous phase. Perform a blank determination.

Calculate the concentration of benzalkonium chloride, in $\mu\text{g/g}$, in the portion of Nasal Spray taken:

$$\text{Result} = TV/W$$

T = titer value of the *Docusate sodium titrant*

V = volume of the *Docusate sodium titrant* used in the titration of the Nasal Spray (mL)

W = weight of the portion of Nasal Spray taken (g)

▲ Buffer: Dissolve 10.8 g of *monobasic sodium phosphate dihydrate* in 90 mL of water, and adjust with *phosphoric acid* to a pH of 2.5. Dilute with water to 100 mL.

Solution A: Mix 50 mL of *Buffer*, 750 mL of water, and 200 mL of *methanol*. Add 5 mL of *triethylamine*. Mix and adjust with *phosphoric acid* to a pH of 2.5.

Solution B: Mix 1 L of *methanol* with 50 mL of *phosphoric acid*.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	55	45
3.0	5	95
3.2	55	45
5.0	55	45

Diluent: 1% Hydrochloric acid (v/v) in methanol

System suitability solution: 0.04 mg/mL of USP Benzalkonium Chloride RS prepared as follows. Transfer the required quantity of USP Benzalkonium Chloride RS to a suitable volumetric flask, and add 30% of the flask volume of water. Dilute with *Diluent* to volume.

Standard stock solution: 200 µg/mL of USP Benzalkonium Bromide RS in water. [Note—A few drops of methanol may be used to resolve the formation of foam prior to dilution.]

Standard solution: 20 µg/mL of USP Benzalkonium Bromide RS. Transfer an aliquot of *Standard stock solution* to a suitable volumetric flask, and add water equal to 30% of the flask volume. Dilute with *Diluent* to volume.

Sample solution: Transfer 1.0 g of the Nasal Spray to a 10-mL volumetric flask. Dilute with *Diluent* to volume. Centrifuge if necessary and use the supernatant.

[Note—Centrifuging at 4000 rpm for 15 min may be suitable. Supernatant may be passed through a suitable filter with a pore size of NMT 0.2 µm.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 3.0-cm; 2.6-µm packing *L1*

Column temperature: 50°

Flow rate: 2 mL/min

Injection volume: 100 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for relative retention times. C₁₀ homolog may not be present.]

Suitability requirements

Resolution: NLT 2.5 between the pairs of C₁₂ and C₁₄ homologs and the C₁₄ and C₁₆ homologs of benzalkonium, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the sum of the corrected benzalkonium peak responses (r_{CU}) in the portion of the *Sample solution* taken:

$$\text{Result} = \sum [r_U \times (1/F)]$$

r_U = peak response of each C_{10} , C_{12} , C_{14} , and C_{16} benzalkonium homolog from the *Sample solution*

F = relative response factor of the corresponding benzalkonium homolog relative to benzalkonium bromide (see *Table 2*)

Table 2

Benzalkonium Chloride Analog	Relative Retention Time	Relative Response Factor
C_{10}	0.65	1.2
C_{12}	1.0	1.1
C_{14}	1.35	1.0
C_{16}	1.59	0.98

Calculate the percentage of benzalkonium chloride in the portion of Nasal Spray taken:

$$\text{Result} = (r_{CU}/r_S) \times C_S \times (V/W) \times 100$$

r_{CU} = sum of the corrected peak responses of the benzalkonium homologs from the *Sample solution*

r_S = peak response of benzalkonium from the *Standard solution*

C_S = concentration of USP Benzalkonium Bromide RS in the *Standard solution* ($\mu\text{g/mL}$)

V = volume of the *Sample solution* (mL)

W = weight of Nasal Spray in the *Sample solution* (g)

▲USP40

Acceptance criteria: 140–220 $\mu\text{g/g}$

PERFORMANCE TESTS

Change to read:

- **Delivered Dose Uniformity** (within container)

Diluent: *Acetonitrile* and 0.001 M hydrochloric acid (60:40)

Buffer: 1.2 g/L of *monobasic ammonium phosphate*. Adjust with *phosphoric acid* to a pH of 3.5 ± 0.05 .

Mobile phase: *Methanol*, *acetonitrile*, and *Buffer* (50:15:35)

System suitability solution: 5 $\mu\text{g/mL}$ of USP Fluticasone Propionate Nasal Spray Resolution Mixture RS

▲USP Fluticasone Propionate RS and 0.5 $\mu\text{g/mL}$ of USP Fluticasone Propionate Related Compound D RS ▲USP40 in *Diluent*

Standard solution: 4 $\mu\text{g/mL}$ of USP Fluticasone Propionate RS in *Diluent*

Sample solution: Wipe the pump clean. Shake the bottle for 30 s, and mechanically prime the bottle. Hold a 25-mL volumetric flask in an inverted position, and discharge the first two actuations (1 dose) into the flask. Turn the flask to the upright position immediately after each actuation. Insert the stopper into the flask after collecting two actuations. Discharge actuations 3–48 (50-spray pack) or 3–118 (120-spray pack) to waste. Wipe the

bottle clean, and collect the last two actuations (49 and 50 or 119 and 120) in a second 25-mL volumetric flask. Turn the flask to the upright position immediately after each actuation, and insert the stopper into the flask. Add 20 mL of *Diluent* to each flask, and shake well for 10 min to disperse the suspension. Dilute with *Diluent* to volume, and mix thoroughly. Allow the flask to stand until the excipients have settled. Inject the clear supernatant. Repeat this procedure with 4 additional bottles.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 25-cm; 5-μm packing *L1*

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for fluticasone propionate and fluticasone propionate related compound D are about 1.0 and 1.10, respectively.]

Suitability requirements

Resolution: NLT 1.5 between fluticasone propionate and fluticasone propionate related compound D, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluticasone propionate (C₂₅H₃₁F₃O₅S) in each dose taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (μg/mL)

C_U = nominal concentration of fluticasone propionate in the *Sample solution* (μg/mL)

Acceptance criteria ~~The mean dose delivered from 10 doses is within 85%–115% of the label claim. NMT 1 dose is outside 80%–120% of the label claim. No doses are outside 75%–125% of the label claim. If 2 or 3 doses are outside 80%–120% of the label claim, test an additional 10 bottles. The mean dose delivered from 30 doses is within 85%–115% of the label claim. NMT 3 doses are outside 80%–120% of the label claim. No doses are outside 75%–125% of the label claim.~~

▲ Tier 1

1. Mean dose delivered from 10 doses is within 85%–115% of the labeled amount of

- fluticasone propionate.
2. NMT 1 dose is outside 80%–120% of the labeled amount of fluticasone propionate.
 3. No doses are outside 75%–125% of the labeled amount of fluticasone propionate.

If the criteria in *Tier 1* cannot be met, proceed to *Tier 2*.

Tier 2: If 2 or 3 doses are outside 80%–120% of the label claim, test an additional 10 bottles.

1. The mean dose delivered from 30 doses is within 85%–115% of the labeled amount of fluticasone propionate.
2. NMT 3 doses are outside 80%–120% of the labeled amount of fluticasone propionate.
3. No doses are outside 75%–125% of the labeled amount of fluticasone propionate.

▲USP40

Change to read:

- **Delivered Dose Uniformity** (within batch)

Diluent, Buffer, Mobile phase, System suitability solution, Standard solution, Chromatographic system, and System suitability: Prepare as directed in the test for *Delivered Dose Uniformity (within container)*.

Sample solution: Wipe the pump clean. Shake the bottle for 30 s, and mechanically prime the bottle. Hold a 25-mL volumetric flask in an inverted position, and discharge the first two actuations into the flask. Turn the flask to the upright position immediately after each actuation. Insert the stopper into the flask after collecting two actuations (1 dose). Repeat this procedure with 9 additional bottles.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in each dose taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of fluticasone propionate in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria ~~The mean dose delivered from 10 doses is within 85%–115% of the label claim. NMT 1 dose is outside 80%–120% of the label claim. No doses are outside 75%–125% of the label claim. If 2 or 3 doses are outside 80%–120% of the label claim, test an additional 20 bottles. The mean dose delivered from 2 actuations in the beginning of the 30 bottles (30 doses) is within 85%–115% of the label claim. NMT 3 doses are outside 80%–120% of the label claim. No doses are outside 75%–125% of the label claim.~~

▲ Tier 1

1. Mean dose delivered from 10 doses is within 85%–115% of the labeled amount of fluticasone propionate.
2. NMT 1 dose is outside 80%–120% of the labeled amount of fluticasone propionate.
3. No doses are outside 75%–125% of the labeled amount of fluticasone propionate.

If the criteria in *Tier 1* cannot be met, proceed to *Tier 2*.

Tier 2: If 2 or 3 doses are outside 80%–120% of the label claim, test an additional 20 bottles.

1. The mean dose delivered from 30 doses is within 85%–115% of the labeled amount of fluticasone propionate.
2. NMT 3 doses are outside 80%–120% of the labeled amount of fluticasone propionate.
3. No doses are outside 75%–125% of the labeled amount of fluticasone propionate.

▲USP40

IMPURITIES**Change to read:**

- **Organic Impurities**

Diluent: *Acetonitrile* and 0.001 M hydrochloric acid (60:40)

Solution A: *Methanol* and *acetonitrile* (77:23)

Buffer: 1.2 g/L of *monobasic ammonium phosphate*. Adjust with *phosphoric acid* to a pH of 3.4. ±0.1.

▲▲USP40

Mobile phase: *Solution A* and *Buffer* (60:40)

System suitability solution: 0.1 mg/mL of USP Fluticasone Propionate Related Compounds Mixture RS and 0.5 mg/mL of USP Phenylethyl Alcohol RS in *Diluent*

▲ USP Fluticasone Propionate RS and 0.05 mg/mL of USP Fluticasone Propionate Related Compound D RS in *Diluent*▲USP40

Control solution

▲ **Identification solution:**▲USP40

0.5 mg/mL of USP Phenylethyl Alcohol RS and 0.08 mg/mL of USP Benzalkonium Chloride RS in a mixture of *Diluent* and water (4:1)

Sample solution: 0.2 g/mL of Nasal Spray in *Diluent*. Shake the flask vigorously to dissolve. Pass through a filter of 0.5-µm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 μL

System suitability

Sample: *System suitability solution*

[Note—See *Table 3* for the relative retention times.]

Suitability requirements

Resolution: ~~NLT 1.5 between fluticasone propionate related compound F and phenylethyl alcohol.~~

▲▲USP40

NLT 2 between fluticasone propionate related compound D and fluticasone propionate

Analysis

Samples: *System suitability solution, Control solution*

▲ *Identification solution*, ▲USP40

and *Sample solution*

Calculate the percentage of each degradation product in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*, excluding the peaks from the *Control solution*

▲ *Identification solution* ▲USP40

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
S-Fluoromethyl 17α-acetyloxy-6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carbothioate; ▲ Fluticasone acetate ^a ▲ <i>USP40</i>	0.7	0.3
S-Fluoromethyl 9α-fluoro-11β-hydroxy-16α-methyl-3,6-dioxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate ▲ 6-Ketofluticasone propionate ^b ▲ <i>USP40</i>		
▲ Fluticasone propionate	1.0	—▲ <i>USP40</i>
Fluticasone propionate related compound D	1.1	0.3
6α,9α-Difluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid-6α,9α-difluoro-17β-(fluoromethylthio)carbonyl-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17α-yl ester ▲ Fluticasone propionate dimer ^c ▲ <i>USP40</i>	2.1	0.3
Any unspecified related impurities ▲ individual unspecified degradation products▲ <i>USP40</i>	—	0.2
Total impurities ▲ degradation products▲ <i>USP40</i>	—	1.5
▲ a S-Fluoromethyl 17 α -acetyloxy-6 α ,9 α -difluoro-11 β -hydroxy-16 α -methyl-3-oxoandrosta-1,4-diene-17 β -carbothioate. b S-Fluoromethyl 9 α -fluoro-11 β -hydroxy-16 α -methyl-3,6-dioxo-17 α -propionyloxyandrosta-1,4-diene-17 β -carbothioate. c 6 α ,9 α -Difluoro-11 β ,17 α -dihydroxy-16 α -methyl-3-oxoandrosta-1,4-diene-17 β -carboxylic acid-6 α ,9 α -difluoro-17 β -(fluoromethylthio)carbonyl-11 β -hydroxy-16 α -methyl-3-oxoandrosta-1,4-dien-17 β -yl ester.▲ <i>USP40</i>		

SPECIFIC TESTS

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): Meets the requirements of the tests for absence of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* species, and *Pseudomonas aeruginosa*. The total aerobic microbial count does not exceed 25 cfu/mL, and the total combined molds and yeasts count does not exceed 25 cfu/mL.
- **pH** (791): 5.0–7.0
- **Particle Size**

Analysis: Remove the pump system after shaking the test bottle to ensure product uniformity. Transfer 1 drop of the Nasal Spray onto a clean microscope slide. Examine 10 random fields of view on the slide using 400 \times magnification. Drug substance particles are irregular in shape, whereas the excipient particles are elongated and angular. Record the number of individual drug substance particles that are less than 5 μ m in diameter, greater than 5 μ m but less than 15 μ m in diameter, and greater than 15 μ m in diameter. Calculate

the percentage of each category by number.

Acceptance criteria: See Table 4.

Table 4

Particle Size (μm)	Acceptance Criteria (%)
<5	NLT 98
>5 to <15	NMT 1.8
>15	NMT 0.2

Delete the following:

▲ ● Foreign Particulates

Analysis: Shake the required number of bottles to ensure uniformity. Remove the pump system carefully to minimize contamination of the sample. Collect about 100 g of Nasal Spray, and pass it through a wetted 250 μm screen. Rinse each bottle with a portion of water equal to twice the volume of each bottle. Pass the rinse through the 250 μm screen. Visually observe the screen and filtrate for any foreign particulates. Also examine the screen under a microscope using transmitted light.

Acceptance criteria: No foreign particulates greater than 250 μm are visible. ▲USP40

Delete the following:

▲ ● Droplet Size Distribution: Determine using a validated laser diffraction technique and method that measures the volume diameters of droplets. In preparation shake the bottle to ensure product uniformity. Prime the pump by discharging a predetermined (refer to the product label) number of actuations to waste, at which time a fine mist should appear. Measure and record the average of three sprays per bottle, and report the mean diameter defining the population of particles, by volume, below 10% (D_{10}), 50% (D_{50}), and 90% (D_{90}) of five bottles. ▲USP40

Delete the following:

▲ ● Spray Pattern: Determine the spray pattern using a validated method that measures the size of the pattern. Gently shake the bottle to ensure product uniformity. Prime the pump by discharging a predetermined number (refer to the product label) of actuations to waste, at which time a fine mist should appear. Measure and record the average of two sprays per bottle and report the longest axis (x axis), and the ratio of longest to shortest axes (x/y ratio) of two bottles. ▲USP40

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store between 4° and 30°.

Change to read:

- **USP Reference Standards** (11)

▲ USP Benzalkonium Bromide RS ▲USP40

USP Benzalkonium Chloride RS

USP Docusate Sodium RS

▲▲USP40

USP Fluticasone Propionate RS ~~USP Fluticasone Propionate Nasal Spray Resolution Mixture RS~~ ~~This Reference Standard is a mixture of fluticasone propionate and fluticasone propionate related compound D, and the chemical names for both are given below:~~~~*Fluticasone propionate*: 5-Fluoromethyl 6 α ,9 α -difluoro-11 β -hydroxy-16 α -methyl-3-oxo-17 α -propionyloxyandrosta-1,4-diene-17 β -carbothioate.~~▲ USP Fluticasone Propionate Related Compound D RS S-Methyl-6 α ,9 α -difluoro-11 β -hydroxy-16 α -methyl-3-oxo-17 α -propionyloxyandrosta-1,4-diene-17 β -carbothioate.

▲USP40

~~USP Fluticasone Propionate Related Compounds Mixture RS~~ ~~This Reference Standard is a mixture of fluticasone propionate and fluticasone propionate related compounds D and F, and the chemical names for all are given below:~~~~*Fluticasone propionate*: 5-Fluoromethyl 6 α ,9 α -difluoro-11 β -hydroxy-16 α -methyl-3-oxo-17 α -propionyloxyandrosta-1,4-diene-17 β -carbothioate.~~~~*Fluticasone propionate related compound D*: S-Methyl 6 α ,9 α -difluoro-11 β -hydroxy-16 α -methyl-3-oxo-17 α -propionyloxyandrosta-1,4-diene-17 β -carbothioate.~~~~*Fluticasone propionate related compound F*: 6 α ,9 α -Difluoro-11 β ,17 α -dihydroxy-16 α -methyl-3-oxoandrosta-1,4-diene-17 β -carboxylic acid.~~

▲▲USP40

USP Phenylethyl Alcohol RS **BRIEFING**

Hard Gelatin Capsule Shell, PF 41(1) [Jan.-Feb. 2015]. On the basis of comments received, it is proposed to cancel the proposal published in PF 41(1) and replace it with the following proposal. Comments and suggestions should be sent to Margareth Marques, Ph.D., at mrm@usp.org not later than Jan. 31, 2016.

(GCDF: M. Marques.)

Correspondence Number—C161795

Comment deadline: January 31, 2016**Add the following:**▲ **Hard Gelatin Capsule Shell****DEFINITION**

Hard Gelatin Capsule Shell consists of two overlapping pieces (cap and body). One end of each piece is rounded and closed while the other is open. The cap overlaps the body and maintains a tight closure. Hard Gelatin Capsule Shell is composed of NF *Gelatin* and water, and may also contain additives such as plasticizers, surfactants, dispersing agents, flavoring agents, antimicrobial agents, and sweeteners. It may contain opacifiers, colorants, and/or processing aids. It may be externally coated and imprinted.

IDENTIFICATION

- **A.**

Sample solution: Dissolve an amount of Hard Gelatin Capsule Shell equivalent to 1 g in 100 mL of *carbon dioxide-free water* at about 55°. Let the solution stand for a period of time to allow any suspended solids to settle to the bottom of the vessel.

Analysis: To 2 mL of the supernatant of the *Sample solution*, add 0.05 mL of a 125-g/L solution of copper sulfate pentahydrate. Mix, and add 0.5 mL of an 85-g/L solution of *sodium hydroxide*.

Acceptance criteria: A violet color is produced.

- **B.**

Sample: A quantity of Capsule Shells equivalent to 0.5 g

Analysis: Place the *Sample* in a test tube of about 15 mm internal diameter and add 10 mL of *water*. Allow to stand for 10 min and then heat to 60° for 15 min. Keep the tube upright and store at 0° for 6 h. Invert the tube.

Acceptance criteria: The content of the tube does not flow out immediately.

SPECIFIC TESTS

- **Loss on Drying** (731)

Sample: 1.5 ± 0.5 g

Analysis: Dry the *Sample* in an oven at 105 ± 2° to constant weight.

Acceptance criteria: 11.0%–16.0%

- **Disintegration** (701)

Medium: *Water*

Analysis: Fill each Hard Gelatin Capsule Shell body to capacity with lactose or other suitable material. Place the cap onto the body and press the cap and body together to lock. Place the Hard Gelatin Capsule Shell in the basket and add disks.

For capsules larger than 18 mm: Use the apparatus described in *Disintegration and Dissolution* (2040).

Acceptance criteria: 15 min

- **Microbial Enumeration Tests** (61) and **Tests for Specified Microorganisms** (62): The total bacteria count does not exceed 10³ cfu/g, and the total yeast and molds count does not exceed 10² cfu/g.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store in tightly closed containers at 15°–25° and at a relative humidity of 35%–65%, unless moisture impermeable containers are used. Protect from the light. ▲USP40

BRIEFING

Heparin Sodium, USP 38 page 3748. In preparation for the omission of the flame tests from *Identification Tests—General* (191), proposed in PF 41(2), it is proposed to delete the reference to (191) under *Identification* test E and include a description of the flame test and requirement in the monograph. Manufacturers are encouraged to submit a replacement wet-chemistry test

or an instrumental procedure for Expert Committee consideration.

Corrections have also been made to the column description under *Molecular Weight Determinations* to make it consistent with the information in the corresponding footnote, and the benzonase concentration was corrected in another footnote to reflect the information in the method validation document.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(BIO3: A. Szajek.)

Correspondence Number—C165050

Comment deadline: January 31, 2016

Heparin Sodium

DEFINITION

Heparin Sodium is the sodium salt of sulfated glycosaminoglycans present as a mixture of heterogeneous molecules varying in molecular weights that retains a combination of activities against different factors of the blood clotting cascade. It is present in mammalian tissues and is usually obtained from the intestinal mucosa or other suitable tissues of domestic mammals used for food by humans. The sourcing of heparin material must be specified in compliance with applicable regulatory requirements. The manufacturing process should be validated to demonstrate clearance and inactivation of relevant infectious and adventitious agents (e.g., viruses, TSE agents). See *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* (1050) for general guidance on viral safety evaluation. The heparin manufacturing process should also be validated to demonstrate clearance of lipids. It is composed of polymers of alternating derivatives of α -D-glucosamido (*N*-sulfated, *O*-sulfated, or *N*-acetylated) and *O*-sulfated uronic acid (α -L-iduronic acid or β -D-glucuronic acid). The component activities of the mixture are in ratios corresponding to those shown by USP Heparin Sodium for Assays RS. Some of these components have the property of prolonging the clotting time of blood. This occurs mainly through the formation of a complex of each component with the plasma proteins antithrombin and heparin cofactor II to potentiate the inactivation of thrombin (factor IIa). Other coagulation proteases in the clotting sequence, such as activated factor X (factor Xa), are also inhibited. The ratio of anti-factor Xa activity to anti-factor IIa potency is between 0.9 and 1.1. The potency of Heparin Sodium, calculated on the dried basis, is NLT 180 USP Heparin Units in each mg.

IDENTIFICATION

● A. ^1H NMR spectrum

(See *Nuclear Magnetic Resonance* (761).)

Standard solution: NLT 20 mg/mL of USP Heparin Sodium Identification RS in deuterium oxide with 0.002% (w/v) deuterated trimethylsilylpropionic (TSP) acid sodium salt

System suitability solution: Prepare 0.3% (w/w) USP Oversulfated Chondroitin Sulfate RS in the *Standard solution*.

Sample solution: NLT 20 mg/mL of Heparin Sodium in deuterium oxide with 0.002% (w/v)

deuterated TSP. [Note—EDTA may be added to the *Sample solution* to NMT 12 µg/mL. In the event that EDTA is added to the *Sample solution*, spectra should be recorded and compared both with and without addition of EDTA.]

Instrumental conditions

(See *Nuclear Magnetic Resonance* (761).)

Mode: NMR, pulsed (Fourier transform)

Frequency: NLT 500 MHz (for ^1H)

Temperature: 20°–30°

System suitability

Samples: *Standard solution* and *System suitability solution*

Using a pulsed (Fourier transform) NMR spectrometer operating at NLT 500 MHz for ^1H , acquire a free induction decay (FID) using NLT 16 scans using a 90° pulse, an acquisition time of NLT 2 s, and at least a 10-s delay. Record the ^1H NMR spectra of the *Standard solution* and the *System suitability solution* at a stable temperature between 20°–30°.

Collect the ^1H NMR spectrum with a spectral window of at least 10 to –2 ppm and without spinning. The number of transients should be adjusted until the signal-to-noise ratio of the *N*-acetyl heparin signal in the *Standard solution* is at least 1000/1 in the region near 2 ppm. The *Standard solution* shall be run at least daily when *Sample solutions* are being run. For all samples, the TSP methyl signal should be set to 0.00 ppm. The chemical shift for the *N*-acetyl resonance of heparin and oversulfated chondroitin sulfate in the *System suitability solution* should be observed at 2.05 ± 0.02 and 2.16 ± 0.03 ppm, respectively. Record the ^1H NMR spectrum of the *Sample solution* at a stable temperature between 20°–30°. Draw a baseline from 8.00 ppm to 0.10 ppm. The ppm values for H1 of GlcNAc/GlcNS, 6S (signal 1), H1 of IdoA2S (signal 2), the H2 of GlcNS (signal 3), and the methyl of GlcNAc (signal 4) of heparin are present at 5.42, 5.21, 3.28 (doublet centered at 3.28 ppm), and 2.05 ppm, respectively.¹ The chemical shifts of these signals do not differ by more than ± 0.03 ppm. Measure the signal heights above the baseline of signal 1 and signal 2, and calculate the mean of these signal heights. Other signals of variable heights and ppm values, attributable to heparin and HOD, may be seen between signal 2 and 4.55 ppm. Residual solvent signals may be observed in the 0.10–3.75 range. Heparin Sodium must meet the requirements stated in *Residual Solvents* (467).

Suitability requirements

Number of transients: Adjust until the signal-to-noise ratio of the *N*-acetyl heparin signal in the *Standard solution* is at least 1000/1 in the region near 2 ppm.

Chemical shift: The TSP methyl signal should be set to 0.00 ppm for all samples.

Chemical shifts (for the *N*-acetyl resonance of heparin and oversulfated chondroitin sulfate in the *System suitability solution*): Should be observed at 2.05 ± 0.02 and 2.16 ± 0.03 ppm, respectively

Analysis

Sample: *Sample solution*

Acceptance criteria: No unidentified signals greater than 4% of the mean of the height of signals 1 and 2 are present in the following ranges: 0.10–2.00, 2.10–3.20, and 5.70–8.00

ppm. No signals greater than 200% of the mean of the height of signals 1 and 2 are present in the 3.75–4.55 ppm for porcine heparin.

• **B. Chromatographic Identity**

Solution A: Dissolve 0.8 g of monobasic sodium phosphate dihydrate in 2 L of water, and adjust with phosphoric acid to a pH of 3.0. Pass the solution through a membrane filter with a 0.45- μm pore size, and degas before use.

Solution B: Dissolve 0.8 g of monobasic sodium phosphate dihydrate and 280 g of sodium perchlorate monohydrate in 2 L of water, and adjust with phosphoric acid to a pH of 3.0. Pass the solution through a membrane filter with a 0.45- μm pore size, and degas before use.

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
30	10	90
31	80	20
45	80	20

Standard solution: NLT 20 mg/mL of USP Heparin Sodium Identification RS in water

System suitability solution: Prepare 0.1% (w/w) USP Oversulfated Chondroitin Sulfate RS and 0.5% (w/w) USP Dermatan Sulfate RS in the *Standard solution*.

Sample solution: NLT 20 mg/mL of Heparin Sodium in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 202 nm

Column: 2-mm \times 25-cm; packing L81²

Guard column: 2-mm \times 5-cm; packing L61

Column temperature: Maintain columns at 40°

Flow rate: 0.22 mL/min

Injection volume: 20 μL

System suitability

Sample: *System suitability solution*

[Note—The retention times for dermatan sulfate, heparin, and oversulfated chondroitin sulfate are about 17, 22, and 30 min, respectively.]

Suitability requirements

Resolution: NLT 1.0 between the dermatan sulfate and heparin peaks, and NLT 1.5 between the heparin and oversulfated chondroitin sulfate peaks

Relative standard deviation: NMT 2% for the heparin peak area determined from three replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms, and measure the retention times for the major peaks.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*.

• **C. Anti-Factor Xa to Anti-Factor IIa Ratio**

Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated and Low Molecular Weight Heparins (208), *Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated Heparin*

Acceptance criteria: 0.9–1.1

Change to read:

• **D. Molecular Weight Determinations**

1 M ammonium acetate solution: Accurately weigh 77.1 g of ammonium acetate, and dissolve in 1 L of water.

1% Sodium azide solution: Dissolve 1 g of sodium azide in 100 mL of water.

Mobile phase: Transfer 100 mL of *1 M ammonium acetate solution* to a 1-L volumetric flask, add 20 mL of *1% sodium azide solution*, and dilute with water to volume. Filter using a nylon membrane with a 0.2- μ m pore size prior to use.

Calibration solution: Prepare by dissolving 10 mg of the USP Heparin Sodium Molecular Weight Calibrant RS in 2 mL of *Mobile phase*, and filter using a nylon membrane with a 0.2- μ m pore size.

System suitability solution: 5 mg/mL of USP Heparin Sodium Identification RS in *Mobile phase*. Filter using a nylon membrane with a 0.2- μ m pore size.

Sample solution: Dissolve about 10 mg of Heparin Sodium sample in 2 mL of *Mobile phase*, and filter using a nylon membrane with a 0.2- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

[Note—The temperature of the refractive index detector must be set at the same temperature as the *Column temperature*.]

Mode: LC

Detector: Refractive index

Columns: One 7.8-mm \times 30-cm, 5

▲ 8▲*USP40*

- μ m packing L59 in series with a 7.8-mm \times 30-cm, 8

▲ 5▲*USP40*

- μ m packing L59³

Guard column: 6-mm \times 4-cm; 7- μ m packing L59

Column temperature: 30°

Flow rate: 0.6 mL/min \pm 0.1%

Column equilibration: 0.6 mL/min for 2 h

Injection volume: 20 μ L

System suitability

Samples: *Calibration solution* and *System suitability solution* (duplicate injections)

Suitability requirements

Weight-average molecular weight (M_w): Take the mean of the calculated M_w from the duplicate injections of the *System suitability solution*, and round to the nearest 100 Da. The chromatographic system is suitable if the M_w of the *System suitability* sample is within 500 Da of the labeled value as stated in the USP Certificate for USP Heparin Sodium Identification RS.

Peak molecular weights (M_p): The peak molecular weights (M_p) of the duplicate injections of the *System suitability solution* do not differ by more than 5% of the upper value.

Resolution: There is baseline resolution between the heparin and salt peaks.

Calibration curve: The linear regression coefficient of the calibration curve fitted to the Broad Standard Table values must be NLT 0.990, using a third-order polynomial equation.

Analysis

Samples: Inject 20 μ L of the *System suitability solution* (duplicate injections), *Sample solution* (duplicate injection), and *Calibration solution* (single injection), and record the chromatograms for a length of time to ensure complete elution, including salt and solvent peaks (about 50 min). [Note—The calibrant, standard, or sample of heparin will give a broad heparin peak between about 20 and 40 min, followed by a later eluting narrow salt peak, as illustrated in the USP Certificate for USP Heparin Sodium Molecular Weight Calibrant RS.]

Calculations: Calculate the total area under the heparin peak in the *Calibration solution* chromatogram, and the cumulative area at each point under the peak as a percent of the total. Do not include the salt peak. Using the Broad Standard Table provided in the USP Certificate for USP Heparin Sodium Molecular Weight Calibrant RS, identify those points in the chromatogram for which the percent cumulative area is closest to the percent fractions listed in the Table, and assign the molecular weight (MW) in the Table to the corresponding retention time (RT) in the chromatogram. For the set of retention times and molecular weights identified, fit $\log(MW)$ vs. RT to a third-order polynomial function using suitable gel permeation chromatography (GPC) software [or: find values of a , b , c , and d such that $\log(MW) = a + b(RT) + c(RT)^2 + d(RT)^3$]. Using the same GPC software, for each of the duplicate chromatograms of the *System suitability solution* and the *Sample solution*, with the calibration function derived as described above, calculate M_w according to the following formula:

$$M_w = \Sigma(RI_i M_i) / \Sigma RI_i$$

where the detector response at each point is defined as RI_i and the MW at each point as M_i . Round the mean value of M_w to the nearest 100 Da.

Using the same GPC software, determine for each of the duplicate *Sample solution* chromatograms: the percentage of heparin with molecular weight in the range 8,000–16,000, $M_{8000-16000}$, the percentage of heparin with molecular weight in the range 16,000–24,000, $M_{16000-24000}$, and the percentage of heparin with molecular weight greater than 24,000, M_{24000} . Round the mean percentage values to the nearest 1%.

Acceptance criteria: M_{24000} is NMT 20%, M_w is between 15,000 Da and 19,000 Da, and

the ratio of $M_{8000-16000}$ to $M_{16000-24000}$ is NLT 1.0.

Delete the following:

- ~~▲ ● E. Identification Tests—General (191), Sodium: It meets the requirements of the flame test for sodium. ▲USP40~~

Add the following:

- ▲ ● E. A solution of Heparin Sodium imparts an intense yellow color to a nonluminous flame. ▲USP40

ASSAY

● **Anti-Factor IIa Potency**

Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated and Low Molecular Weight Heparins (208), Anti-Factor IIa Activity for Unfractionated Heparin

Acceptance criteria: The potency of Heparin Sodium, calculated on the dried basis, is NLT 180 USP Heparin Units in each mg.

OTHER COMPONENTS

- **Nitrogen Determination (461), Method I:** 1.3%–2.5%, calculated on the dried basis, using the procedure for *Nitrates and Nitrites Absent*

IMPURITIES

- **Residue on Ignition (281):** 28.0%–41.0%

Delete the following:

- ~~* ● Heavy Metals (231), Method II: NMT 30 ppm (Official 1-Jan-2018)~~

- **Limit of Galactosamine in Total Hexosamine** (a measure of dermatan sulfate and other galactosamine containing impurities)

Mobile phase: 14 mM potassium hydroxide

Glucosamine standard solution: 1.6 mg/mL of USP Glucosamine Hydrochloride RS in 5 N hydrochloric acid

Galactosamine standard solution: 16 µg/mL of USP Galactosamine Hydrochloride RS in 5 N hydrochloric acid

Standard solution: Mix equal volumes of the *Glucosamine standard solution* and the *Galactosamine standard solution*.

Hydrolyzed standard solution: Transfer 5 mL of the *Standard solution* to a 7-mL screw-cap test tube, cap, and heat for 6 h at 100°. Cool to room temperature, and dilute with water (1 in 100).

Sample solution: Transfer 12 mg of Heparin Sodium to a 7-mL screw-cap test tube, dissolve in 5 mL of 5 N hydrochloric acid, and cap.

Hydrolyzed sample solution: Heat the *Sample solution* for 6 h at 100°. Cool to room temperature, and dilute with water (1 in 100).

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: HPIC

Detector: Pulsed amperometric detector, set to the following waveform. See *Table 2*.

Table 2

Step	Time (s)	Potential (V)	Integration
1	0.00	+0.1	—
2	0.20	+0.1	Begins
3	0.40	+0.1	Ends
4	0.41	-2.0	—
5	0.42	-2.0	—
6	0.43	+0.6	—
7	0.44	-0.1	—
8	0.50	-0.1	—

Column: 3-mm × 3-cm amino acid trap column in series with a 3-mm × 3-cm guard column and a 3-mm × 15-cm column that contains packing L69

Column temperature: 30°

Flow rate: 0.5 mL/min

Pre-equilibration: At least 60 min with *Mobile phase*

Injection volume: 10 µL

Elution: 10 min with *Mobile phase*

Column cleaning: At least 10 min with 100 mM potassium hydroxide

Equilibration: At least 10 min with *Mobile phase* before each injection

System suitability

Sample: *Hydrolyzed standard solution*

Suitability requirements

Resolution: NLT 2 between the galactosamine and glucosamine peaks

Column efficiency: NLT 2000 theoretical plates for glucosamine

Tailing factor: Between 0.8 and 2.0 for the galactosamine and glucosamine peaks

Analysis

Samples: *Hydrolyzed standard solution* and *Hydrolyzed sample solution*

Record the chromatograms, and measure the responses for the peaks at the retention time of galactosamine and glucosamine. Calculate the response ratio of galactosamine to glucosamine ($GalN_R$) in the *Hydrolyzed standard solution*:

$$\text{Result} = (GalN_B / GalN_W) \times (GlcN_W / GlcN_B)$$

$GalN_B$ = peak area of galactosamine from the *Hydrolyzed standard solution*

$GalN_W$ = weight of galactosamine for the *Standard solution*

$GlcN_W$ = weight of glucosamine for the *Standard solution*

$GlcN_B$ = peak area of glucosamine from the *Hydrolyzed standard solution*

Calculate the percentage of galactosamine in the portion of total hexosamine taken:

$$\text{Result} = \{[(GalN_U/GalN_R)]/[(GalN_U/GalN_R) + GlcN_U]\} \times 100$$

$GalN_U$ = peak area of galactosamine from the *Hydrolyzed sample solution*

$GalN_R$ = response ratio of galactosamine

$GlcN_U$ = peak area of glucosamine from the *Hydrolyzed sample solution*

Acceptance criteria: The percent galactosamine peak area of the total hexosamine of the *Hydrolyzed sample solution* must be NMT 1%.

Change to read:

• **Nucleotidic Impurities**

Solution A: Dissolve 3.08 g of ammonium acetate in 2 L of water, and adjust with glacial acetic acid to a pH of 4.4 ± 0.2 . Degas for 2 min under vacuum with sonication before use.

Solution B: 100% acetonitrile. Degas for 1 min under vacuum with sonication before use.

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	98	2
5.00	98	2
15.00	80	20
20.00	80	20
20.10	98	2
25.00	98	2

Nucleoside identification solution: Accurately weigh and transfer about 25 mg each of uridine, guanosine, cytidine, thymidine, 2'-deoxyadenosine, 2'-deoxyguanosine, 2'-deoxycytidine, and 5-methyl-2'-deoxycytidine into a 200-mL volumetric flask, add approximately 185 mL of water, and dissolve with sonication and vortexing, if necessary. Dilute with water to volume, and mix. Transfer 2.0 mL of this solution into a 100-mL volumetric flask, dilute with water to volume, and mix.

Adenosine stock solution: Accurately weigh and transfer 25 mg of USP Adenosine RS into a 100-mL volumetric flask, add approximately 85 mL of water, and dissolve with sonication and vortexing, if necessary. Dilute with water to volume, and mix.

Standard solution: Transfer 2.0 mL of the *Adenosine stock solution* into a 200-mL volumetric flask, dilute with water, and mix.

System suitability solution: Transfer 2.0 mL of the *Standard solution* into a 100-mL volumetric flask, dilute with water to volume, and mix.

Reaction buffer: Accurately weigh and transfer 0.41 g of magnesium chloride hexahydrate,

0.24 g of tris (hydroxymethyl)amino methane, and 0.58 g of sodium chloride into a 100-mL volumetric flask, dissolve in 75 mL of water, and mix. Adjust with 1 N hydrochloric acid to a pH of 7.9 ± 0.1 . Dilute with water to volume, and mix.

PDE I diluent: Transfer 5.0 mL of glycerol and 5.0 mL of the *Reaction buffer* into a 20-mL flask, and vortex to mix.

PDE I solution: 0.1 Unit/ μL of phosphodiesterase I (PDE I) in *PDE I diluent*. Store at -20° .

Enzyme digest solution: Add 10 μL of Benzonase⁴, 222 Units of alkaline phosphatase (AP), and 125 μL of *PDE I solution* to 5.0 mL of *Reaction buffer*. Store at -20° .

Blank: Transfer 100 μL of water and 100 μL of *Enzyme digest solution* into a 250- μL HPLC vial, and mix with a micropipette. Incubate NLT 60 min in the autosampler at 37° before injection.

Sample solution: Accurately weigh and transfer 400 mg of Heparin Sodium into a 20-mL volumetric flask, dilute with water to volume, and mix. Transfer 100 μL of this solution and 100 μL of *Enzyme digest solution* into a 250- μL HPLC vial, and mix. Incubate NLT 60 min in the autosampler at 37° before injection.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Column: 4.6-mm \times 15-cm; 4- μm packing L1

Detector: UV 260 nm

Autosampler temperature: $37 \pm 1^\circ$

Column temperature: $20 \pm 3^\circ$

Flow rate: 1 mL/min

Injection volume: 10 μL

Run time: 25 min

System suitability

Samples: *Nucleoside identification solution*, *Standard solution*, and *System suitability solution*

Suitability requirements

Resolution: The resolution between the 2'-deoxycytidine peak and the uridine peak is NLT 1.3 for the injection of the *Nucleoside identification solution*.

Relative standard deviation: Inject six replicates of the *Standard solution*, and record the chromatograms. The percent relative standard deviation (%RSD) of the areas of the adenosine peak is NMT 10%.

Signal-to-noise ratio: The *S/N* of the adenosine peak in the *System suitability solution* is NLT 10.

Analysis

Samples: *Water*, *Blank*, *Nucleoside identification solution*, *Standard solution*, *System suitability solution*, and *Sample solution*

Record the chromatograms. Calculate the area reject value, *Q*:

$$Q = (10 \times A_{SSS})/(S/N)$$

A_{SSS} = peak area of adenosine in the *System suitability solution*

S/N = signal-to-noise ratio of the adenosine peak in the *System suitability solution*

For the *Standard solution*, calculate the concentration of adenosine, in mg/mL:

$$C_s = W_s/DF$$

C_s = concentration of adenosine in the *Standard solution* (mg/mL)

W_s = weight of USP Adenosine RS (mg)

DF = 10,000 (dilution factor)

Calculate the percentage of nucleotidic impurities:

$$\text{Result} = \Sigma[(C_s/A_s) \times A_i \times (MW_{ratio}/RRF_i)] \times (DF/W_{sample}) \times 100$$

C_s = concentration of adenosine in the *Standard solution* (mg/mL)

A_s = average peak area ($n = 6$) of adenosine in the *Standard solution*

A_i = peak area of each impurity above Q in the *Sample solution*

MW_{ratio} = see *Table 4*

RRF_i = relative response factor for the corresponding peak (see *Table 4*)

DF = dilution factor, 40

W_{sample} = sample weight of Heparin Sodium (mg)

Table 4

Name	Relative Retention Time	Relative Response Factor	MW_{ratio}
Cytidine	0.28	0.53	1.2548
2'-Deoxycytidine	0.38	0.56	1.2727
Uridine	0.40	0.75	1.2537
5-Methyl-2'-deoxycytidine	0.66	0.25	1.2569
Guanosine	0.81	0.74	1.2188
2'-Deoxyguanosine	0.89	0.83	1.2319
Thymidine	0.92	0.68	1.2558
Adenosine	1.00	1.00	1.2319
2'-Deoxyadenosine	1.04	1.09	1.2466
Others	—	1.00	1.0000

Acceptance criteria: NMT 0.1% (w/w) is found.

- **Absence of Oversulfated Chondroitin Sulfate**

A. Proceed as directed in *Identification* test *A*. No features associated with oversulfated chondroitin sulfate are found between 2.12 and 3.00 ppm.

B. Proceed as directed in *Identification* test *B*. No peaks corresponding to oversulfated chondroitin sulfate should be detected eluting after the heparin peak.

- **Protein Impurities**

[Note—Treatment for interfering substances is only required for samples previously tested with

a protein content greater than 0.1%. *Spiked sample* should be prepared and assayed only if the treatment for interfering substances is performed.]

Standard stock solution: 2.0 mg/mL of bovine serum albumin in water

Standard solutions: Dilute portions of the *Standard stock solution* with water to obtain NLT 5 standard solutions having concentrations between 0.010 and 0.050 mg/mL of bovine serum albumin, the concentrations being evenly spaced.

System suitability standard: Dilute a portion of the *Standard stock solution* with water to obtain a solution containing 0.030 mg/mL of bovine serum albumin.

Sample solution: 30 mg/mL of Heparin Sodium in water. Prepare in triplicate.

Spiked sample: Using an appropriate dilution scheme and the *Standard stock solution*, prepare a *Spiked sample* containing 30 mg/mL Heparin Sodium and 0.030 mg/mL bovine serum albumin in water.

Blank: Water

Lowry reagent A: Prepare a solution of 10 g/L of sodium hydroxide in water and a solution of 50 g/L of sodium carbonate in water. Mix equal volumes (2V:2V) of each solution, and dilute with water to 5V.

Lowry reagent B: Prepare a solution of 29.8 g/L of disodium tartrate dihydrate in water. Prepare a solution of 12.5 g/L of cupric sulfate in water. Mix equal volumes of both solutions (2V:2V), and dilute with water to 5V.

Lowry reagent C: Mix 50 volumes of *Lowry reagent A* with 1 volume of *Lowry reagent B*. Prepare fresh daily.

Diluted Folin–Ciocalteu’s phenol reagent: Dilute Folin–Ciocalteu’s phenol reagent 1–2 times with water. The dilution should be chosen such that the pH of the samples (i.e., *Standard solution* and *Sample solution* after addition of *Lowry reagent C* and the *Diluted Folin–Ciocalteu’s phenol reagent*) is 10.3 ± 0.3 .

Sodium deoxycholate reagent: Prepare a solution of sodium deoxycholate in water having a concentration of 150 mg in 100 mL.

Trichloroacetic acid reagent: Prepare a solution of trichloroacetic acid in water having a concentration of 72 g in 100 mL.

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

To 1 mL each of *Standard solution*, *Sample solution*, and *Blank*, add 5 mL of *Lowry reagent C*. Mix. Allow to stand at room temperature for 10 min. Add 0.5 mL of *Diluted Folin–Ciocalteu’s phenol reagent* to each solution, mix immediately, and allow to stand at room temperature for NLT 30 min. Determine the absorbance at the wavelength of maximum absorbance at 750 nm with a suitable spectrophotometer, using the solution from the *Blank* to set the instrument to zero and ensuring that all samples and standards absorbances are measured after the same final incubation time. To remove interfering substances, add 0.1 mL of *Sodium deoxycholate reagent* to 1 mL of a solution of the protein under test. Mix on a vortex mixer, and allow to stand at room temperature for 10 min. Add 0.1 mL of *Trichloroacetic acid reagent*, and mix on a vortex mixer. Centrifuge at a speed that ensures removal of visible particulate matter. [Note—NLT 14,100 RCF should be used. Appropriate centrifuge speed should be determined by each laboratory.] The supernatant should be essentially free of visible particulates. A pellet may not be

visible. If the interfering substances method is used, dissolve the protein residue in 1 mL of *Lowry reagent C*.

Calculations

Using the linear regression method, plot the absorbances of the solutions from the *Standard solutions* versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the absorbance of the *Sample solution*, determine the concentration of protein in the *Sample solution*.

System suitability: The correlation coefficient (r) for a linear fit of all standards is NLT 0.99. The percent RSD between triplicate sample results is NMT 10%. If the sample absorbances are lower than the standard curve, the percent RSD specification is not required. The percent recovery of the *System suitability standard* is 90%–110%. If the interfering substances treatment is performed, the percent recovery of the *Spiked sample* is 85%–115%.

Acceptance criteria: NMT 0.1% (w/w) is found.

SPECIFIC TESTS

- **Bacterial Endotoxins Test** (85): It contains NMT 0.03 USP Endotoxin Unit/USP Heparin Unit.
- **Loss on Drying** (731)
 - Analysis:** Dry a sample in a vacuum at 60° for 3 h.
 - Acceptance criteria:** It loses NMT 5.0% of its weight.
- **pH** (791): 5.0–7.5 in a solution (1 in 100)
- **Sterility Tests** (71): Where it is labeled as sterile, it meets the requirements.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store below 40°, preferably at room temperature.
- **Labeling:** Label it to indicate the tissue and the animal species from which it is derived.
- **USP Reference Standards** (11)
 - USP Adenosine RS
 - USP Oversulfated Chondroitin Sulfate RS
 - USP Dermatan Sulfate RS
 - USP Endotoxin RS
 - USP Galactosamine Hydrochloride RS
 - USP Glucosamine Hydrochloride RS
 - USP Heparin Sodium for Assays RS
 - USP Heparin Sodium Identification RS
 - USP Heparin Sodium Molecular Weight Calibrant RS

¹ GlcNAc, *N*-acetylated glucosamine; GlcNS, *N*-sulfated glucosamine; S, sulfate; IdoA, iduronic acid; GlcN, glucosamine; GalN, galactosamine.

² L81—A hydroxide-selective, strong anion-exchange resin consisting of a highly cross-linked core of 9 µm porous particles having a pore size of 2000 Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene with a latex coating composed of 70 nm diameter microbeads (6% crosslinked) bonded with alkanol quaternary ammonium ions (βfa suitable column is Dionex IonPac AS11-HC available from www.thermofisher.com).

³ The method was validated using a guard column TSK SWXL 6-mm × 4-cm, 7-μm in series with two analytical columns: TSK G4000 SWXL 7.8- × 30-cm, 8-μm in series with a TSK G3000 SWXL 7.8- × 30-cm, 5-μm diameter.

⁴ A suitable ultrapure *Serratia marcescens* nuclease (EC 3.1.30.2) must be ≥99% containing ≥~~250~~

~~▲ 25 ▲ USP40~~
units/μL.

BRIEFING

Histidine, *USP 38* page 3760. As part of USP monograph modernization efforts, the following changes are proposed:

1. Replace the titrimetric *Assay* and the TLC *Related Compounds* tests with an HPLC procedure to address the specificity issue and to improve the characterization of the impurities. The liquid chromatographic procedures in the *Assay* and in the test for *Related Compounds* are based on analyses performed with the GL Sciences Inertsil ODS-3V brand of L1 column. The typical retention times for alanine, biotin, histidine, lysine, arginine, and thiamine are 4.4, 9.8, 13.7, 15.8, 16.7, and 21.1 min, respectively.
2. Add *Identification test B* based on the retention time comparison of the main peaks obtained from the *Assay*.
3. Revise the *USP Reference Standards* section to include the new Reference Standards used in the revised tests.

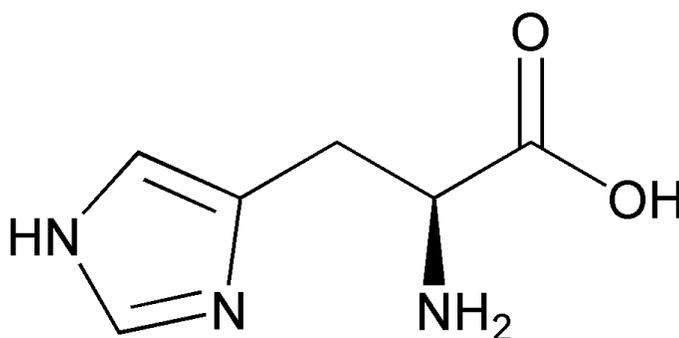
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(NBDS: H. Dinh.)

Correspondence Number—C154577

Comment deadline: January 31, 2016

Histidine



C₆H₉N₃O₂ 155.15

L-Histidine [71-00-1].

DEFINITION

Change to read:

Histidine contains NLT ~~98.5%~~

▲ 98.0%▲*USP40*
and NMT 101.5%
▲ 102.0%▲*USP40*

of L-histidine ($C_6H_9N_3O_2$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K): The sample and USP L-Histidine RS are previously recrystallized from 80% alcohol.

Add the following:

- ▲ ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲*USP40*

ASSAY

Change to read:

- **Procedure**

Sample: ~~150 mg of Histidine~~

Blank: ~~Mix 3 mL of formic acid and 50 mL of glacial acetic acid.~~

Titrimetric system

(See *Titrimetry* (541).)

Mode: ~~Direct titration~~

Titrant: ~~0.1 N perchloric acid VS~~

Endpoint detection: ~~Potentiometric~~

Analysis: ~~Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate very slowly with the *Titrant*. Perform the *Blank* determination.~~

~~Calculate the percentage of histidine ($C_6H_9N_3O_2$) in the *Sample* taken:~~

$$\text{Result} = \left\{ \frac{(V_S - V_B) \times N \times F}{W} \right\} \times 100$$

V_S = ~~*Titrant* volume consumed by the *Sample* (mL)~~

V_B = ~~*Titrant* volume consumed by the *Blank* (mL)~~

N = ~~actual normality of the *Titrant* (mEq/mL)~~

F = ~~equivalency factor, 155.2 mg/mEq~~

W = ~~*Sample* weight (mg)~~

Acceptance criteria: ~~98.5%–101.5% on the dried basis~~

▲ **Solution A:** 10 mM octanesulfonic acid sodium salt in water, adjusted with phosphoric acid to a pH of 2.5

Solution B: Acetonitrile

Mobile phase: Gradient elution. See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
5	85	15
25	65	35
30	85	15
35	85	15

Standard solution: Transfer 30 mg of USP L-Histidine RS to a 100-mL volumetric flask, dissolve, and dilute with water to volume. Transfer 5.0 mL of the resulting solution to a 50-mL volumetric flask and dilute with water to volume.

System suitability solution: Transfer 10 mg of USP L-Lysine Hydrochloride RS to a 10-mL volumetric flask, add about 6 mL of the *Standard solution*, sonicate to dissolve, and dilute with the *Standard solution* to volume.

Sample solution: Transfer 30 mg of Histidine to a 100-mL volumetric flask, dissolve, and dilute with water to volume. Transfer 5.0 mL of the resulting solution to a 50-mL volumetric flask and dilute with water to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 5.0 between the histidine and lysine peaks, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of histidine ($C_6H_9N_3O_2$) in the portion of Histidine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP L-Histidine RS in the *Standard solution* (mg/mL)

C_U = concentration of Histidine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ▲ *USP41*

IMPURITIES

- **Residue on Ignition** (281): NMT 0.4%
- **Chloride and Sulfate** (221), *Chloride*
Standard solution: 0.50 mL of 0.020 N hydrochloric acid
Sample: 0.73 g of Histidine
Acceptance criteria: NMT 0.05%
- **Chloride and Sulfate** (221), *Sulfate*
Standard solution: 0.10 mL of 0.020 N sulfuric acid
Sample: 0.33 g of Histidine
Acceptance criteria: NMT 0.03%
- **Iron** (241): NMT 30 ppm

Delete the following:

- * • **Heavy Metals** (231), *Method I* NMT 15 ppm ★

Test preparation: Transfer 1.33 g of Histidine to a 50-mL color-comparison tube. Dissolve in and dilute with 1 N *acetic acid* to 25 mL. Proceed as directed for *Method I*, starting with "Using a pH meter or short-range pH indicator paper as external indicator".

Acceptance criteria: NMT 15 ppm

▲USP40 ★ (Official 1-Jan-2018)

Change to read:

- **Related Compounds**

System suitability solution: 0.4 mg/mL each of USP I Histidine RS and USP I Proline RS

Standard solution: 0.05 mg/mL of USP I Histidine RS in water. [Note—This solution has a concentration equivalent to 0.5% of the *Sample solution*.]

Sample solution: 10 mg/mL of Histidine in water

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)

Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2-N acetic acid (95:5)

System suitability

Sample: *System suitability solution*

Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

Analysis

Samples: ~~System suitability solution, Standard solution, and Sample solution~~

After air-drying the plate, spray with ~~Spray reagent~~, and heat between 100° and 105° for 15 min. Examine the plate under white light.

Acceptance criteria: Any secondary spot of the ~~Sample solution~~ is not larger or more intense than the principal spot of the ~~Standard solution~~.

Individual impurities: ~~NMT 0.5%~~

Total impurities: ~~NMT 2.0%~~

▲ **Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

Alanine standard solution: 2.5 µg/mL of USP I-Alanine RS in water

Biotin standard solution: 2.5 µg/mL of USP Biotin RS in water

Lysine standard solution: 3.1 µg/mL of USP I-Lysine Hydrochloride RS in water

Arginine standard solution: 2.5 µg/mL of USP I-Arginine RS in water

Thiamine standard solution: 3.2 µg/mL of USP Thiamine Hydrochloride RS in water

Sample solution: 0.5 mg/mL of Histidine in water

Analysis

Samples: Standard solutions and *Sample solution*

Calculate the percentage of each amino acid impurity in the portion of Histidine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of alanine, biotin, or arginine from the *Sample solution*

r_S = peak response of alanine, biotin, or arginine from the corresponding Standard solution

C_S = concentration of USP I-Alanine RS, USP Biotin RS, or USP I-Arginine RS in the corresponding Standard solution (mg/mL)

C_U = concentration of Histidine in the *Sample solution* (mg/mL)

Calculate the percentage of lysine in the portion of Histidine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of lysine from the *Sample solution*

r_S = peak response of lysine from *Lysine standard solution*

C_S = concentration of USP I-Lysine Hydrochloride RS in *Lysine standard solution* (mg/mL)

C_U = concentration of Histidine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of lysine, 146.19

M_{r2} = molecular weight of lysine hydrochloride, 182.65

Calculate the percentage of thiamine in the portion of Histidine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of thiamine from the *Sample solution*

r_S = peak response of thiamine from *Thiamine standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in *Thiamine standard solution* (mg/mL)

C_U = concentration of Histidine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of thiamine, 265.35

M_{r2} = molecular weight of thiamine hydrochloride, 337.27

Calculate the percentage of any unspecified impurity in the portion of Histidine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Alanine	0.3	0.5
Biotin	0.6	0.5
Histidine	1.00	—
Lysine	1.1	0.5
Arginine	1.2	0.5
Thiamine	1.5	0.5
Any unspecified impurity	—	0.1
Total impurities	—	2.0

▲USP40

SPECIFIC TESTS

- **Optical Rotation** (781S), *Procedures, Specific Rotation*

Sample solution: 110 mg/mL in 6 N hydrochloric acid

Acceptance criteria: +12.6° to +14.0°

- **pH** (791)

Sample solution: 20-mg/mL solution

Acceptance criteria: 7.0–8.5

- **Loss on Drying** (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** (11)

USP I-Proline RS

▲ USP I-Alanine RS

USP I-Arginine RS

USP Biotin RS

▲*USP40*

USP I-Histidine RS

▲ USP I-Lysine Hydrochloride RS

USP Thiamine Hydrochloride RS ▲*USP40*

BRIEFING

Hydrocortisone Sodium Succinate, *USP 38* page 3793. In preparation for the omission of the flame tests from *Identification Tests—General* (191), proposed in *PF 41(2)* [Mar.–Apr. 2015], the reference to *Sodium* in *Identification* test *C* is deleted and a complete description for the *Sodium* test is included in the monograph. Manufacturers are encouraged to submit an instrumental procedure for the Expert Committee's consideration.

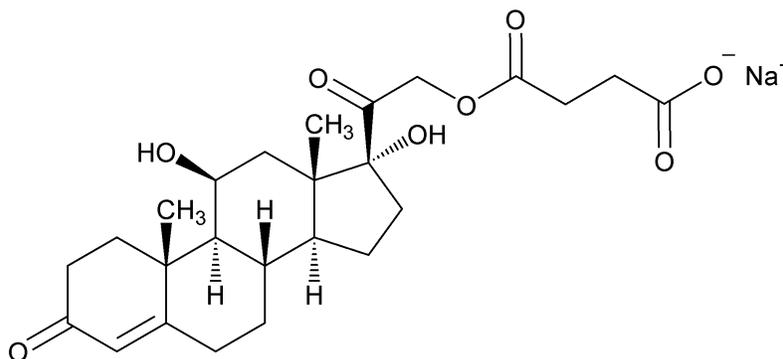
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM5: D. Min.)

Correspondence Number—C123868

Comment deadline: January 31, 2016

Hydrocortisone Sodium Succinate



$C_{25}H_{33}NaO_8$ 484.51

Pregn-4-ene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-11,17-dihydroxy-, monosodium salt, (11 β)-;

Cortisol 21-(sodium succinate) [125-04-2].

DEFINITION

Hydrocortisone Sodium Succinate contains NLT 97.0% and NMT 102.0% of total steroids, calculated as hydrocortisone sodium succinate ($C_{25}H_{33}NaO_8$), on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption**

Sample: Transfer 100 mg of Hydrocortisone Sodium Succinate to a suitable container, and dissolve in 10 mL of water. In rapid succession, add 1 mL of 3 N *hydrochloric acid*, shake briefly, immediately decant the aqueous layer, and wash the precipitate with two additional 10-mL portions of water, each time removing the water by decanting. Remove as much of the water as possible, spread the precipitate in a suitable container, and dry under vacuum at 60° for 3 h.

Acceptance criteria: The IR spectrum of a mineral oil dispersion of the precipitate so obtained exhibits maxima only at the same wavelengths as those of a similar preparation of USP Hydrocortisone Hemisuccinate RS.

- **B. Ultraviolet Absorption** (197U)

Sample solution: 20 µg/mL in *methanol*

Analytical wavelength: 242 nm

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

Delete the following:

~~▲ ● **C. Identification Tests—General, Sodium** (191): It meets the requirements of the flame test. ▲USP40~~

Add the following:

▲ ● **C. Sodium compounds impart an intense yellow color to a nonluminous flame.** ▲USP40

ASSAY

- **Assay for Steroids** (351)

Blue tetrazolium solution: 5 mg/mL of blue tetrazolium in *alcohol*

Tetramethylammonium hydroxide solution: *Tetramethylammonium hydroxide TS* in *alcohol* (1 in 10)

Standard solution: 12.5 µg/mL of USP Hydrocortisone Hemisuccinate RS in *alcohol*

Sample solution: 12.5 µg/mL of Hydrocortisone Sodium Succinate in *alcohol*

Blank solution: *Alcohol*

Analysis

Samples: *Standard solution, Sample solution, and Blank solution*

Transfer 20.0-mL aliquots of the *Samples* to separate glass-stoppered, 50-mL conical flasks. To each flask add 2.0 mL of *Blue tetrazolium solution*, mix, and add 4.0 mL of *Tetramethylammonium hydroxide solution*. Allow to stand in the dark for 90 min, and add 1.0 mL of *glacial acetic acid*. Concomitantly determine the absorbances of the solutions from the *Sample solution* and the *Standard solution* at about 525 nm, with a suitable spectrophotometer, against the blank.

Calculate the percentage of total steroids, as hydrocortisone sodium succinate (C₂₅H₃₃NaO₈), in the portion of Hydrocortisone Sodium Succinate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Hydrocortisone Hemisuccinate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of Hydrocortisone Sodium Succinate in the *Sample solution* ($\mu\text{g/mL}$)

M_{r1} = molecular weight of hydrocortisone sodium succinate, 484.51

M_{r2} = molecular weight of hydrocortisone hemisuccinate, 462.53

Acceptance criteria: 97.0%–102.0% on the dried basis

SPECIFIC TESTS

• Sodium Content

Sample: 1 g

Analysis: Dissolve the *Sample*, with gentle heating, in 75 mL of *glacial acetic acid*. Add 20 mL of *dioxane*, then add crystal violet TS, and titrate with 0.1 N *perchloric acid* VS. Each mL of 0.1 N *perchloric acid* is equivalent to 2.299 mg of sodium (Na).

Acceptance criteria: 4.60%–4.84% on the dried basis

• Optical Rotation (781S), Procedures, Specific Rotation

Sample solution: 10 mg/mL in *alcohol*

Acceptance criteria: +140° to +150°

• Loss on Drying (731)

Sample: Dry at 105° for 3 h.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

- **USP Reference Standards (11)**

USP Hydrocortisone Hemisuccinate RS

BRIEFING

Iodine Topical Solution, USP 38 page 3890. In preparation for the omission of the flame tests from *Identification Tests—General* (191), proposed in PF 41(2) [Mar.–Apr. 2015], it is proposed to delete the reference to *Sodium* under *Identification* test B, and include a complete description of the flame test in the monograph.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

(CMP: J. Sun.)

Correspondence Number—C164384

Comment deadline: January 31, 2016

Iodine Topical Solution

DEFINITION

Iodine Topical Solution contains NLT 1.8 g and NMT 2.2 g of iodine (I) and NLT 2.1 g and NMT 2.6 g of sodium iodide (NaI) in each 100 mL.

Iodine	20 g
Sodium Iodide	24 g
Purified Water, a sufficient quantity to make	1000 mL

Dissolve *Iodine* and *Sodium Iodide* in 50 mL of *Purified Water*, then add sufficient *Purified Water* to bring to final volume.

IDENTIFICATION• **A.**

Analysis: Add 1 drop to a mixture of 1 mL of starch TS and 9 mL of water.

Acceptance criteria: A deep blue color is produced.

Change to read:• **B. Identification Tests—General** (191), *Iodide* and ~~*Sodium*~~

▲▲USP40

Sample: Evaporate a few mL on a steam bath to dryness.

Acceptance criteria: The residue meets the requirements of the test for *Iodide*. and the flame test for ~~*Sodium*~~.

▲▲USP40

Add the following:▲ • **C. Sodium**

Acceptance criteria: The *Sample* obtained in *Identification B* imparts an intense yellow color to a nonluminous flame. ▲USP40

ASSAY• **Iodine**

Sample: 10.0 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a glass-stoppered 500-mL flask, and dilute with 10 mL of water. Titrate with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Each mL of *Titrant* is equivalent to 12.69 mg of iodine (I).

Acceptance criteria: 1.8–2.2 g in 100 mL of Topical Solution

• **Sodium Iodide**

Sample: 10.0 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.05 M potassium iodate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a glass-stoppered 500-mL flask, add 30 mL of water and 50 mL of hydrochloric acid, cool to room temperature, and titrate with *Titrant* until the dark brown solution that is produced becomes pale brown. Add 1 mL of amaranth TS, and continue the titration slowly until the red color just changes to yellow. The difference in volume, in mL, between the *Titrant* used and half the volume of 0.1 N sodium thiosulfate VS used in the *Assay for Iodine*, multiplied by 14.99, represents the number of mg of sodium iodide (NaI) in the portion of Topical Solution taken.

Acceptance criteria: 2.1–2.6 g in 100 mL of Topical Solution

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant containers. Store at a temperature not exceeding 35°.

BRIEFING

Iodine Tincture, *USP 38* page 3891. In preparation for the omission of the flame tests from *Identification Tests—General* (191), proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to delete the reference to *Sodium* under *Identification* test *B*, and include a complete description of the flame test in the monograph.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CMP: J. Sun.)

Correspondence Number—C164384

Comment deadline: January 31, 2016

Iodine Tincture

DEFINITION

Iodine Tincture contains NLT 1.8 g and NMT 2.2 g of iodine (I) and NLT 2.1 g and NMT 2.6 g of sodium iodide (NaI) in each 100 mL.

Prepare Iodine Tincture as follows.

Iodine	20 g
Sodium Iodide	24 g
Alcohol	500 mL
Purified Water, a sufficient quantity to make	1000 mL

Dissolve *Iodine* and *Sodium Iodide* in *Alcohol*. Add *Purified Water* to bring to final volume.

IDENTIFICATION

- **A.**

Analysis: Add 1 drop to a mixture of 1 mL of starch TS and 9 mL of water.

Acceptance criteria: A deep blue color is produced.

Change to read:

● **B. Identification Tests—General** (191), *Iodide and Sodium*

▲▲USP40

Sample: Evaporate a few mL on a steam bath to dryness.

Acceptance criteria: The residue meets the requirements of the test for *Iodide*. and the flame test for *Sodium*

▲▲USP40

Add the following:

- ▲● **C. Sodium:** The *Sample* obtained in *Identification B* imparts an intense yellow color to a nonluminous flame.▲USP40

ASSAY

● **Iodine**

Sample: 10 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a glass-stoppered 500-mL flask, and dilute with 10 mL of water. Titrate with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Each mL of *Titrant* is equivalent to 12.69 mg of iodine (I).

Acceptance criteria: 1.8–2.2 g in 100 mL of Tincture

● **Sodium Iodide**

Sample: 10 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.05 M potassium iodate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a glass-stoppered 500-mL flask, add 30 mL of water and 50 mL of hydrochloric acid, cool to room temperature, and titrate with *Titrant* until the dark brown solution that is produced becomes pale brown. Add 1 mL of amaranth TS, and continue the titration slowly until the red color just changes to yellow. The difference in volume, in mL, between the *Titrant* used and half the volume of the 0.1 N sodium thiosulfate used in the *Assay* for *Iodine*, multiplied by 14.99, represents the number of mg of sodium iodide (NaI) in the portion of Tincture taken.

Acceptance criteria: 2.1–2.6 g in 100 mL of Tincture

OTHER COMPONENTS

- **Alcohol Determination** (611): 44.0%–50.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight containers.

BRIEFING

Strong Iodine Tincture, *USP 38* page 3891. In preparation for the omission of the flame tests from *Identification Tests—General* (191), proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to delete the reference to *Potassium* under *Identification* test *B*, and include a complete description of the flame test in the monograph.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CMP: J. Sun.)

Correspondence Number—C164384

Comment deadline: January 31, 2016

Strong Iodine Tincture

DEFINITION

Strong Iodine Tincture contains NLT 6.8 g and NMT 7.5 g of iodine (I) and NLT 4.7 g and NMT 5.5 g of potassium iodide (KI) in each 100 mL.

Prepare Strong Iodine Tincture as follows.

Potassium Iodide	50 g
Iodine	70 g
Purified Water	50 mL
Alcohol, a sufficient quantity to make	1000 mL

Dissolve *Potassium Iodide* in *Purified Water*. Add *Iodine* and agitate until the solution is effected. Add *Alcohol* to bring to final volume.

IDENTIFICATION

- **A.**

Analysis: Add 1 drop to a mixture of 1 mL of starch TS and 9 mL of water.

Acceptance criteria: A deep blue color is produced.

Change to read:

- **B. Identification Tests—General** (191), *Potassium* and

▲▲*USP40*

Iodide

Analysis: Evaporate a few mL on a steam bath to dryness.

Acceptance criteria: The residue meets the requirements of ~~the flame test for *Potassium*~~

and of

▲▲USP40

the test for *Iodide*.

Add the following:

▲ ● **C. Potassium**

Acceptance criteria: The *Sample* obtained in *Identification B* imparts a violet color to a nonluminous flame. The presence of small quantities of sodium masks the color unless the yellow color produced by sodium is screened out by viewing through a blue filter that blocks the emission at 589 nm (sodium). It is transparent to the emission at 404 nm (potassium).

[Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

▲USP40

ASSAY

● **Iodine**

Sample: 10 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a glass-stoppered 500-mL flask and add 10 mL of water. Titrate with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Each mL of *Titrant* is equivalent to 12.69 mg of iodine (I).

Acceptance criteria: 6.8–7.5 g of iodine (I) in 100 mL

● **Potassium Iodide**

Sample: 10 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.05 M potassium iodate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a glass-stoppered 500-mL flask, add 30 mL of water and 50 mL of hydrochloric acid, cool to room temperature, and titrate with *Titrant* until the dark brown solution that is produced becomes pale brown. Add 1 mL of amaranth TS, and continue the titration slowly until the red color just changes to yellow. The difference between the volume of *Titrant* used and half the volume of 0.1 N sodium thiosulfate used in the *Assay for Iodine*, in mL, multiplied by 16.60, represents the number of mg of potassium iodide (KI) in the portion of Tincture taken.

Acceptance criteria: 4.7–5.5 g of potassium iodide (KI) in 100 mL

OTHER COMPONENTS

- **Alcohol Determination** (611): 82.5%–88.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant containers.

BRIEFING

Iothalamate Sodium Injection, *USP 38* page 3922. It is proposed to omit this monograph for the following reasons:

1. This dosage form is not used for human use in the United States.
2. This dosage form is currently not used in veterinary medicine in the United States.

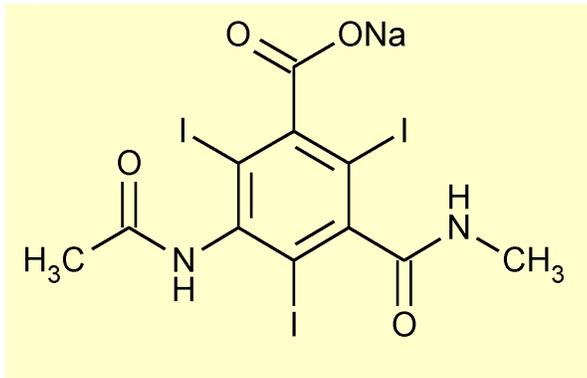
(CHM4: R. Ravichandran.)

Correspondence Number—C162942

Comment deadline: January 31, 2016

Delete the following:

▲ **Iothalamate Sodium Injection**



$C_{11}H_8I_3N_2NaO_4$

635.90

Benzoic acid, 3-(acetamino)-2,4,6-triiodo-5-[(methylamino)carbonyl]-, monosodium salt.
Monosodium 5-acetamido-2,4,6-triiodo-N-methylisophthalamate [[1225-20-3]]:

» Iothalamate Sodium Injection is a sterile solution of Iothalamic Acid in Water for Injection prepared with the aid of Sodium Hydroxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of iothalamate sodium ($C_{11}H_8I_3N_2NaO_4$). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium or Edetate Disodium as a stabilizer. Iothalamate Sodium Injection intended for intravascular use contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light.

Labeling—Label containers of the Injection intended for intravascular injection to direct the

user to discard any unused portion remaining in the container. Label containers of the Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.

~~USP Reference standards (11)~~

~~USP 5-Amino-2,4,6-triiodo-N-methylisophthalamic Acid RS~~

~~C₉H₇I₃N₂O₃ 571.88~~

~~USP Endotoxin RS~~

~~USP Iothalamic Acid RS~~

Identification

A: Dilute 3 mL of Injection with water to 100 mL, add an excess of 3-N hydrochloric acid, and filter. Wash the precipitated iothalamic acid with four 10-mL portions of water, and dry at 105° for 4 hours: the dried iothalamic acid responds to *Identification* tests A and B under *Iothalamate Meglumine Injection*.

B: It responds to the flame test for *Sodium* (191).

~~Bacterial endotoxins (85)~~—It contains not more than 3.35 USP Endotoxin Units per mL.

~~pH (791):~~ between 6.5 and 7.7.

Free aromatic amine—Dilute a suitable volume of Injection with water to yield a solution containing 100 mg of iothalamate sodium per mL. Proceed as directed in the test for *Free aromatic amine* under *Iothalamic Acid*, beginning with "Pipet 5 mL of this solution into a 50-mL volumetric flask."

Iodine and iodide—Dilute a volume of Injection, equivalent to about 2 g of iothalamate sodium, with 20 mL of water in a 50-mL beaker, add 5 mL of 2-N sulfuric acid, stir, and filter into a glass-stoppered, 50-mL cylinder. Proceed as directed for *Procedure* in the test for *Iodine and iodide* under *Iothalamic Acid*, beginning with "To the filtrate add 5 mL of toluene."

Heavy metals (231)—In a 50-mL color-comparison tube, mix a volume of Injection, equivalent to 1.0 g of iothalamate sodium, with 5 mL of 1-N sodium hydroxide, dilute with water to 40 mL, and mix. Using this as the *Test preparation*, proceed as directed in the test for *Heavy metals* under *Diatrizoate Meglumine*: the limit is 0.002%.

Other requirements—It meets the requirements under *Injections* (1).

Assay—Proceed with Injection as directed in the *Assay* under *Iothalamate Meglumine Injection*. Each mL of 30.05-N silver nitrate is equivalent to 10.60 mg of C₁₁H₈I₃N₂NaO₄. ▲USP40

BRIEFING

Iron Sucrose Injection, USP 38 page 3944. On the basis of comments received, it is proposed to revise the final concentration of the Injection after dilution in the *Labeling* statement from 0.5–2.0 mg/mL of elemental iron to 1.0–2.0 mg/mL of elemental iron to be consistent with the FDA-approved label content.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM2: S. Ramakrishna.)

Correspondence Number—C116655

Comment deadline: January 31, 2016

Iron Sucrose Injection

DEFINITION

Iron Sucrose Injection is a sterile, colloidal solution of ferric hydroxide in complex with Sucrose in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of iron. Sodium Hydroxide may be added to adjust the pH. It contains no antimicrobial agent, chelating agent, dextran, gluconate, or other added substances.

IDENTIFICATION

- **A. Iron**

To 2.5 mL of Injection add 17.5 mL of water and 5 mL of hydrochloric acid. Mix and heat the solution for 5 min in a boiling water bath. Cool, add dropwise 13.5 N ammonium hydroxide until no further precipitation of ferric hydroxide occurs, and filter. Wash the precipitate with water to remove excess ammonium hydroxide, dissolve the precipitate in a minimum volume of 2 N hydrochloric acid, and add sufficient water to make a volume of 20 mL. To 3 mL of the solution add 1 mL of 2 N hydrochloric acid and 1 mL of potassium thiocyanate TS: the resulting solution (Solution 1) is red. To 1 mL of Solution 1 add 5 mL of amyl alcohol or ethyl ether, shake, and allow to stand: the organic layer is pink. To a separate 1-mL aliquot of Solution 1 add 2 mL of mercuric chloride TS: a red color is discharged [iron (III) salts].

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Sucrose*.

- **C. Molecular Weight Determination**

Mobile phase: Dissolve 7.12 g of dibasic sodium phosphate dihydrate, 5.52 g of monobasic sodium phosphate, and 0.40 g of sodium azide in 2 L of water.

System suitability solution: Dissolve 200 mg of high molecular weight dextran and 100 mg of glucose in 20 mL of *Mobile phase*.

Standard solutions: Transfer about 20 mg of each polysaccharide molecular weight standard (5,000–400,000 Da) to separate 5-mL volumetric flasks. Add 4 mL of *Mobile phase* to each flask, and allow each aliquot to stand at or below 25° for a minimum of 12 h. After the agglomerate particles of each *Standard solution* have swelled to their fullest extent, gently swirl each *Standard solution* until dissolved.

[Note—The chromatograms of freshly prepared *Standard solutions* regularly show a small, unidentified secondary peak following the main peak. Discard the *Standard solutions* if the secondary peak reaches half the height of the main peak.]

Sample solution: Transfer 5.0 mL of Injection to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: Refractive index, maintained at a constant temperature of 45°

Columns: Two 7.8-mm × 30-cm; packing L39 with pore sizes of 1000 and 120 Å, respectively

Column temperature: $45 \pm 2^\circ$

Flow rate: 0.5 mL/min

Injection volume: 25 μ L

System suitability

Samples: *System suitability solution* and *Standard solutions*

Suitability requirements

Resolution: NLT 4.0 between dextran and glucose, *System suitability solution*

Correlation coefficient: NLT 0.98 for the calibration curve generated using a suitable program, plotting the retention times of the *Standard solutions* and their molecular weights to generate a third order (cubic) calibration curve

Analysis

Samples: *System suitability solution*, *Standard solutions*, and *Sample solution*

The molecular weight of the complex is calculated from the calibration curve. The molecular weight distribution curve of the sample is sliced into fractions. Calculate the weight-average molecular weight (M_W) as follows:

$$\text{Result} = \Sigma(A_T M_T) / \Sigma A_T$$

Calculate the number-average molecular weight (M_N) as follows:

$$\text{Result} = \Sigma(A_T) / \Sigma(A_T / M_T)$$

A_T = area of each fraction of the sample distribution

M_T = corresponding mean molecular weight of each fraction as determined from its retention time on the calibration curve

Acceptance criteria: The molecular weight distribution curve of the Injection conforms to the following parameters.

M_W : 34,000–60,000 Da

M_N : NLT 24,000 Da

M_W/M_N : NMT 1.7

ASSAY

• Sucrose

Mobile phase: Acetonitrile and water (79:21)

Standard solutions: Individual solutions of 13, 16, 18, 21, and 23 mg/mL of sucrose from USP Sucrose RS, in water

Sample solution: Transfer about 1.875 g of Injection to a 25-mL flask. Add 1.25 mL of water and mix. Add 1.25 mL of a monobasic sodium phosphate solution, prepared by dissolving 30 g in 50 mL, and mix. Allow the resulting solution to stand for 10 min to precipitate the colloidal ferric hydroxide. Dilute with water to volume. Centrifuge this solution at 3000 rpm for 15 min. Pass the resulting solution through a filter, discarding the first 2 mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 4-mm × 25-cm; packing L8

Temperatures

Detector: 20–25° (±2°)

Column: 20–25° (±2°)

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solutions*

[Note—The retention time for sucrose is about 8 min.]

Suitability requirements

Correlation coefficient: NLT 0.998 from the linear regression of the *Standard solutions*

Analysis

Samples: *Standard solutions* and *Sample solution*

Plot the peak area for each *Standard solution* versus concentration of sucrose in mg/mL, and draw the straight line best fitting the five plotted points. From the graph, determine the concentration of sucrose, in mg/mL, in the *Sample solution*.

Calculate the quantity of sucrose, in mg, in each mL of Injection taken:

$$\text{Result} = (C_U \times D \times G)/W$$

C_U = concentration of sucrose in the *Sample solution* (mg/mL)

D = dilution volume of the *Sample solution* (mL)

G = density of Injection taken (g/mL)

W = weight of Injection taken (g)

Acceptance criteria: 260–340 mg/mL

Change to read:

- **Iron**

Solution A: Transfer 2.64 g of calcium chloride to a 1000-mL volumetric flask, add 500 mL of water, and swirl to dissolve. Add 5.0 mL of hydrochloric acid, and dilute with water to volume.

Standard stock solution: 50 µg/mL of iron prepared as follows. Transfer about 350 mg of ferrous ammonium sulfate to a 1000-mL volumetric flask. Add water to dissolve, dilute with water to volume, and mix.

Standard solutions: Individual solutions containing 2.0, 4.0, 6.0, 8.0, and 10.0 µg/mL of iron in *Solution A* from the *Standard stock solution*

Sample stock solution: Using a “to contain” pipet, transfer 2.0 mL of Injection to a 100-

mL volumetric flask. Rinse the pipet several times with *Solution A*. Add 5 mL of hydrochloric acid, and swirl until the solution turns yellow. After the solution has cooled to room temperature, dilute with *Solution A* to volume, and mix.

Sample solution: Nominally 8.0 µg/mL of iron prepared as follows. Pipet 2.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with *Solution A* to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 248.3 nm iron emission line

Lamp: Iron hollow-cathode

Flame: Air-acetylene

Blank: *Solution A*

Analysis

Samples: *Standard solutions* and *Sample solution*

Plot the absorbance of each *Standard solution* versus concentration, in µg/mL, of iron, and draw the straight line best fitting the five plotted points. From the graph, determine the concentration, in µg/mL, of iron in the *Sample solution*.

Calculate the quantity, in mg, of iron in each mL of the Injection taken:

$$\text{Result} = 5C/V$$

C = concentration of iron in the *Sample solution* (µg/mL)

V = volume of Injection taken

▲ Calculate the percentage of the labeled amount of iron in each mL of Injection taken:

$$\text{Result} = (C_A/C_U) \times 100$$

C_A = actual concentration of iron in the *Sample solution* determined from the calibration curve (µg/mL)

C_U = nominal concentration of iron in the *Sample solution* (µg/mL) ▲USP40

Acceptance criteria: 95.0%–105.0%

OTHER COMPONENTS

• Content of Chloride

Sample: About 12 g of Injection

Analysis: Transfer the *Sample* to a 50-mL beaker. Add 40 mL of water and 0.3 mL of 65% nitric acid, and, while stirring, titrate with 0.01 N silver nitrate VS, determining the endpoint potentiometrically with silver-glass electrodes.

Calculate the chloride content, in mg, of Injection taken. Each mL of 0.01 N silver nitrate consumed is equal to 0.3545 mg of chloride (Cl).

Acceptance criteria: 0.012%–0.025%

IMPURITIES

- **Limit of Iron [Fe(II)]**

Supplementary electrolyte solution: Dissolve 15.0 g of sodium acetate in 100 mL of water and adjust with 0.1 N acetic acid to a pH of 7.0.

Sample solution: Volume of Injection equivalent to 20–120 µg/mL of elemental iron

Analysis: Transfer a suitable amount of *Supplementary electrolyte solution* to a polarographic cell equipped with a mercury drop electrode. With the electrode submerged in the liquid, bubble nitrogen through the liquid for 5 min. Avoiding any undue exposure to air, immediately transfer the *Sample solution* to the polarographic cell. The sample must be analyzed immediately upon opening the container.

Record the polarogram from 0 mV and –1700 mV. The iron [Fe(III)/Fe(II)] peak is detected at -750 ± 50 mV and the iron [Fe(II)/Fe(0)] peak is detected at -1400 ± 50 mV. Measure the iron [Fe(II)/Fe(III)] peak responses obtained from the polarogram, and perform a blank determination.

Calculate the Fe(II) content, in % w/v, in the volume of Injection taken:

$$\text{Result} = [1 - (2/R)] \times C_T$$

R = peak response ratio of iron [Fe(II)]/iron [Fe(III)]

C_T = total iron concentration of the Injection (% w/v)

Acceptance criteria: NMT 0.4%

SPECIFIC TESTS

- **pH (791):** 10.5–11.1 at 20°

- **Turbidity**

Sample solution: Transfer 0.5 g of Injection to a 150-mL beaker. Add 100 mL of water and, with constant stirring, adjust with 0.1 N hydrochloric acid VS to a pH of 6.0.

Analysis: Remove the pH electrode from the solution. Adjust a light source such that the beam hits the beaker at a parallel angle 2 cm below the surface of the liquid. The light must shine through to the surface, and the solution must not have any turbidity. Measurement must be carried out in a room as dark as possible. Slowly add 0.1 N hydrochloric acid VS, dropwise, until a slight but lasting turbidity develops. Record the pH of the solution as the turbidity point of the Injection.

Acceptance criteria: 4.4–5.3

- **Absence of Low-Molecular Weight Iron [Fe(II) and Fe(III)] Complexes:** In the polarograms obtained in the test for *Limit of Iron [Fe(II)]*, no additional peaks are found.

- **Alkalinity**

Sample solution: 5 mL of Injection

Analysis: Titrate the *Sample solution* with 0.1 N hydrochloric acid VS with constant stirring to a pH of 7.4. Record the volume of 0.1 N hydrochloric acid VS consumed, and calculate the alkalinity of the Injection as the volume of acid, in mL, consumed per mL of Injection.

Acceptance criteria: 0.5–0.8 mL of 0.1 N hydrochloric acid VS is consumed per mL of Injection.

- **Osmolality and Osmolarity** (785), *Osmolarity*
Sample solution: Dilute Injection in water (1 in 10).
Acceptance criteria: 1150–1350 mOsmol/L
- **Specific Gravity** (841): 1.135–1.165 at 20°
- **Particulate Matter in Injections** (788), *Method 1 Light Obscuration Particle Count Test*
Sample solution: Prepare a solution of Injection (1 in 40) using water that has been passed through a filter having a 1.2- μm or finer pore size.
Acceptance criteria: Meets the requirements for small-volume injections
- **Bacterial Endotoxins Test** (85): NMT 3.7 USP Endotoxin Units/mg of iron contained in the Iron Sucrose Injection
- **Other Requirements:** Meets the requirements in *Injections* (1)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose containers of Type I glass. Store at controlled room temperature. Do not freeze.

Change to read:

- **Labeling:** Label it to indicate that it is for intravenous use only, and that when administered by intravenous infusion, the Injection must be diluted with 0.9% Sodium Chloride Injection to a concentration of 0.5
▲ 1.0 ▲ USP40
–2.0 mg/mL of elemental iron. Label it also to state the total osmolality of the solution expressed in mOsmol/L.
- **USP Reference Standards** (11)
USP Endotoxin RS
USP Sucrose RS

BRIEFING

Ixabepilone. Because there is no existing *USP* monograph for this drug substance, a new monograph based on validated methods of analysis is proposed. The liquid chromatographic procedure for the *Assay* and test for *Organic Impurities* is based on analyses performed with the Phenomenex Gemini C18 brand of L1 column. The typical retention time for ixabepilone is about 8.5 min.

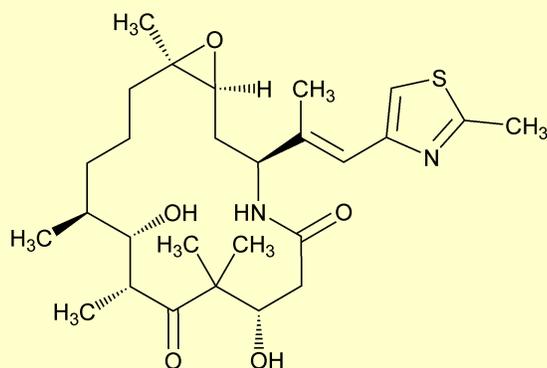
(CHM3: F. Mao.)

Correspondence Number—C139175

Comment deadline: January 31, 2016

Add the following:

▲ **Ixabepilone**



$C_{27}H_{42}N_2O_5S$ 506.70

17-Oxa-4-azabicyclo[14.1.0]heptadecane-5,9-dione, 7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[(1*E*)-1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-, (1*S*,3*S*,7*S*,10*R*,11*S*,12*S*,16*R*)-; (1*S*,3*S*,7*S*,10*R*,11*S*,12*S*,16*R*)-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[(*E*)-1-(2-methylthiazol-4-yl)prop-1-en-2-yl]-17-oxa-4-azabicyclo[14.1.0]heptadecane-5,9-dione. [219989-84-1].

DEFINITION

Ixabepilone contains NLT 97.5% and NMT 102.0% of ixabepilone ($C_{27}H_{42}N_2O_5S$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** (197)
[Note—Methods described in (197K) or (197A) may be used.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• Procedure

Buffer stock solution: 100 mM *tris(hydroxymethyl)aminomethane* in water prepared as follows. Dissolve 12.1 g of *tris(hydroxymethyl)aminomethane* in 1 L of water and adjust to a pH of 8.5.

Buffer: 5 mM *tris(hydroxymethyl)aminomethane* in water, from *Buffer stock solution*

Solution A: *Acetonitrile* and *Buffer* (10:90)

Solution B: *Acetonitrile* and *Buffer* (90:10)

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for 6 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
2.5	70	30
10	60.6	39.4
20	35	65
22	12.5	87.5
25	12.5	87.5

Standard solution: 1.0 mg/mL of USP Ixabepilone RS in *acetonitrile*. Sonicate to assist the dissolution.

Sample solution: 1.0 mg/mL of Ixabepilone in *acetonitrile*. Sonicate to assist the dissolution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; 5-μm packing *L1*

Autosampler temperature: 4°

Flow rate: 1.8 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 1.0% for six injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ixabepilone (C₂₇H₄₂N₂O₅S) in the portion of Ixabepilone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ixabepilone RS in the *Standard solution* (mg/mL)

C_U = concentration of Ixabepilone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.0% on the anhydrous basis

IMPURITIES

- **Organic Impurities**

Mobile phase, Standard solution, Sample solution, and Chromatographic system:

Proceed as directed in the *Assay*.

System suitability solution: 1.0 mg/mL of USP Ixabepilone System Suitability Mixture RS in *acetonitrile*. Sonicate to assist the dissolution.

Sensitivity solution: 0.3 µg/mL of USP Ixabepilone RS in *acetonitrile* from *Standard solution*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.0 between ixabepilone oxazine analog and desmethyl ixabepilone peaks, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Ixabepilone taken:

$$\text{Result} = r_U / \{ \Sigma [r_U \times (1/F)] + r_T \} \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

F = relative response factor for each individual impurity (see *Table 2*)

r_T = peak response of ixabepilone from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.03%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT(%)
Ixabepilone diol	0.47	1.0	0.15
Ixabepilone oxazine analog	0.79	1.0	0.15
Desmethyl ixabepilone	0.85	1.0	0.4
Ixabepilone	1.0	—	—
(Z)-Ixabepilone	1.19	0.83	0.15
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.8

SPECIFIC TESTS

- **Water Determination** (921), *Method 1c*: NMT 0.2%
- **Bacterial Endotoxins Test** (85): It contains NMT 0.32 USP Endotoxin Units/mg of ixabepilone.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Protect from light.

- **USP Reference Standards** <11>

USP Endotoxin RS

USP Ixabepilone RS

USP Ixabepilone System Suitability Mixture RS

It contains ixabepilone and small amounts of:

Ixabepilone diol;

(4*S*,7*R*,8*S*,9*S*,13*R*,14*R*,16*S*)-4,8,13,14-Tetrahydroxy-5,5,7,9,13-pentamethyl-16-[(*E*)-1-(2-methylthiazol-4-yl)prop-1-en-2-yl]azacyclohexadecane-2,6-dione.

C₂₇H₄₄N₂O₆S 524.72

Ixabepilone oxazine analog;

(1*R*,2*R*,6*S*,7*S*,8*R*,11*S*,15*S*)-2,7,11-Trihydroxy-2,6,8,10,10-pentamethyl-15-[(*E*)-1-(2-methylthiazol-4-yl)prop-1-en-2-yl]-17-oxa-14-azabicyclo[11.3.1]heptadec-13-en-9-one.

C₂₇H₄₂N₂O₅S 506.70

Desmethyl ixabepilone;

(1*S*,3*S*,7*S*,10*R*,11*S*,12*S*,16*R*)-7,11-Dihydroxy-8,8,10,12-tetramethyl-3-[(*E*)-1-(2-methylthiazol-4-yl)prop-1-en-2-yl]-17-oxa-4-azabicyclo[14.1.0]heptadecane-5,9-dione.

C₂₆H₄₀N₂O₅S 492.68

(*Z*)-Ixabepilone;

(1*S*,3*S*,7*S*,10*R*,11*S*,12*S*,16*R*)-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[(*Z*)-1-(2-methylthiazol-4-yl)prop-1-en-2-yl]-17-oxa-4-azabicyclo[14.1.0]heptadecane-5,9-dione.

C₂₇H₄₂N₂O₅S 506.70

[Note—It may contain other impurities.]

▲USP40

BRIEFING

Methylprednisolone Acetate, USP 38 page 4353. As part of USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Replace the liquid chromatographic procedure that requires the use of tetrahydrofuran in the test for *Organic Impurities* with a chromatographic procedure that specifies and identifies impurities. The liquid chromatographic procedure was validated using the Phenomenex Kinetex phenyl-hexyl brand of L11 column. The typical retention time for methylprednisolone acetate is about 15 min.
2. Replace the current HPLC procedure in the *Assay* that requires the use of an internal standard in chloroform with the same chromatographic parameters as those in the proposed test for *Organic Impurities*.
3. Revise *Identification* test A for flexible IR sample preparations.
4. Replace *Identification* test B based on UV absorption with the retention time agreement in the proposed *Assay*.
5. Revise the storage condition in the *Packaging and Storage* section to be consistent with the description in *Packaging and Storage Requirements* <659>.
6. Add USP Dexamethasone Acetate RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

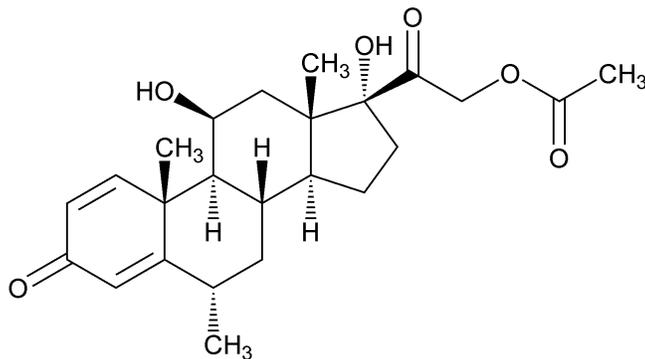
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM5: D. Min.)

Correspondence Number—C154743

Comment deadline: January 31, 2016

Methylprednisolone Acetate



$C_{24}H_{32}O_6$ 416.51

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-11,17-dihydroxy-6-methyl-, (6 α ,11 β)-; 11 β ,17,21-Trihydroxy-6 α -methylpregna-1,4-diene-3,20-dione 21-acetate [53-36-1].

DEFINITION

Methylprednisolone Acetate contains NLT 97.0% and NMT 103.0% of methylprednisolone acetate ($C_{24}H_{32}O_6$), calculated on the dried basis.

IDENTIFICATION

Delete the following:

~~▲ ● A. Infrared Absorption (197K) ▲_{USP40}~~

Add the following:

▲ ● A. Infrared Absorption (197): [Note—Methods described in (197K) or (197A) may be used.] ▲_{USP40}

Delete the following:

~~▲ ● B. Ultraviolet Absorption (197U)~~

~~Analytical wavelength: 243-nm~~

~~Standard solution: 10 μ g/mL of USP Methylprednisolone Acetate RS in alcohol~~

~~Sample solution: 10 μ g/mL of Methylprednisolone Acetate in alcohol~~

~~Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%. ▲_{USP40}~~

Add the following:

▲ ● B. The retention time of the major peak of the *Sample solution* corresponds to that of the

Standard solution, as obtained in the Assay. ▲USP40

ASSAY

Change to read:

• Procedure

Mobile phase: ~~*n-Butyl chloride*, water saturated *n-butyl chloride*, *tetrahydrofuran*, *methanol*, and *glacial acetic acid* (95:95:14:7:6)~~

Internal standard solution: ~~6 mg/mL of prednisone prepared as follows. Transfer an appropriate amount of prednisone to a suitable volumetric flask. Add 3% of the flask volume of *glacial acetic acid*, and sonicate. Dilute with *chloroform* to volume, slowly adding the *chloroform*. Sonicate, and shake to dissolve.~~

Standard solution: ~~0.2 mg/mL of USP Methylprednisolone Acetate RS prepared as follows. Transfer an appropriate amount of USP Methylprednisolone Acetate RS to a suitable volumetric flask, and add 5% of the flask volume of the *Internal standard solution*. Dilute with *chloroform* to volume, and shake to dissolve.~~

Sample solution: ~~0.2 mg/mL of Methylprednisolone Acetate prepared as follows. Transfer an appropriate amount of Methylprednisolone Acetate to a suitable volumetric flask, and add 5% of the flask volume of the *Internal standard solution*. Dilute with *chloroform* to volume, and shake to dissolve.~~

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: ~~LC~~

Detector: ~~UV 254 nm~~

Column: ~~4 mm × 25 cm; packing L3~~

Flow rate: ~~1 mL/min~~

Injection volume: ~~10 µL~~

System suitability

Sample: ~~*Standard solution*~~

[Note—The relative retention times for methylprednisolone acetate and prednisone are about 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: ~~NLT 2.5 between methylprednisolone acetate and prednisone~~

Relative standard deviation: ~~NMT 2.0%~~

Analysis

Samples: ~~*Standard solution* and *Sample solution*~~

Calculate the percentage of methylprednisolone acetate ($C_{24}H_{32}O_6$) in the portion of Methylprednisolone Acetate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak height ratio of methylprednisolone acetate to prednisone from the *Sample solution*

R_S = peak height ratio of methylprednisolone acetate to prednisone from the *Standard solution*

C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Methylprednisolone Acetate in the *Sample solution* (mg/mL)

▲ Solution A: Formic acid and water (0.2: 99.8)

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
3	70	30
22	62	38
27	20	80
34	20	80
35	70	30
40	70	30

Diluent: Solution A and Solution B (50:50)

Standard solution: 0.1 mg/mL of USP Methylprednisolone Acetate RS in *Diluent*

Sample solution: 0.1 mg/mL of Methylprednisolone Acetate in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 2.6-µm packing L11

Temperatures

Autosampler: 5°

Column: 45°

Flow rate: 0.8 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.10%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylprednisolone acetate ($C_{24}H_{32}O_6$) in the portion of Methylprednisolone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methylprednisolone acetate from the *Sample solution*

r_S = peak response of methylprednisolone acetate from the *Standard solution*

C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Methylprednisolone Acetate in the *Sample solution* (mg/mL)

▲USP40

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.2%

Change to read:

- **Organic Impurities**

Mobile phase: ~~Tetrahydrofuran and water (51:149)~~

Diluent: ~~Tetrahydrofuran, acetonitrile, glacial acetic acid, and water (250:250:1:499)~~

Standard solution: ~~20 µg/mL of USP Methylprednisolone Acetate RS in Diluent. Sonicate, if necessary, to dissolve.~~

Sample solution: ~~1 mg/mL of Methylprednisolone Acetate in Diluent. Sonicate, if necessary, to dissolve.~~

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: ~~LC~~

Detector: ~~UV 254 nm~~

Column: ~~4.6 mm × 25 cm; packing L1~~

Flow rate: ~~1 mL/min~~

Injection volume: ~~20 µL~~

System suitability

Sample: ~~Standard solution~~

Suitability requirement

Relative standard deviation: ~~NMT 5.0%~~

Analysis

Samples: ~~Standard solution and Sample solution~~

Calculate the percentage of each impurity in the portion of Methylprednisolone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response for methylprednisolone acetate from the *Standard solution*

C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Methylprednisolone Acetate in the *Sample solution* (mg/mL)

Acceptance criteria

Any individual impurity: NMT 1.0%

Total impurities: NMT 2.0%

▲ Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system:

Proceed as directed in the *Assay*.

Standard solution: 0.5 µg/mL each of USP Methylprednisolone Acetate RS and USP Dexamethasone Acetate RS in *Diluent*

Sample solution: 500 µg/mL of Methylprednisolone Acetate in *Diluent*

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between the methylprednisolone acetate and dexamethasone acetate peaks

Relative standard deviation: NMT 3.0% for methylprednisolone acetate

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified impurity in the portion of Methylprednisolone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*

r_S = peak response of methylprednisolone acetate from the *Standard solution*

C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (µg/mL)

C_U = concentration of Methylprednisolone Acetate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methylprednisolone acetate	1.0	—
Dexamethasone acetate ^a	1.1	—
Any individual unspecified impurity	—	1.0
Total impurities	—	2.0
^a Used only for resolution measurement and not to be included in the calculation of total impurities.		

▲USP40

SPECIFIC TESTS

- **Optical Rotation** (781S), *Procedures, Specific Rotation*

Sample solution: 10 mg/mL in *dioxane*

Acceptance criteria: +97° to +105°

- **Loss on Drying** (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at 25°₇, excursions permitted between 15° and 30°
▲ controlled room temperature. ▲USP40

Change to read:

- **USP Reference Standards** (11)

▲ USP Dexamethasone Acetate RS

9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-acetate.

C₂₄H₃₁FO₆ 434.51 ▲USP40

USP Methylprednisolone Acetate RS

BRIEFING

Methylprednisolone Acetate Injectable Suspension, USP 38 page 4354. As part of the USP monograph modernization efforts, it is proposed to revise the monograph as follows:

1. Add a new liquid chromatographic procedure in the test for *Organic Impurities*. The HPLC procedure was validated using the Phenomenex Kinetex phenyl-hexyls brand of L11 column. The typical retention time for methylprednisolone acetate is about 15 min.
2. Replace the current HPLC procedure in the *Assay* that requires the use of an internal standard in chloroform with a chromatographic procedure using the same parameters as those in the proposed test for *Organic Impurities*.
3. Revise *Identification* test A for flexible IR sample preparations.
4. Add *Identification* test B based on the retention time agreement in the proposed *Assay*.
5. Add additional requirements in the *Packaging and Storage* section based on the

information from the drug product packaging insert.

6. Add USP Dexamethasone Acetate RS to the *USP Reference Standards* section to support the proposed test for *Organic Impurities*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM5: D. Min.)

Correspondence Number—C158404

Comment deadline: January 31, 2016

Methylprednisolone Acetate Injectable Suspension

DEFINITION

Methylprednisolone Acetate Injectable Suspension is a sterile suspension of Methylprednisolone Acetate in a suitable aqueous medium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of methylprednisolone acetate ($C_{24}H_{32}O_6$).

IDENTIFICATION

Delete the following:

▲ ● A. Infrared Absorption ~~(197K)~~

Sample: Nominally 100 mg of methylprednisolone acetate from Injectable Suspension

Analysis: Filter the *Sample* through paper. Wash the residue with several 5-mL portions of water, and dry at 105° for 3 h.

Acceptance criteria: Meets the requirements ▲*USP40*

Add the following:

▲ ● A. Infrared Absorption (197)

[Note—Methods described in (197K) or (197A) may be used.]

Sample: Nominally 100 mg of methylprednisolone acetate from Injectable Suspension

Analysis: Filter the *Sample* through paper. Wash the residue with several 5-mL portions of water, and dry at 105° for 3 h.

Acceptance criteria: Meets the requirements ▲*USP40*

Add the following:

- ▲ ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP40*

ASSAY

Change to read:

- **Procedure**

Mobile phase: ~~*n*-Butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)~~

Internal standard solution: ~~6 mg/mL of prednisone prepared as follows. Transfer an appropriate amount of prednisone to a suitable volumetric flask. Add 3% of the flask volume of *glacial acetic acid*, and sonicate. Dilute with *chloroform* to volume, slowly adding the *chloroform*. Sonicate, and shake to dissolve.~~

Standard solution: ~~0.2 mg/mL of USP Methylprednisolone Acetate RS prepared as follows. Transfer an appropriate amount of USP Methylprednisolone Acetate RS to a suitable volumetric flask, and add 5% of the flask volume of the *Internal standard solution*. Dilute with *chloroform* to volume, and shake to dissolve.~~

Sample solution: ~~Swirl Injectable Suspension to ensure uniformity before analysis. Transfer a suitable quantity of Injectable Suspension equivalent to 40 mg of methylprednisolone acetate to a 25 mL volumetric flask, add 10.0 mL of the *Internal standard solution*, dilute with *chloroform* to volume, and shake for 15 min or until the aqueous layer is clear. Transfer 4.0 mL of the *chloroform* layer to a suitable vial, add 30 mL of *chloroform* and a small quantity (about 400 mg) of *anhydrous sodium sulfate*, shake for 5 min. Use the clear solution.~~

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4 mm × 25 cm; packing L3

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for methylprednisolone acetate and prednisone are 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 2.5 between methylprednisolone acetate and prednisone

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylprednisolone acetate ($C_{24}H_{32}O_6$) in the portion of Injectable Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak height ratio of methylprednisolone acetate to prednisone from the *Sample solution*

R_S = peak height ratio of methylprednisolone acetate to prednisone from the *Standard solution*

C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methylprednisolone acetate in the *Sample solution* (mg/mL)

▲ Solution A: Formic acid and water (0.2: 99.8)

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
3	70	30
22	62	38
27	20	80
34	20	80
35	70	30
40	70	30

Diluent: Solution A and Solution B (50:50)

Standard solution: 0.1 mg/mL of USP Methylprednisolone Acetate RS in Diluent

Sample stock solution: Nominally 4 mg/mL of methylprednisolone acetate from Injectable Suspension prepared as follows. Transfer 40 mg of methylprednisolone acetate from a portion of Injectable Suspension to a suitable volumetric flask. Add Diluent to 50% of the flask volume and sonicate. Dilute with Diluent to volume. Centrifuge and use the supernatant. [Note—May centrifuge at 3000 rpm for 10 min.]

Sample solution: Nominally 0.1 mg/mL of methylprednisolone acetate from Sample stock solution in Diluent

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 2.6-µm packing L11

Temperatures

Autosampler: 5°

Column: 45°

Flow rate: 0.8 mL/min

Injection volume: 10 µL

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylprednisolone acetate ($C_{24}H_{32}O_6$) in the portion of Injectable Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methylprednisolone acetate from the *Sample solution*

r_S = peak response of methylprednisolone acetate from the *Standard solution*

C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methylprednisolone acetate in the *Sample solution* (mg/mL)

▲USP40

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Uniformity of Dosage Units** (905): Meets the requirements

IMPURITIES

Add the following:

▲ • Organic Impurities

Solution A, Solution B, Mobile phase, Diluent, Sample stock solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.5 $\mu\text{g/mL}$ each of USP Methylprednisolone Acetate RS and USP Dexamethasone Acetate RS in *Diluent*

Sample solution: Nominally 500 $\mu\text{g/mL}$ of methylprednisolone acetate from *Sample stock solution* in *Diluent*

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between the methylprednisolone acetate and dexamethasone acetate peaks

Relative standard deviation: NMT 3.0% for methylprednisolone acetate

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified degradation product in the portion of Injectable Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of methylprednisolone acetate from the *Standard solution*

C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of methylprednisolone acetate in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.10%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methylprednisolone acetate	1.0	—
Dexamethasone acetate ^a	1.1	—
Any individual unspecified degradation product	—	1.0
Total impurities	—	2.0

^a Used for resolution measurement and not to be included in the calculation of total degradation products.

▲USP40

SPECIFIC TESTS

- **pH** (791): 3.0–7.0
- **Particle Size**

Analysis: Transfer 1 drop to a microscope slide, and spread it evenly, diluting with water if necessary, to decrease the density of the field. Examine the slide under a microscope equipped with a calibrated ocular micrometer, using 400× magnification. Scan the entire slide, and note the size of the individual particles.

Acceptance criteria: NLT 99% of the particles are less than 20 μm in length when measured along the longest axis; and NLT 75% of the particles are less than 10 μm .

- **Other Requirements:** It meets the requirements in *Injections* (1).

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
▲ Store at controlled room temperature. ▲USP40

Change to read:

- **USP Reference Standards** (11)
▲ USP Dexamethasone Acetate RS
9-Fluoro-11 β ,17,21-trihydroxy-16 α -methylpregna-1,4-diene-3,20-dione 21-acetate.
 $\text{C}_{24}\text{H}_{31}\text{FO}_6$ 434.51 ▲USP40
USP Methylprednisolone Acetate RS

BRIEFING

Morphine Sulfate Extended-Release Capsules, *USP 38* page 4450. On the basis of comments received, the following revisions are proposed.

1. Replace the IR procedure for *Identification* test A, which lacks specified sample preparation, with the UV spectra agreement from the *Assay*. The *Detector* for the *Assay* is updated accordingly.
2. Revise the calculation for the *Assay* to include the molecular weight conversion to be consistent with the label information of USP Morphine Sulfate RS.
3. Revise the preparation of the *Sample stock solution* in the *Assay* to make it more flexible and less formulation specific.
4. Update the sensitivity requirement in the test for *Organic Impurities* to add a limit for *Signal-to-noise ratio* based on supporting data.
5. Revise the test for *Organic Impurities* by using the external standard approach for specified impurities to address the comments on the relative response factors. Quantitation of individual unspecified impurity is revised to indicate that peak responses are from the *Sample solution* to be consistent with the FDA-approved specifications.
6. Update the disregard limits in the *Organic Impurities* based on the supporting data.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM2: H. Cai.)

Correspondence Number—C122244

Comment deadline: January 31, 2016

Morphine Sulfate Extended-Release Capsules

DEFINITION

Morphine Sulfate Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$.

IDENTIFICATION

Change to read:

- **A. Infrared Absorption** ~~(197K)~~

▲ **Standard solution** and **Sample solution:** Prepare as directed in the *Assay*.

Analysis: Inject 10 µL each of the *Standard solution* and the *Sample solution* using the *Chromatographic system* except for the injection volume in the *Assay*.

Acceptance criteria: The UV absorption spectrum of the morphine peak of the *Sample solution* and of the *Standard solution* exhibits maxima and minima at the same wavelengths, as obtained in the *Assay*. ▲*USP40*

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

Diluent: Water. Adjust with *phosphoric acid* to a pH of 3.6.

Buffer solution: 13.8 mg/mL of *monobasic sodium phosphate*

Solution A: Acetonitrile, *triethylamine*, *Buffer solution*, and water (25: 0.5: 100: 874.5).
Adjust with *phosphoric acid* to a pH of 3.6.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
33	100	0
44	85	15
54	85	15
55	100	0
65	100	0

System suitability solution: 400 µg/mL of USP Morphine Sulfate RS and 10 µg/mL each of USP Morphine Related Compound A RS and USP Morphine Related Compound B RS (pseudomorphine) in *Diluent*

Standard solution: 1.0 mg/mL of USP Morphine Sulfate RS in *Diluent*

Sample stock solution: Transfer a weighed portion of the contents from NLT 20 Capsules, nominally equivalent to 250 mg of morphine sulfate, to a 100-mL volumetric flask. Add 5 mL of methanol and mix well for ~~30 min~~

▲ NLT 30 min ▲ USP40

with gentle swirling ~~every 5 min~~

▲ about every 5 min. ▲ USP40

Add *Diluent* up to half of the flask volume and sonicate for ~~5 min~~

▲ NLT 5 min ▲ USP40

to dissolve. Dilute with *Diluent* to volume.

Sample solution: Nominally 1.0 mg/mL of morphine sulfate from the *Sample stock solution* in *Diluent*. Pass through a suitable filter and use the clear filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 245 nm.

▲ For *Identification test A*, use a diode-array detector in the range of 200–400 nm.

▲ USP40

Columns

Guard: Packing *L1*

Analytical: 3.9-mm × 30-cm; 10-µm packing *L1*

Flow rate: 2 mL/min

Injection volume: 40 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between the morphine related compound A and morphine sulfate peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

▲

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100 \quad \blacktriangle_{USP40}$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Morphine Sulfate RS in the *Standard solution* (mg/mL),
▲ calculated on the anhydrous basis ▲_{USP40}

C_U = nominal concentration of
▲ morphine sulfate pentahydrate from ▲_{USP40}
the *Sample solution* (mg/mL)

▲ M_{r1} = molecular weight of morphine sulfate pentahydrate, 758.83 ▲_{USP40}

▲ M_{r2} = molecular weight of anhydrous morphine sulfate, 668.77 ▲_{USP40}

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• Dissolution (711)

pH 7.5 Phosphate buffer: 6.8 mg/mL of *monobasic potassium phosphate* and 1.6 mg/mL of *sodium hydroxide*. Adjust with *phosphoric acid* or 2 N *sodium hydroxide* to a pH of 7.5.

Medium: Proceed as directed for *Dissolution (711)*, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B*, observing the following exceptions. Perform *Acid Stage* testing, using 500 mL of 0.1 N *hydrochloric acid* for 1 h; and perform *Buffer Stage* testing, using 500 mL of *pH 7.5 Phosphate buffer* for NLT 8 h.

Apparatus 1: 100 rpm

Times: 1, 4, 6, and 9 h

Determine the amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ dissolved by using the following method.

Mobile phase: Methanol, *glacial acetic acid*, and water (280:10:720), containing 0.73 g of *sodium 1-heptanesulfonate* for each 1.01 L of the solvent mixture

System suitability solution: 0.1 mg/mL each of *phenol* and USP Morphine Sulfate RS in

Mobile phase

Standard solution: USP Morphine Sulfate RS in *pH 7.5 Phosphate buffer* to obtain a solution with a known concentration corresponding to that of the *Sample solution*.

Sample solution: Sample per *Dissolution* (711).

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 284 nm

Column: 3.9-mm × 30-cm; 10-μm packing *L1*

Flow rate: 2 mL/min

Injection volume: 25 μL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for phenol and morphine sulfate are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the phenol and morphine sulfate peaks

Tailing factor: NMT 2.0 for the morphine sulfate peak

Relative standard deviation: NMT 2.0%

▲ for the morphine sulfate peak▲*USP40*

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
1	NMT 10
4	25–50
6	50–90
9	NLT 85

The percentage of the labeled amount of morphine sulfate pentahydrate [(C₁₇H₁₉NO₃)₂·H₂SO₄·5H₂O] dissolved in 1 h conforms to *Dissolution* (711), *Acceptance Table 3*. The percentages of the labeled amount of morphine sulfate pentahydrate [(C₁₇H₁₉NO₃)₂·H₂SO₄·5H₂O] dissolved at the other times specified conform to *Dissolution* (711), *Acceptance Table 2*.

- **Uniformity of Dosage Units** (905): Meet the requirements

IMPURITIES**Change to read:**

- **Organic Impurities**

Diluent, Solution A, System suitability solution, ~~Standard solution,~~▲▲*USP40***Chromatographic system, and Sample solution:** Proceed as directed in the Assay.**Sensitivity solution:** 0.5 µg/mL of USP Morphine Sulfate RS in *Diluent*▲ **Standard solution:** 0.002 mg/mL of USP Morphine Sulfate RS and 0.005 mg/mL each of USP Morphine Related Compound A RS and USP Morphine Related Compound B RS (pseudomorphine) in *Diluent*▲*USP40***System suitability****Samples:** *System suitability solution, Standard solution, and Sensitivity solution***Suitability requirements****Resolution:** NLT 2.0 between the morphine related compound A and morphine sulfate peaks, *System suitability solution***Sensitivity:**▲ **Signal-to-noise ratio:**▲*USP40*

Morphine peak is detectable

▲ NLT 10 for morphine sulfate,▲*USP40**Sensitivity solution***Relative standard deviation:** NMT 2.0%▲ 5% for morphine related compound A, morphine sulfate, and morphine related compound B,▲*USP40**Standard solution***Analysis****Samples:** ~~*Diluent and Sample solution*~~[~~Note—Disregard the peaks corresponding to those obtained in the chromatogram of the *Diluent*.~~]

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

 ~~r_U = peak response for each impurity from the *Sample solution*~~ ~~r_S = peak response for morphine sulfate from the *Standard solution*~~ ~~F = relative response factor (See *Table 3*)~~▲ **Samples:** *Diluent, Standard solution, and Sample solution*

Calculate the percentage of morphine related compound A and morphine related compound B in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of morphine related compound A or morphine related compound B from the *Sample solution* r_S = peak response of USP Morphine Related Compound A RS or USP Morphine Related Compound B RS from the *Standard solution* C_S = concentration of USP Morphine Related Compound A RS or USP Morphine Related Compound B RS in the *Standard solution* (mg/mL) C_U = nominal concentration of morphine sulfate pentahydrate in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*

r_T = peak response of morphine sulfate from the *Sample solution* ▲USP40

Acceptance criteria: See *Table 3*

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
Morphine related compound A ^a	1.4	1.0	0.5
Morphine sulfate	1.0	—	—
Morphine related compound B ^b	2.3	2.1	0.5
Any unspecified impurity	—	—	0.2
Total impurities	—	—	1.5

a 7,8-Didehydro-4,5 α -epoxy-17-methylmorphinan-3,6 α -diol, *N*-oxide.
b 2,2'-Bimorphine.

▲ **Acceptance criteria:** See *Table 3*. Disregard any peaks below 0.05% and the peaks corresponding to those from the *Diluent*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Morphine related compound A ^a	1.4	0.5
Morphine sulfate	1.0	—
Morphine related compound B ^b	2.3	0.5
Any unspecified impurity	—	0.2
Total impurities	—	1.5

a 7,8-Didehydro-4,5 α -epoxy-17-methylmorphinan-3,6 α -diol, *N*-oxide.
b 2,2'-Bimorphine.

▲USP40

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP Reference Standards** (11)
USP Morphine Related Compound A RS
7,8-Didehydro-4,5 α -epoxy-17-methylmorphinan-3,6 α -diol, *N*-oxide.
C₁₇H₁₉NO₄ 301.34

USP Morphine Related Compound B RS

2,2'-Bimorphine.

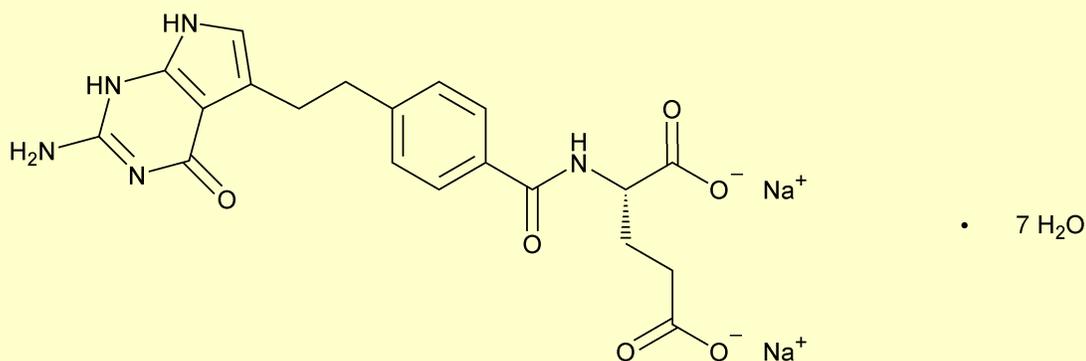
C₃₄H₃₆N₂O₆ 568.66USP Morphine Sulfate RS **BRIEFING**

Pemetrexed Disodium. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods of analysis, is proposed.

1. The liquid chromatographic procedure used in the *Assay* is based on analyses performed with the Zorbax SB-C8 brand of L7 column. The typical retention time for pemetrexed is about 3 min.
2. The liquid chromatographic procedure used in the test for *Organic Impurities* is also based on analyses performed with the Zorbax SB-C8 brand of L7 column. The typical retention time for pemetrexed is about 29 min.
3. The liquid chromatographic procedure used in the *Enantiomeric Purity* test is based on analyses performed with the Phenomenex IB-SIL C18 brand of L1 column. The typical retention time for pemetrexed is about 33 min.

(CHM3: F. Mao.)

Correspondence Number—C146273

Comment deadline: January 31, 2016**Add the following:****▲ Pemetrexed Disodium**C₂₀H₁₉N₅Na₂O₆·7H₂O 597.49

L-Glutamic acid, *N*-[4-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-, disodium salt, heptahydrate;

Disodium *N*-{*p*-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-*L*-glutamate heptahydrate [357166-29-1].

Anhydrous C₂₀H₁₉N₅Na₂O₆ 471.37

[150399-23-8].

Pemetrexed (free acid) C₂₀H₂₁N₅O₆ 427.42

[137281-23-3].

DEFINITION

Pemetrexed Disodium contains NLT 97.5% and NMT 102.0% of pemetrexed disodium ($C_{20}H_{19}N_5Na_2O_6$), calculated on the anhydrous and solvent-free basis.

[**Caution**—Handle pemetrexed disodium with great care as it alters genetic material and may be irritating to the eyes and skin.]

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Enantiomeric Purity* test.
- **C. Identification Tests—General** (191), *Sodium*

ASSAY• **Procedure**

Buffer: 0.17% (v/v) of *Glacial acetic acid* in water. Adjust with a 50% *sodium hydroxide* solution to a pH of 5.3 ± 0.1 .

Mobile phase: *Acetonitrile* and *Buffer* (11:89)

Standard solution: 0.15 mg/mL of USP Pemetrexed Disodium RS in water

Sample solution: 0.15 mg/mL of Pemetrexed Disodium in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm \times 7.5-cm; 3.5- μ m packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pemetrexed disodium ($C_{20}H_{19}N_5Na_2O_6$) in the portion of Pemetrexed Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Pemetrexed Disodium RS in the *Standard solution* (mg/mL)

C_U = concentration of Pemetrexed Disodium in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• Organic Impurities

Buffer: 1.45 g/L of *ammonium formate* in water. Adjust with *formic acid* to a pH of 3.5 ± 0.1 .

Solution A: *Acetonitrile* and *Buffer* (5:95)

Solution B: *Acetonitrile* and *Buffer* (30:70)

Mobile phase: See *Table 1*. [Note—After each injection, re-equilibrate the chromatographic system at the initial condition for a minimum of 13 min.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
45	0	100
47	100	0

System suitability stock solution: Prepare 3 mg/mL of USP Pemetrexed Disodium RS in 0.1 N *sodium hydroxide*. Heat this solution at 70° for 40 min.

[Note—The preparation degrades pemetrexed and generates the pemetrexed *R*-dimer and pemetrexed *S*-dimer as follows:

Pemetrexed *R*-dimer: (2*S*,2'*S*)-2,2'-{[(*R*)-2,2'-Diamino-4,4',6-trioxo-1,4,4',6,7,7'-hexahydro-1'*H*,5*H*-5,6'-bipyrrolo[2,3-*d*]pyrimidine-5,5'-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)}diglutamic acid.

Pemetrexed *S*-dimer: (2*S*,2'*S*)-2,2'-{[(*S*)-2,2'-Diamino-4,4',6-trioxo-1,4,4',6,7,7'-hexahydro-1'*H*,5*H*-5,6'-bipyrrolo[2,3-*d*]pyrimidine-5,5'-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)}diglutamic acid.]

System suitability solution: Transfer 1 mL of the *System suitability stock solution* to a 10-mL volumetric flask and dilute with water to volume.

Sensitivity solution: 0.1 µg/mL of USP Pemetrexed Disodium RS in water

Sample solution: 0.2 mg/mL of Pemetrexed Disodium in water. Do not sonicate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing *L7*

Autosampler temperature: 2°–8°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[Note—The relative retention times for the pemetrexed *R*-dimer and pemetrexed *S*-dimer peaks are 0.87 and 0.88, respectively.]

Suitability requirements

Peak-to-valley ratio: The ratio of the height of the pemetrexed *R*-dimer peak to the height of the valley between the pemetrexed *R*-dimer and pemetrexed *S*-dimer is NLT 1.5, *System suitability solution*.

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Pemetrexed Disodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_T = total peak areas from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
<i>N</i> -Methyl pemetrexed ^a	0.82	0.15
Pemetrexed glutamide ^b	0.90	0.15
Pemetrexed	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.60
^a {4-[2-(2-Amino-1-methyl-4-oxo-4,7-dihydro-1 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-5-yl)ethyl]benzoyl}-l-glutamic acid. ^b {4-[2-(2-Amino-1-methyl-4-oxo-4,7-dihydro-1 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-5-yl)ethyl]benzoyl}-4-l-glutamyl-l-glutamic acid.		

- Enantiomeric Purity**

Buffer: Dissolve 8 g of anhydrous *beta cyclodextrin* in 1 L of water. Add 15 mL of *triethylamine* to this solution and mix. Add about 6 mL of *phosphoric acid* and adjust with additional *phosphoric acid* to a pH of 6.0.

Mobile phase: *Acetonitrile* and *Buffer* (5:95)

Standard solution: 0.24 mg/mL of USP Pemetrexed Disodium RS in water

Sensitivity solution: 0.12 µg/mL of USP Pemetrexed Disodium RS in water from the *Standard solution*

Sample solution: 0.24 mg/mL of Pemetrexed Disodium in water

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-μm packing *L1*

Temperatures

Autosampler: 2°–8°

Column: 40°

Flow rate: 1 mL/min

Injection volume: 50 μL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[Note—USP Pemetrexed Disodium RS contains a small amount of pemetrexed enantiomer disodium (disodium *N*-{*p*-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-*d*-glutamate). The relative retention times for pemetrexed enantiomer and pemetrexed are about 0.94 and 1.0, respectively.]

Suitability requirements

Peak-to-valley ratio: The ratio of the height of the pemetrexed enantiomer peak to the height of the valley between the pemetrexed enantiomer and pemetrexed is NLT 5.0, *Standard solution*

Signal-to-noise ratio: NLT 10 for the pemetrexed peak, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of pemetrexed enantiomer in the portion of Pemetrexed Disodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of pemetrexed enantiomer from the *Sample solution*

r_T = total peak areas of pemetrexed enantiomer and pemetrexed from the *Sample solution*

Acceptance criteria: NMT 0.3%

SPECIFIC TESTS

- **Water Determination** <921>, *Method Ic*

Sample: 25 mg

Acceptance criteria: 19.5%–22.1%

- **pH** <791>

Sample: 56 mg/mL in water

Acceptance criteria: 7.5–8.4

- **Bacterial Endotoxins Test** (85): It contains less than 0.17 USP Endotoxin Units/mg of pemetrexed.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at room temperature.
- **USP Reference Standards** (11)
 - USP Endotoxin RS
 - USP Pemetrexed Disodium RS

▲USP40

BRIEFING

Pemetrexed for Injection. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is proposed.

1. The liquid chromatographic procedure used in the *Assay* is based on analyses performed with the Zorbax SB-C8 brand of L7 column. The typical retention time for pemetrexed is about 3 min.
2. The liquid chromatographic procedure used in the test for *Organic Impurities* is also based on analyses performed with the Zorbax SB-C8 brand of L7 column. The typical retention time for pemetrexed is about 22 min.

(CHM3: F. Mao, R. Tirumalai.)

Correspondence Number—C146703

Comment deadline: January 31, 2016

Add the following:

▲ **Pemetrexed for Injection**

DEFINITION

Pemetrexed for Injection is a sterile, lyophilized mixture of pemetrexed disodium and suitable added substances. It contains NLT 90.0% and NMT 110.0% of the labeled amount of pemetrexed ($C_{20}H_{21}N_5O_6$). [**Caution**—Handle pemetrexed disodium with great care as it alters genetic material and may be irritating to the eyes and skin.]

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: 0.17% (v/v) *Glacial acetic acid* in water. Adjust with a 50% *sodium hydroxide*

solution to a pH of 5.3.

Mobile phase: *Acetonitrile and Buffer (11:89)*

Standard solution: 0.14 mg/mL of USP Pemetrexed Disodium RS in water

Sample solution: Nominally equivalent to 0.1 mg/mL of pemetrexed in water, prepared from a composite of at least 3 vials of Pemetrexed for Injection

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm × 7.5-cm; 3.5-μm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of the labeled amount of pemetrexed ($C_{20}H_{21}N_5O_6$) in the portion of Pemetrexed for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Pemetrexed Disodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pemetrexed in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of pemetrexed, 427.42

M_{r2} = molecular weight of pemetrexed disodium, 597.49

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Uniformity of Dosage Units** (905), *Weight Variation*: Meets the requirements

IMPURITIES

- **Organic Impurities**

Buffer: 0.17% (v/v) *Glacial acetic acid* in water. Adjust with a 50% *sodium hydroxide* solution to a pH of 5.5.

Solution A: Acetonitrile and Buffer (3:97)

Solution B: Acetonitrile and Buffer (12.5: 87.5)

Mobile phase: See Table 1. [Note—After each injection, re-equilibrate the chromatographic system at the initial condition for a minimum of 8 min.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40	0	100
45	0	100
47	100	0

System suitability stock solution: 2.8 mg/mL of USP Pemetrexed Disodium RS prepared as follows. Transfer USP Pemetrexed Disodium RS to a suitable volumetric flask and add a 3% hydrogen peroxide solution equivalent to 10% of the final volume. Dilute with water to volume. Mix and heat this solution at 75° for 2–5 h and allow it to come to room temperature. [Note—The solution preparation forms ketopemetrexed, pemetrexed *R*-dimer, and pemetrexed *S*-dimer.

Pemetrexed *R*-dimer: (2*S*,2'*S*)-2,2'-{[(*R*)-2,2'-Diamino-4,4',6-trioxo-1,4,4',6,7,7'-hexahydro-1'*H*,5*H*-5,6'-bipyrrolo[2,3-*d*]pyrimidine-5,5'-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)}diglutamic acid.

Pemetrexed *S*-dimer: (2*S*,2'*S*)-2,2'-{[(*S*)-2,2'-Diamino-4,4',6-trioxo-1,4,4',6,7,7'-hexahydro-1'*H*,5*H*-5,6'-bipyrrolo[2,3-*d*]pyrimidine-5,5'-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)}diglutamic acid.]

System suitability solution: Transfer 5 mL of the *System suitability stock solution* to a 50-mL volumetric flask and dilute with water to volume.

Sensitivity solution: 0.14 µg/mL of USP Pemetrexed Disodium RS in water

Sample solution: Nominally equivalent to 0.2 mg/mL of pemetrexed in water, prepared from 1 vial of Pemetrexed for Injection

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Temperatures

Autosampler: 2°–8°

Column: 35°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[Note—The relative retention times for the pemetrexed *R*-dimer and pemetrexed *S*-dimer peaks are 0.67 and 0.71, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the pemetrexed *R*-dimer and pemetrexed *S*-dimer peaks,
System suitability solution

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Pemetrexed for Injection taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_T = total peak areas from the *Sample solution*

F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ketopemetrexed ^a	0.31	0.61	0.60
Pemetrexed	1.0	—	—
Any individual unspecified impurity	—	1.0	0.24
Total Impurities	—	—	1.30

^a (4-{2-[(*RS*)-2-Amino-4,6-dioxo-4,5,6,7-tetrahydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl]ethyl}benzoyl)-l-glutamic acid.

SPECIFIC TESTS

- **Bacterial Endotoxins Test** (85): NMT 0.17 USP Endotoxin Units/mg of pemetrexed
- **Sterility Tests** (71): Meets the requirements
- **Particulate Matter in Injections** (788): Meets the requirements for small-volume injections
- **pH** (791)
 - Sample:** A constituted solution prepared as directed in the labeling
 - Acceptance criteria:** 6.6–7.8
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* (1)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*, *Sterile solids packaging*. Store at controlled room temperature.
- **USP Reference Standards** (11)
 - USP Endotoxin RS
 - USP Pemetrexed Disodium RS

BRIEFING

Penicillin G Procaine, USP 38 page 4789. The following revisions are proposed:

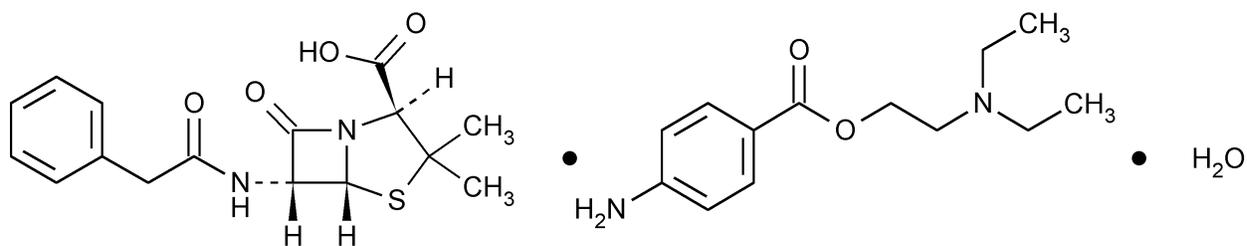
1. In preparation for the revisions to *Packaging and Storage Requirements* (659) proposed in PF 41(3) [May–June 2015], it is proposed to revise the *Packaging and Storage* section to indicate that the requirement applies only to those products that are intended for use in preparing injectable dosage forms.
2. Added a reference to *Antibiotics—Microbial Assays* (81) to clarify the preparation of *Buffer B.1* in the *Assay*.
3. References to USP Penicillin V Potassium RS are added to the test for *Content of Penicillin G and Procaine* and the *USP Reference Standards* sections; this Reference Standard is used to evaluate system suitability.
4. The column dimensions in the test for *Content of Penicillin G and Procaine* are revised to the correct inner diameter for the Waters μ Bondapak C18 column that was used in the original validation of the procedure. Relative retention times for the procaine, penicillin G, and penicillin V are revised based on supporting data. The reagents used in the test are revised for clarity.
5. The *Sample solution* in the test for *pH* is revised for clarity.
6. The chemical names are updated to align with each other.
7. The requirement in *Sterility Tests* is revised to make it more flexible.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM1: M. Puderbaugh.)

Correspondence Number—C158692

Comment deadline: January 31, 2016

Penicillin G Procaine**Change to read:**

$C_{16}H_{18}N_2O_4S \cdot C_{13}H_{20}N_2O_2 \cdot H_2O$ 588.72

$C_{16}H_{18}N_2O_4S \cdot C_{13}H_{20}N_2O_2$ 570.71

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino-], 2S-(2 α ,5 α ,6 β)-, ~~compd.~~

▲ compound ▲USP40

with 2-(diethylamino)ethyl 4-aminobenzoate (1:1) monohydrate;

(2S,5R,6R)-3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-

carboxylic acid compound with 2-(diethylamino)ethyl *p*-aminobenzoate (1:1) monohydrate [6130-64-9].
Anhydrous [54-35-3].

DEFINITION

Penicillin G Procaine has a potency of NLT 900 Penicillin G Units/mg and NMT 1050 Penicillin G Units/mg.

IDENTIFICATION

• A. Thin-Layer Chromatography

Solution A: Acetone, 0.1 M citric acid, and 0.1 M sodium citrate (2:1:1)

Standard solution 1: Prepare a solution containing the equivalent of 12,000 Penicillin G Units/mL, from USP Penicillin G Potassium RS in *Solution A*.

Standard solution 2: 5 mg/mL of USP Procaine Hydrochloride RS in *Solution A*

Sample solution: Nominally 12,000 Penicillin G Units/mL from Penicillin G Procaine in *Solution A*

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 μ L

Developing solvent system: Toluene, dioxane, and glacial acetic acid (90:25:4)

Spray reagent 1: Starch TS

Spray reagent 2: Iodine TS diluted 1 in 10 with water

Spray reagent 3: 50 mg/mL of *p*-dimethylaminobenzaldehyde in methanol

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Proceed as directed in the chapter. Develop the chromatogram until the solvent has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow to air-dry. Examine the plate under short- and long-wavelength UV light, noting the positions of the spots. Spray the plate with *Spray reagent 1* followed by *Spray reagent 2*. Penicillin G appears as a white spot on a purple background. Spray the location of the spots visualized with UV light with *Spray reagent 3*. Procaine appears as a bright yellow spot.

Acceptance criteria: The R_F value of the penicillin G spot from the *Sample solution* corresponds to that from *Standard solution 1*. The R_F value of the procaine spot from the *Sample solution* corresponds to that from *Standard solution 2*.

ASSAY

Change to read:

• Procedure

Standard solution: Prepare as directed in *Iodometric Assay—Antibiotics* (425), *Standard Preparation*, using USP Penicillin G Potassium RS.

Sample solution: Prepare as directed in *Iodometric Assay—Antibiotics* (425), *Assay Preparation*, except dissolve 100 mg of Penicillin G Procaine in 2.0 mL of *methanol*, and dilute with *Buffer B.1*

▲ (see *Antibiotics—Microbial Assays* (81)) ▲ USP40

to obtain a solution containing 2000 Penicillin G Units/mL.

Analysis: Pipet 2 mL of the *Sample solution* into each of two glass-stoppered, 125-mL conical flasks. Use one of these to perform the *Blank Determination*. Proceed as directed in *Iodometric Assay—Antibiotics* (425), *Procedure*.

Calculate the potency, in Penicillin G Units/mg, of the Penicillin G Procaine taken:

$$\text{Result} = (B - I) \times F \times 1/(D \times V) \times 100$$

B = volume of 0.01 N sodium thiosulfate consumed in the *Blank Determination* (mL)

I = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation and Titration* (mL)

F = equivalency factor as calculated in (Penicillin G Unit/mL of 0.01 N sodium

(425)

thiosulfate consumed by the *Standard solution*)

D = nominal concentration of Penicillin G in the *Sample solution* (Penicillin G Units/mL)

V = volume of the *Sample solution* used for the *Inactivation and Titration* (mL)

Acceptance criteria: 900–1050 Penicillin G Units/mg

SPECIFIC TESTS

Change to read:

• **Content of Penicillin G and Procaine**

Solution A: Dilute phosphoric acid (1 in 10)

▲ Phosphoric acid diluted 1 in 10 with water ▲ USP40

Mobile phase: Dissolve 14 g of *monobasic potassium phosphate* and 6.5 g of *tetrabutylammonium hydroxide solution* (4 in 10)

▲ *tetrabutylammonium hydroxide, 40% in water*, ▲ USP40

in 700 mL of water. Adjust with 1 N *potassium hydroxide* to a pH of 7.0, and dilute with water to 1000 mL. Mix 500 mL of this solution, 250 mL of *acetonitrile*, and 250 mL of water. Adjust with 1 N *potassium hydroxide* or *Solution A* to a pH of 7.5 ± 0.05, and pass through a suitable filter.

Standard solution: 0.8 mg/mL of USP Penicillin G Potassium RS and 0.54 mg/mL of USP Procaine Hydrochloride RS in *Mobile phase*

System suitability solution: 2.4 mg/mL of penicillin V potassium

▲ USP Penicillin V Potassium RS ▲ USP40

in *Mobile phase*. Mix the resultant solution with *Standard solution* (1:3).

Sample solution: Transfer 70 mg of Penicillin G Procaine to a 50-mL volumetric flask. Add

30 mL of *Mobile phase*, sonicate to dissolve, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4-mm

▲ 3.9-mm ▲ USP40

× 30-cm; 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for procaine and penicillin G are about 1.0 and 2.2, respectively.

▲ The relative retention times for procaine, penicillin G, and penicillin V are about 0.4, 1.0, and 1.5, respectively. ▲ USP40

]

Suitability requirements

Resolution: NLT 2.0 between penicillin G and penicillin V, *System suitability solution*

Relative standard deviation: NMT 3.0% for penicillin G potassium, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of penicillin G ($C_{16}H_{18}N_2O_4S$) in the portion of Penicillin G Procaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times G_S$$

r_U = peak response of penicillin G from the *Sample solution*

r_S = peak response of penicillin G from the *Standard solution*

C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin G Procaine in the *Sample solution* (mg)

G_S = content of penicillin G in USP Penicillin G Potassium RS (%)

Calculate the percentage of procaine ($C_{13}H_{20}N_2O_2$) in the portion of Penicillin G Procaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of procaine from the *Sample solution*

r_S = peak response of procaine from the *Standard solution*

C_S = concentration of USP Procaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin G Procaine in the *Sample solution* (mg)

M_{r1} = molecular weight of procaine, 236.32

M_{r2} = molecular weight of procaine hydrochloride, 272.78

Acceptance criteria: See Table 1.

Table 1

Penicillin G	51.0%–59.6%
Procaine	37.5%–43.0%

- **Bacterial Endotoxins Test** (85): Where the label states that Penicillin G Procaine is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.01 USP Endotoxin Unit/100 Penicillin G Units.

Change to read:

- **Sterility Tests** (71): Where the label states that Penicillin G Procaine is sterile, it meets the requirements. ~~when tested as directed under *Test for Sterility of the Product to Be Examined, Membrane Filtration*, except use *Fluid A* to which has been added sufficient sterile penicillinase to inactivate the penicillin G, and swirl the vessel until solution is complete before filtering~~
▲ If the test for *Membrane Filtration* is used, perform the procedure as directed in the chapter with the following exceptions. Use *Fluid A* to which has been added sufficient sterile penicillinase to inactivate the penicillin G, and swirl the vessel until solution is complete before filtering. ▲USP40
- **Crystallinity** (695): Meets the requirements

Change to read:

- **pH** (791)
Sample solution: A (saturated)
▲ saturated ▲USP40
solution containing about 300 mg/mL of Penicillin G Procaine in water
Acceptance criteria: 5.0–7.5
- **Water Determination** (921), *Method I*: 2.8%–4.2%

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** ~~Preserve as described under *Injections* (1), *Containers for Sterile Solids*.~~
▲ Where it is intended for use in preparing injectable dosage forms, preserve as directed in *Packaging and Storage Requirements* (659), *Injection Packaging*, *Packaging for Constitution*. ▲USP40
- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

Change to read:

- **USP Reference Standards** (11)
USP Endotoxin RS
USP Penicillin G Potassium RS
▲ USP Penicillin V Potassium RS

▲USP40

USP Procaine Hydrochloride RS

BRIEFING

Pentazocine and Acetaminophen Tablets, *USP 38* page 4803. As part of the USP monograph modernization initiative and based on correspondence from the FDA regarding modernization, the addition of an HPLC procedure for monitoring 4-aminophenol, based on *4-Aminophenol in Acetaminophen-Containing Drug Products (227)*, is proposed. This liquid chromatographic procedure is based on analyses performed with the Dionex Acclaim Mixed Mode WCX-1 brand of L85 column. The typical retention time for 4-aminophenol is about 4.2–5.3 min. The proposed limit of this nephrotoxin is 0.15% and is based on input provided by the FDA and the pharmaceutical industry to the USP Acetaminophen Expert Panel, which reports to the Small Molecules-2 Expert Committee.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM6: C. Anthony.)

Correspondence Number—C161687

Comment deadline: January 31, 2016

Pentazocine and Acetaminophen Tablets**DEFINITION**

Pentazocine and Acetaminophen Tablets contain an amount of Pentazocine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of pentazocine ($C_{19}H_{27}NO$) and NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$).

IDENTIFICATION**• A. Thin-Layer Chromatographic Identification Test (201)**

Diluent: Chloroform and methanol (1:1)

Standard solution A: 1 mg/mL of USP Pentazocine RS in *Diluent*

Standard solution B: 26 mg/mL of USP Acetaminophen RS in *Diluent*

Sample solution: Transfer a quantity of finely powdered Tablets, nominally equivalent to about 5 mg of pentazocine and 130 mg of acetaminophen, to a suitable flask. Add 5 mL of *Diluent*, shake, and allow the solids to settle. Use the supernatant.

Chromatographic system

Developing solvent system: Ethyl acetate, methanol, and formic acid (90:5:5)

Spray reagent: Dissolve 300 mg of platinum chloride in 100 mL of water and add 100 mL of potassium iodide solution (6 in 100).

Analysis: Evaporate the solvents in cool, circulating air. After developing and examining the spots, spray the plate with *Spray reagent*.

Acceptance criteria: The R_F values, size, and intensity of color of the two principal spots of the *Sample solution* correspond to those of *Standard solution A* and *Standard solution B*.

B.

ASSAY**Change to read:**● **Pentazocine****Mobile phase:** Chloroform, methanol, and isopropylamine (960:40:2)**Diluent:** Methanol and 0.035 N sulfuric acid (1:1)**Standard stock solution:** 0.5 mg/mL of USP Pentazocine RS in *Diluent***Standard solution:** Transfer 10.0 mL of the *Standard stock solution* to a 125-mL separator. Add 30 mL of water and 5 mL of sodium carbonate solution (1:10). Extract with 60 mL of chloroform and pass the chloroform layer through filter paper, collecting the filtrate in a 100-mL volumetric flask. Dilute with chloroform to volume and mix.**Sample solution:** Transfer an amount nominally equivalent to 25 mg of pentazocine, from NLT 20 finely powdered Tablets, to a 50-mL glass-stoppered cylinder. Add 50.0 mL of *Diluent* and shake intermittently for 15 min. Sonicate for about 2 min, allow the solids to settle, and transfer 10.0 mL of the supernatant to a 125-mL separator. [Note—Save the remainder of the supernatant for use in the *Assay for Acetaminophen*. Minimize the waiting period before this test is performed to prevent significant hydrolysis of acetaminophen to *p*-aminophenol.]

Add 30 mL of water and 5 mL of sodium carbonate solution (1:10) to the separator and mix. Extract with 60 mL of chloroform and pass the chloroform layer through filter paper, collecting the filtrate in a 100-mL volumetric flask. Dilute with chloroform to volume and mix.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 280 nm**Column:** 4.6-mm × 25-cm; 10-μm packing L3**Flow rate:** 1.2 mL/min**Injection volume:** 20 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Column efficiency:** ~~NLT 1000 theoretical plates~~

▲▲USP40

Tailing factor: NMT 3.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of pentazocine (C₁₉H₂₇NO) in the portion

of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pentazocine from the *Sample solution*

r_S = peak response of pentazocine from the *Standard solution*

C_S = concentration of USP Pentazocine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pentazocine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

• **Acetaminophen**

[Note—Minimize the time between the addition of the *Diluent* and the injection of the *Sample solution* to prevent significant hydrolysis of acetaminophen to *p*-aminophenol.]

Mobile phase: Chloroform, methanol, and isopropylamine (960:40:2)

Diluent: Methanol and 0.035 N sulfuric acid (1:1)

Standard stock solution: 13 mg/mL of USP Acetaminophen RS in *Diluent*

Standard solution: Dilute 2.0 mL of the *Standard stock solution* with ethyl acetate to 200 mL.

Sample solution: Dilute 2.0 mL of the supernatant reserved from the *Assay* for *Pentazocine* immediately with ethyl acetate to volume in a 200-mL volumetric flask to minimize hydrolysis of acetaminophen to *p*-aminophenol and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 10-μm packing L3

Flow rate: 1.4 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

~~**Column efficiency:** NLT 1000 theoretical plates~~

▲▲USP40

Tailing factor: NMT 3.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen (C₈H₉NO₂) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*

r_S = peak response of acetaminophen from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• Uniformity of Dosage Units (905)

Procedure for content uniformity

Diluent: Acetonitrile and 0.035 N sulfuric acid (6:4)

Mobile phase: Tetrahydrofuran, phosphoric acid, and 0.005 M monobasic sodium phosphate (50:1:950)

Pentazocine standard stock solution: 0.25 mg/mL of USP Pentazocine RS in *Diluent*

System suitability stock solution: 0.325 mg/mL of USP Acetaminophen RS in *Diluent*

System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* to a 100-mL volumetric flask. Add 5.0 mL of the *Pentazocine standard stock solution*, dilute with *Mobile phase* to volume, and mix.

Standard solution: Transfer a quantity of USP Acetaminophen RS to a suitable volumetric flask. Add a sufficient volume of *Pentazocine standard stock solution* and mix to dissolve the acetaminophen. Dilute with *Mobile phase* to volume. Mix to obtain known concentrations of 0.0125 and 0.325 mg/mL of pentazocine and acetaminophen, respectively.

Sample stock solution: Transfer 1 Tablet to a 100-mL volumetric flask, add 50 mL of *Diluent*, and sonicate for 30 min. Dilute with *Diluent* to volume, and mix. Pass a portion of this solution through a paper filter, covering the funnel with a watch glass and discarding the first few mL of the filtrate.

Sample solution: Dilute 5.0 mL of the filtrate with *Mobile phase* to 100 mL and pass this solution through a membrane filter of 0.5- μ m or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 9.4-mm \times 10-cm; 5- μ m packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for acetaminophen and pentazocine are 0.2 and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 7 between pentazocine and acetaminophen**Relative standard deviation:** NMT 2.0% for the pentazocine and acetaminophen peaks**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of pentazocine (C₁₉H₂₇NO) and acetaminophen (C₈H₉NO₂) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of pentazocine or acetaminophen from the *Sample solution* r_S = peak response of pentazocine or acetaminophen from the *Standard solution* C_S = concentration of the appropriate USP Reference Standard (USP Pentazocine RS or USP Acetaminophen RS) in the *Standard solution* (mg/mL) C_U = nominal concentration of pentazocine or acetaminophen in the *Sample solution* (mg/mL)**Acceptance criteria:** Meet the requirements**IMPURITIES****Add the following:**

- ▲ ● **4-Aminophenol in Acetaminophen-Containing Drug Products** <227>: Meet the requirements ▲^{USP40}

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** <11>
 - USP Acetaminophen RS
 - USP Pentazocine RS

BRIEFING

Phenoxybenzamine Hydrochloride Capsules, USP 38 page 4842. On the basis of comments received, the following changes are proposed:

1. *Identification* test *A* is replaced with the matching of the UV absorption spectra of the phenoxybenzamine peak from the *Sample solution* and the *Standard solution*, as obtained in *Assay*.
2. An orthogonal *Identification* test *B* is included based on the retention time agreement of the phenoxybenzamine peak from the *Sample solution* and the *Standard solution*, as obtained in *Assay*.
3. The *Detector* in the *Assay* is revised to include the diode array range for performing *Identification* test *A*.
4. The *Procedure for content uniformity* under the test for *Uniformity of Dosage Units* is deleted to provide flexibility for using either of the procedures described in *Uniformity of*

Dosage Units (905).

5. The *Column temperature* in the *Assay* and *Dissolution* test is deleted as it is listed in *Chromatography* (621).
6. The relative retention times of known degradation products is included under *System suitability* in the *Assay*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM2: S. Ramakrishna.)

Correspondence Number—C156294

Comment deadline: January 31, 2016

Phenoxybenzamine Hydrochloride Capsules

DEFINITION

Phenoxybenzamine Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$).

IDENTIFICATION

Delete the following:

▲ ● **A. Ultraviolet Absorption**

Analytical wavelengths: 268 and 272 nm

Sample solution: 0.15 mg/mL of phenoxybenzamine hydrochloride in acidic alcohol (1 in 1000 solution of hydrochloric acid in alcohol)

Acceptance criteria: The ratio A_{268}/A_{272} of the maximum at 268 ± 2 nm and the minimum at 272 ± 2 nm is between 1.75 and 1.95. ▲*USP40*

Add the following:

- ▲ ● **A.** The UV absorption spectra of the phenoxybenzamine peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak from the *Standard solution*, as obtained in the *Assay*. ▲*USP40*

Add the following:

- ▲ ● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP40*

ASSAY

Change to read:

● **Procedure**

Solution A: 2.2 mg/mL of *anhydrous monobasic sodium phosphate* in water. Adjust with concentrated

▲ ▲*USP40*

phosphoric acid to a pH of 3.0.

Mobile phase: Filtered and degassed mixture of *Solution A* and *acetonitrile* (45:55)

Standard solution: 0.2 mg/mL of USP Phenoxybenzamine Hydrochloride RS in *acetonitrile*.
[Note—Sonicate if necessary.]

System suitability solution: 10 mL of the *Standard solution* and 0.5 mL of 0.1 N *sodium hydroxide* taken in a vial. [Note—Basic solutions of phenoxybenzamine hydrochloride will produce the known degradant, tertiary amine phenoxybenzamine—the second major peak that elutes before the phenoxybenzamine peak and has a relative retention time of about 0.3 and an unknown related substance. Severe degradation of the drug substance will be observed if the solution is allowed to stand for more than 1 h.]

Sample solution: 0.2 mg/mL of phenoxybenzamine hydrochloride in *acetonitrile* prepared as follows. Remove, as completely as possible, the contents of NLT 20 Capsules. Transfer a portion of the mixed powder, equivalent to about 10 mg of phenoxybenzamine hydrochloride, to a 50-mL volumetric flask. Add about 40 mL of *acetonitrile*, and sonicate for 15 min with occasional swirling. Cool, and dilute with *acetonitrile* to volume to obtain the concentration, based on the label claim. Allow the sample to stand undisturbed for 30 min such that the undissolved material settles to the bottom. Transfer the top clear solution into HPLC vials, and use as the *Sample solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 268 nm

▲ Detector

Assay: UV 268 nm

Identification A: Diode array, UV 240–340 nm

▲ USP40

Column: 4.6-mm × 150-cm; packing L7

Column temperature: Maintained at room temperature

▲ ▲ USP40

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

▲ [Note—The relative retention times for the phenoxybenzamine peak and the known degradant, tertiary amine phenoxybenzamine, peak are about 1.0 and 0.3, respectively.]

▲ USP40

Suitability requirements

Resolution: NLT 4 between phenoxybenzamine and the unknown peak eluting after the phenoxybenzamine peak (at about 9.4 min), *System suitability solution*

Relative standard deviation: NMT 2%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Phenoxybenzamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phenoxybenzamine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **Dissolution** (711)

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 1: 100 rpm

Time: 45 min

Buffer: 2.2 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.00 ± 0.05 .

Mobile phase: Buffer and acetonitrile (9:11)

Standard solution: 0.02 mg/mL of USP Phenoxybenzamine Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 268 nm

Column: 4.6-mm \times 150-cm; packing L7

Column temperature: Maintained at room temperature

▲▲USP40

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2%

Calculate the percentage of the labeled amount of phenoxybenzamine hydrochloride dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the USP Phenoxybenzamine Hydrochloride RS from the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of *Medium*, 500 mL

Tolerances: NLT 75% (Q) of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$) is dissolved.

Change to read:

- **Uniformity of Dosage Units** (905)

Procedure for content uniformity

Solution A, Mobile phase, Standard solution, and Chromatographic system:

~~Proceed as directed in the Assay.~~

Sample solution: ~~0.2 mg/mL of phenoxybenzamine hydrochloride in water prepared as follows: Carefully open 10 Capsules, and transfer each immediately into separate volumetric flasks, including the Capsule shells. Add acetonitrile, to about 60% of the volume of the flask, and sonicate for 15 min with occasional stirring. Cool the flask, and dilute with acetonitrile to volume to achieve a concentration of 0.2 mg/mL. [Note—The Capsule shell does not dissolve. Cool, dilute with acetonitrile to volume, mix, and pass through a nylon membrane filter of 0.45- μ m pore size, discarding the first few mL of the filtrate.]~~

Analysis

~~**Samples:** *Standard solution* and *Sample solution*~~

Calculate the quantity of phenoxybenzamine hydrochloride in the Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Phenoxybenzamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of phenoxybenzamine hydrochloride in the *Sample solution*, based on the labeled quantity per Capsule and the extent of dilution (mg/mL)

F = labeled quantity of phenoxybenzamine hydrochloride in the Capsules taken (mg)



Acceptance criteria: Meet the requirements

IMPURITIES

- **Organic Impurities**

Solution A, Mobile phase, Standard solution, System suitability solution, Sample

solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of the individual impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

F = relative response factor, 1.1 for phenoxybenzamine tertiary amine, and 1.0 for all other individual impurities

Acceptance criteria

Individual impurities: NMT 0.5% of phenoxybenzamine tertiary amine; NMT 0.1% of any other specified or unspecified individual impurity (degradant)

Total impurities: NMT 0.5%, includes both specified and unspecified

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

- **USP Reference Standards** <11>

USP Phenoxybenzamine Hydrochloride RS

BRIEFING

Prompt Phenytoin Sodium Capsules, *USP 38* page 4869. It is proposed omit this dosage form monograph from *USP* for the following reasons:

1. This dosage form is not used for human use in the United States.
2. This dosage form is not used currently in veterinary medicine in the United States.

(CHM4: R. Ravichandran.)

Correspondence Number—C162941

Comment deadline: January 31, 2016

Delete the following:

▲ Prompt Phenytoin Sodium Capsules

» Prompt Phenytoin Sodium Capsules contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{15}H_{11}N_2NaO_2$.

Packaging and storage—Preserve in tight containers.

Labeling—Label the Capsules with the statement "Not for once-a-day dosing," printed immediately under the official name, in a bold and contrasting color and/or enclosed within a box.

~~USP Reference standards (11)~~~~USP Phenytoin RS~~~~USP Phenytoin Sodium RS~~~~Identification~~~~**A:** The contents of Capsules respond to *Identification test A* under *Phenytoin Sodium*.~~~~**B:** The contents of Capsules respond to the flame test for *Sodium* (191).~~~~Dissolution (711)~~~~Medium: water; 900 mL.~~~~Apparatus 1: 50 rpm.~~~~Time: 30 minutes.~~~~**Procedure**— Determine the amount of $C_{15}H_{11}N_2NaO_2$ dissolved by measuring the UV absorbance at 258 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium* if necessary, in comparison with a Standard solution having a known concentration of *USP Phenytoin Sodium RS* in the same *Medium*.~~~~**Tolerances**— Not less than 85% (Q) of the labeled amount of $C_{15}H_{11}N_2NaO_2$ is dissolved in 30 minutes.~~~~**Uniformity of dosage units** (905): meet the requirements.~~~~**Procedure for content uniformity**— Proceed as directed in the test for *Uniformity of dosage units* under *Extended Phenytoin Sodium Capsules*.~~~~**Assay**— Proceed with Capsules as directed in the *Assay* under *Extended Phenytoin Sodium Capsules*. ▲USP40~~**BRIEFING**

Potassium Gluconate, USP 38 page 4941. In preparation for the omission of the flame tests from *Identification Tests—General* (191), proposed in PF 41(2) [Mar.–Apr. 2015], it is proposed to delete the reference to (191) under *Identification test B*, and replace it with a standalone potassium flame test. It is proposed to delete the test for *Heavy Metals* (231) under *Impurities*.

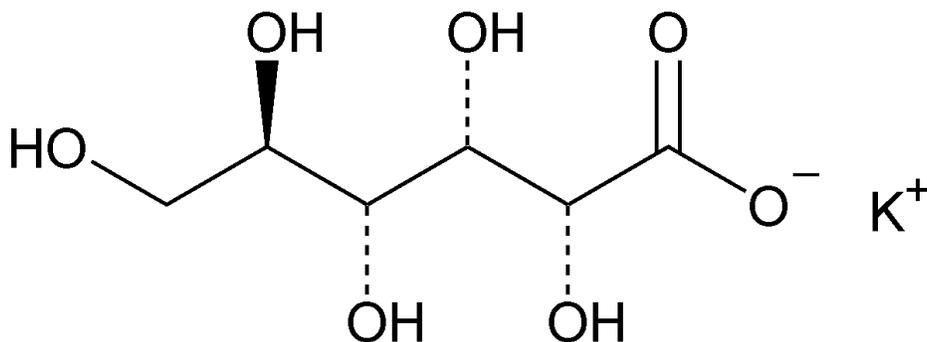
Additionally, minor editorial changes have been made to update the monograph to current USP style.

(NBDS: H. Dinh.)

Correspondence Number—C164268

Comment deadline: January 31, 2016

Potassium Gluconate



$C_6H_{11}KO_7$ 234.25
 $C_6H_{11}KO_7 \cdot H_2O$ 252.26

d-Gluconic acid, monopotassium salt;
 Monopotassium d-gluconate.
 Anhydrous [299-27-4].
 Monohydrate [35398-15-3].

DEFINITION

Potassium Gluconate is anhydrous or contains one molecule of water of hydration. It contains NLT 97.0% and NMT 103.0% of anhydrous potassium gluconate ($C_6H_{11}KO_7$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197M)

Delete the following:

- ▲ • ~~**B. Identification Tests—General, Potassium** (191): Meets the requirements of the flame test~~ ▲*USP40*

Add the following:

- ▲ • **B.** Potassium Gluconate imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.] ▲*USP40*

- **C. Thin-Layer Chromatography**

Standard solution: 10 mg/mL of USP Potassium Gluconate RS

Sample solution: 10 mg/mL of Potassium Gluconate

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 μ L

Developing solvent system: *Alcohol, ethyl acetate, ammonium hydroxide, and water* (50:10:10:30)

Spray reagent: Dissolve 2.5 g of *ammonium molybdate* in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of *ceric sulfate*, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: *Standard solution and Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with the *Spray reagent*. Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F to that of the *Standard solution*.

ASSAY

• Procedure

Standard stock solution: Transfer 190.7 mg of *potassium chloride*, previously dried at 105° for 2 h, to a 1000-mL volumetric flask, add sufficient *water* to dissolve, and dilute with *water* to volume. Transfer 100.0 mL of this solution to a 1000-mL volumetric flask, and dilute with *water* to volume. This solution contains 10 µg/mL of potassium (equivalent to 19.07 µg/mL of potassium chloride).

Standard solutions: Transfer 10.0, 15.0, and 20.0 mL of *Standard stock solution* to separate 100-mL volumetric flasks. Add 2.0 mL of a 200-mg/mL *sodium chloride* solution and 1.0 mL of *hydrochloric acid* to each flask. Dilute with *water* to volume and mix. The *Standard solutions* contain 1.0, 1.5, and 2.0 µg/mL of potassium, respectively.

Sample stock solution: 0.18 mg/mL of Potassium Gluconate in *water*. Filter the solution.

Sample solution: Transfer 5.0 mL of the filtrate from the *Sample stock solution* to a 100-mL volumetric flask. Add 2.0 mL of a 200-mg/mL *sodium chloride* solution and 1.0 mL of *hydrochloric acid*, and dilute with *water* to volume.

Blank: *Water*

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 766.5 nm

Lamp: Potassium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions and Sample solution*

Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbance of the *Standard solutions* versus their concentrations, in µg/mL, of potassium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C_K , in µg/mL, of potassium in the *Sample solution*.

Calculate the percentage of potassium gluconate ($C_6H_{11}KO_7$) in the portion of Potassium Gluconate taken:

$$\text{Result} = (C_K/C_U) \times (M_r/A_r) \times 100$$

C_K = determined concentration of potassium in the *Sample solution* ($\mu\text{g/mL}$)

C_U = concentration of Potassium Gluconate in the *Sample solution* ($\mu\text{g/mL}$)

M_r = molecular weight of potassium gluconate, 234.25

A_r = atomic weight of potassium, 39.10

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

Delete the following:

- **Heavy Metals, Method I (231)**

Test preparation: 1.0 g in 10 mL of water. Add 6 mL of 3 N hydrochloric acid, and dilute with water to 25 mL.

Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

- **Reducing Substances**

Sample: 1.0 g of Potassium Gluconate

Blank: 10 mL of water

Titrimetric system

(See *Titrimetry* (541).)

Mode: Residual titration

Titrant: *Iodine*

Back titrant: *Sodium thiosulfate*

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 250-mL conical flask, dissolve in 10 mL of *water*, and add 25 mL of *alkaline cupric citrate TS*. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N *acetic acid*, 10.0 mL of *Titrant*, and 10 mL of 3 N *hydrochloric acid*, and titrate with *Back titrant*, adding 3 mL of *starch TS* as the endpoint is approached. Perform the *Blank* determination.

Calculate the percentage of reducing substances (as dextrose) in the portion of the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F]/W\} \times 100$$

V_B = *Back titrant* volume consumed by the *Blank* (mL)

V_S = *Back titrant* volume consumed by the *Sample* (mL)

N = actual *Back titrant* normality (mEq/mL)

F = equivalency factor, 27 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

- **Loss on Drying** (731)

Analysis: Dry under vacuum at 105° for 4 h.

Acceptance criteria

Anhydrous: NMT 3.0%

Monohydrate: 6.0%–7.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label it to indicate whether it is the anhydrous or the monohydrate form.
- **USP Reference Standards** (11)

USP Potassium Gluconate RS

BRIEFING

Potassium Gluconate Oral Solution, *USP 38* page 4943. In preparation for the omission of the flame tests from *Identification Tests—General* (191), proposed in *PF 41(2)* [Mar.–Apr. 2015], the reference to (191) in *Identification* test *A* is deleted and a complete description of the flame test is included.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(NBDS: N. Davydova.)

Correspondence Number—C164837

Comment deadline: January 31, 2016

Potassium Gluconate Oral Solution**DEFINITION**

Potassium Gluconate Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of potassium gluconate (C₆H₁₁KO₇).

IDENTIFICATION**Delete the following:**

- ▲ ● ~~**A. Identification Tests—General**, *Potassium* (191): Meets the requirements of the flame test ▲*USP40*~~

Add the following:

- ▲ ● **A.** Potassium Gluconate Oral Solution imparts a violet color to a nonluminous flame. The presence of small quantities of sodium can mask the color unless the yellow color produced by sodium is screened out by viewing through a blue filter that blocks emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.] ▲*USP40*

- **B.**

Analysis: Evaporate 5 mL on a steam bath to dryness.

Acceptance criteria: A mineral oil dispersion of the residue exhibits an IR absorption maximum in the spectral region of 6.2–6.25 μm (carboxylic acid salt).

ASSAY

• Procedure

Standard stock solution: Transfer 190.7 mg of *potassium chloride*, previously dried at 105° for 2 h, to a 1000-mL volumetric flask, add sufficient water to dissolve, and dilute with water to volume. Transfer 100.0 mL of this solution to a 1000-mL volumetric flask, and dilute with water to volume. This solution contains 10 $\mu\text{g/mL}$ of potassium (equivalent to 19.07 $\mu\text{g/mL}$ of potassium chloride).

Standard solutions: Transfer 10.0, 15.0, and 20.0 mL of *Standard stock solution* to separate 100-mL volumetric flasks. To each flask add 2.0 mL of a 200-mg/mL sodium chloride solution and 1.0 mL of hydrochloric acid. Dilute with water to volume, and mix. The *Standard solutions* contain 1.0, 1.5, and 2.0 $\mu\text{g/mL}$ of potassium, respectively.

Sample solution: Transfer an accurately measured volume of Oral Solution, equivalent to 1.8 g of potassium gluconate, to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of the solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Blank: Water

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 766.5 nm

Lamp: Potassium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions* and *Sample solution*

Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbance of the *Standard solutions* versus their concentrations, in $\mu\text{g/mL}$, of potassium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C_K , in $\mu\text{g/mL}$, of potassium in the *Sample solution*. Calculate the percentage of the labeled amount of potassium gluconate ($\text{C}_6\text{H}_{11}\text{KO}_7$) in the portion of Oral Solution taken:

$$\text{Result} = (C_K/C_U) \times (M_r/A_r) \times 100$$

C_K = concentration of potassium in the *Sample solution*, determined from the linear regression line ($\mu\text{g/mL}$)

C_U = nominal concentration of potassium gluconate in the *Sample solution* ($\mu\text{g/mL}$)

M_r = molecular weight of potassium gluconate, 234.25

A_r = atomic weight of potassium, 39.10

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

- **Alcohol Determination** (611), *Method II*: 4.5%–5.5% of alcohol (C₂H₅OH)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

BRIEFING

Potassium Gluconate Tablets, *USP 38* page 4943. In preparation for the omission of the flame tests from *Identification Tests—General* (191), proposed in *PF 41(2)* [Mar.–Apr. 2015], it is proposed to delete the reference to (191) under *Identification* test *B*, and replace it with a complete description of the flame test.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(NBDS: N. Davydova.)

Correspondence Number—C164838

Comment deadline: January 31, 2016

Potassium Gluconate Tablets

DEFINITION

Potassium Gluconate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of potassium gluconate (C₆H₁₁KO₇).

IDENTIFICATION

- **A. Infrared Absorption** (197M): The IR absorption spectrum of potassium gluconate extracted from finely powdered Tablets exhibits maxima only at the same wavelengths as those of a similar preparation of USP Potassium Gluconate RS.

Delete the following:

- ▲ • ~~**B. Identification Tests—General**, *Potassium* (191)~~

~~**Sample solution:** Triturate a portion of powdered Tablets with a few mL of water, and filter.~~

~~**Acceptance criteria:** The filtrate meets the requirements of the flame test. ▲*USP40*~~

Add the following:

- ▲ • **B.**

Sample solution: Triturate a portion of powdered Tablets with a few mL of water, and filter.

Acceptance criteria: The *Sample solution* imparts a violet color to a nonluminous flame; the presence of small quantities of sodium masks the color, unless the yellow color

produced by sodium is screened out by viewing through a blue filter that blocks the emission at 589 nm (sodium); it is transparent to the emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]▲USP40

ASSAY

• Procedure

Standard stock solution: 19.07 µg/mL of potassium chloride in water (equivalent to 10 µg/mL of potassium), prepared from *potassium chloride* previously dried at 105° for 2 h

Standard solutions: 1.0, 1.5, and 2.0 µg/mL of potassium from suitably diluted *Standard stock solution*, in a solution containing 4 mg/mL of sodium chloride and 1 mL of hydrochloric acid per 100 mL

Sample stock solution: Filtered water solution containing 0.18 mg/mL of potassium gluconate from NLT 20 finely powdered Tablets

Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 100-mL volumetric flask. Add 2.0 mL of a 200-mg/mL sodium chloride solution and 1.0 mL of hydrochloric acid, and dilute with water to volume.

Blank: Water

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 766.5 nm

Lamp: Potassium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions* and *Sample solution*

Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbance of the *Standard solutions* versus their concentrations, in µg/mL, of potassium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C , in µg/mL, of potassium in the *Sample solution*. Calculate the percentage of the labeled amount of potassium gluconate ($C_6H_{11}KO_7$) in the portion of Tablets taken:

$$\text{Result} = (C/C_U) \times (M_r/A_r) \times 100$$

C = determined concentration of potassium in the *Sample solution* (µg/mL)

C_U = nominal concentration of potassium gluconate in the *Sample solution* (µg/mL)

M_r = molecular weight of potassium gluconate, 234.25

A_r = atomic weight of potassium, 39.10

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **Dissolution** <711>

Medium: Water; 900 mL

Apparatus 2: 100 rpm

Time: 45 min

Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium* if necessary

Analysis: Proceed as directed in the *Assay*.

Calculate the percentage of the labeled amount of potassium gluconate ($C_6H_{11}KO_7$) dissolved:

$$\text{Result} = (C \times D \times V/L) \times (M_r/A_r) \times 100$$

C = determined concentration of potassium in the *Sample solution* (mg/mL)

D = dilution factor for the *Sample solution*

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

M_r = molecular weight of potassium gluconate, 234.25

A_r = atomic weight of potassium, 39.10

Tolerances: NLT 75% (Q) of the labeled amount of potassium gluconate ($C_6H_{11}KO_7$) is dissolved.

- **Uniformity of Dosage Units** <905>: Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

- **USP Reference Standards** <11>

USP Potassium Gluconate RS

BRIEFING

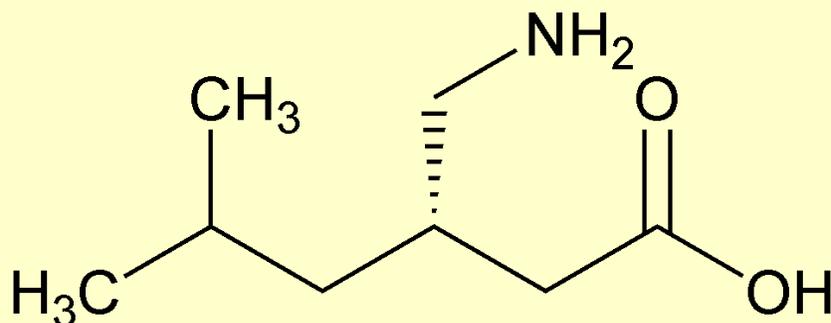
Pregabalin. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods of analysis, is proposed.

1. The liquid chromatographic procedure in the *Assay* and the test for *Organic Impurities* is based on analyses performed with the Inertsil ODS-3V brand of L1 column. The typical retention times for pregabalin in the *Assay* and the test for *Organic Impurities* are about 12 and 11 min, respectively.
2. The liquid chromatographic procedure in the test for *Enantiomeric Purity* is based on analyses performed with the Hypersil BDS C18 brand of L1 column. The typical retention time for pregabalin is about 9.5–11.5 min.

(CHM2: H. Cai.)

Correspondence Number—C136074; C137864

Comment deadline: January 31, 2016

Add the following:**▲ Pregabalin**

$C_8H_{17}NO_2$ 159.23

(S)-3-(Aminomethyl)-5-methylhexanoic acid [148553-50-8].

DEFINITION

Pregabalin contains NLT 98.0% and NMT 102.0% of pregabalin ($C_8H_{17}NO_2$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Mobile phase: Acetonitrile and water (5:95)

Standard solution: 2.0 mg/mL of USP Pregabalin RS in *Mobile phase*

Sample solution: 2.0 mg/mL of Pregabalin in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 25-cm; 5- μ m packing *L1*

Temperatures

Autosampler: 10°

Column: 25°

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

Run time: NLT 2.5 times the retention time of pregabalin

System suitability**Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 0.73%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of pregabalin ($C_8H_{17}NO_2$) in the portion of Pregabalin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of pregabalin from the *Sample solution* r_S = peak response of pregabalin from the *Standard solution* C_S = concentration of USP Pregabalin RS in the *Standard solution* (mg/mL) C_U = concentration of Pregabalin in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the dried basis**IMPURITIES**

- **Residue on Ignition** (281): NMT 0.1%
- **Chloride and Sulfate** (221), *Chloride*

Standard solution: Proceed as directed in the chapter using 10.0 mL.**Sample solution:** Proceed as directed in the chapter using 0.10 g of Pregabalin.**Acceptance criteria:** The turbidity produced in the *Sample solution* is NMT that produced in the *Standard solution* (0.1%).

- **Organic Impurities**

Buffer: 0.04 M dibasic ammonium phosphate. Adjust with *phosphoric acid* to a pH of 6.5.**Solution A:** Methanol and *Buffer* (20:80)**Solution B:** Methanol and acetonitrile (10:90)**Mobile phase:** See *Table 1*.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	98	2
5	98	2
16	80	20
33	80	20
34	98	2
45	98	2

Standard solution: 0.01 mg/mL of USP Mandelic Acid RS and 0.05 mg/mL each of USP Pregabalin RS and USP Pregabalin Related Compound C RS in water

Sample solution: 10.0 mg/mL of Pregabalin in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 35°

Flow rate: 1.0 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5% for mandelic acid, pregabalin, and pregabalin related compound C

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mandelic acid and pregabalin related compound C in the portion of Pregabalin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mandelic acid or pregabalin related compound C from the *Sample solution*

r_S = peak response of mandelic acid or pregabalin related compound C from the *Standard solution*

C_S = concentration of USP Mandelic Acid RS or USP Pregabalin Related Compound C RS in the *Standard solution* (mg/mL)

C_U = concentration of Pregabalin in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Pregabalin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of pregabalin from the *Standard solution*

C_S = concentration of USP Pregabalin RS in the *Standard solution* (mg/mL)

C_U = concentration of Pregabalin in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peaks below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mandelic acid	0.66	—	0.10
Isobutyglutaric acid ^a	0.85	2.1	0.15
Pregabalin	1.0	1.0	—
Isobutyglutarmonoamide ^b	1.52	3.3	0.15
Pregabalin related compound C	3.95	—	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.8
^a 3-Isobutylpentanedioic acid. ^b 3-(2-Amino-2-oxoethyl)-5-methylhexanoic acid.			

• Enantiomeric Purity

Buffer: *Triethylamine* and water (7.2: 1000). Adjust with 50% (v/v) phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (38:62)

Derivatizing reagent solution: 3 mg/mL of *Marfey's reagent* in *acetone*

Standard stock solution: 1 mg/mL of USP Pregabalin RS and 0.05 mg/mL of USP Pregabalin Related Compound A RS prepared as follows. Transfer appropriate amounts of USP Pregabalin RS and USP Pregabalin Related Compound A RS to a suitable volumetric flask. Dissolve in 50% of the final volume of water and dilute with *acetone* to volume.

Standard solution: Transfer 0.5 mL of the *Standard stock solution* to a 5.0-mL volumetric flask, add 0.3 mL of the *Derivatizing reagent solution* and 50 µL of 1 M sodium bicarbonate, and heat at 55° for about 1 h. Allow to cool to room temperature and dilute with water to volume.

Sample stock solution: 1 mg/mL of Pregabalin prepared as follows. Transfer an appropriate amount of Pregabalin to a suitable volumetric flask. Dissolve in 50% of the final volume of water and dilute with *acetone* to volume.

Sample solution: Transfer 0.5 mL of the *Sample stock solution* to a 5.0-mL volumetric flask, add 0.3 mL of the *Derivatizing reagent solution* and 50 µL of 1 M sodium bicarbonate, and heat at 55° for about 1 h. Allow to cool to room temperature and dilute with water to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 340 nm

Column: 4.6-mm × 25-cm; 5-µm packing *L1*

Temperatures

Autosampler: 10°

Column: 25°

Flow rate: 2.0 mL/min

Injection volume: 20 µL

Run time: NLT 3.8 times the retention time of pregabalin

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between pregabalin and pregabalin related compound A

Relative standard deviation: NMT 5.0% for pregabalin related compound A

Analysis

Sample: *Sample solution*

Calculate the percentage of pregabalin related compound A in the portion of Pregabalin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of pregabalin related compound A from the *Sample solution*

r_T = sum of peak responses of pregabalin and pregabalin related compound A from the *Sample solution*

Acceptance criteria: NMT 0.15%

SPECIFIC TESTS

- **Loss on Drying** (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Protect from light and store at NMT 25°.

- **USP Reference Standards** (11)

USP Mandelic Acid RS

USP Pregabalin RS

USP Pregabalin Related Compound A RS

(*R*)-3-(Aminomethyl)-5-methylhexanoic acid.

C₈H₁₇NO₂ 159.23

USP Pregabalin Related Compound C RS

4-Isobutylpyrrolidin-2-one.

C₈H₁₅NO 141.21

▲USP40

BRIEFING

Pseudoephedrine Sulfate, *USP 38* page 5080. As part of the USP monograph modernization initiative, and on the basis of comments received, it is proposed to make the following changes:

1. Add the chemical structure of pseudoephedrine sulfate to the monograph.

2. Revise the upper limit of the *Definition* from 100.5% to 102.0% to be consistent with the customary range afforded to HPLC assays of bulk active ingredients.
3. Replace the nonselective titration *Assay* with a stability-indicating HPLC-based method. The proposed liquid chromatographic procedure is based on analyses performed with the Pinnacle DB Biphenyl brand of L11 column. The typical retention time for pseudoephedrine is about 4 min.
4. Replace *Identification* test *B* that is based on spectroscopic absorption with an orthogonal test based on HPLC retention time in the *Assay*.
5. Replace the test for *Ordinary Impurities* with a stability-indicating HPLC procedure. The proposed liquid chromatographic procedure is based on analyses performed with the Pinnacle DB Biphenyl brand of L11 column. The typical retention times for ephedrine and pseudoephedrine are about 3.2 and 3.9 min, respectively.
6. Delete the test for *Melting Range or Temperature* because the proposed test for *Organic Impurities* and other tests adequately establish the identity, purity, and quality of the drug substance.
7. USP Ephedrine Sulfate RS is added to the *USP Reference Standards* section to support the proposed procedures.

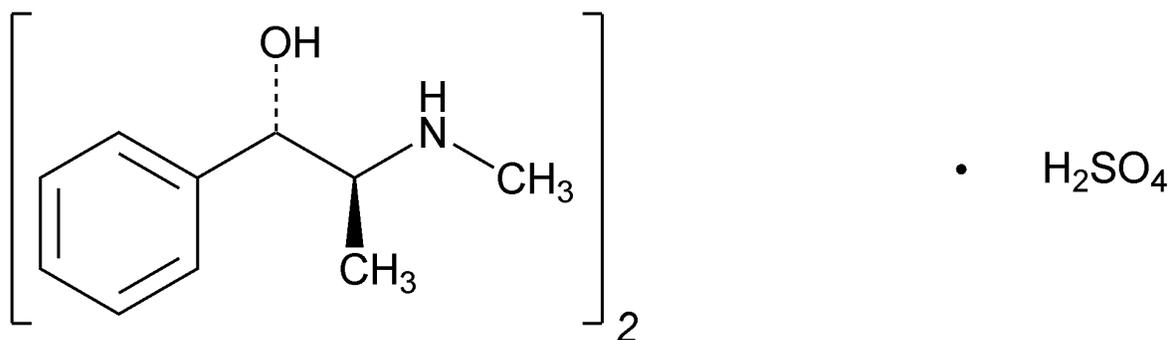
Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM6: A. Potts.)

Correspondence Number—C109903

Comment deadline: January 31, 2016

Pseudoephedrine Sulfate



$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ 428.54

Benzenemethanol, α -[1-(methylamino)ethyl]-, [*S*-(*R**,*R**)]-, sulfate (2:1) (salt);
(+)-Pseudoephedrine sulfate (2:1) (salt) [7460-12-0].

DEFINITION

Change to read:

Pseudoephedrine Sulfate contains NLT 98.0% and NMT ~~100.5%~~

~~▲~~ 102.0% ~~▲~~ *USP40*

of pseudoephedrine sulfate $[(C_{10}H_{15}NO)_2 \cdot H_2SO_4]$, calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)

Delete the following:

- ▲ • ~~**B. Ultraviolet Absorption** (197U)~~

~~**Standard solution:** 500 µg/mL of USP Pseudoephedrine Sulfate RS~~

~~**Sample solution:** 500 µg/mL of Pseudoephedrine Sulfate~~

~~**Acceptance criteria:** Absorptivities at 257 nm, calculated on the dried basis, differ by NMT 3.0%. ▲*USP40*~~

Add the following:

- ▲ • **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP40*

- **C. Identification Tests—General** (191), *Sulfate*: A 100-mg/mL solution meets the requirements.

ASSAY**Change to read:**

- **Procedure**

~~**Sample solution:** Dissolve about 150 mg of Pseudoephedrine Sulfate in 50 mL of glacial acetic acid~~

~~**Titrimetric system**~~

~~**Mode:** Direct titration~~

~~**Titrant:** 0.1 N perchloric acid VS~~

~~**Endpoint detection:** Potentiometric~~

~~**Analysis:** Titrate the *Sample solution* with *Titrant* and determining the endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 42.85 mg of pseudoephedrine sulfate ($C_{10}H_{15}NO$)₂·H₂SO₄.~~

~~**Acceptance criteria:** 98.0%–100.5% on the dried basis~~

▲ **Solution A:** 0.4% Triethylamine, pH 4.0. Add 4.0 mL of *triethylamine* to a 1-L volumetric flask and dilute with approximately 900 mL of water. Adjust to a pH of 4.0 with *phosphoric acid*. Dilute with water to volume, and filter.

Mobile phase: *Acetonitrile* and *Solution A* (1:99)

System suitability solution: 0.01 mg/mL of USP Ephedrine Sulfate RS and 0.1 mg/mL of USP Pseudoephedrine Sulfate RS in water

Standard solution: 0.1 mg/mL of USP Pseudoephedrine Sulfate RS in water

Sample solution: 0.1 mg/mL of Pseudoephedrine Sulfate in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210

Column: 2.1-mm × 10-cm; 1.9-µm packing *L11*

Flow rate: 0.4 mL/min

Injection volume: 5 µL

Run time: NLT 2 times the retention time of pseudoephedrine

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between ephedrine and pseudoephedrine peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pseudoephedrine sulfate [(C₁₀H₁₅NO)₂·H₂SO₄] in the portion of Pseudoephedrine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pseudoephedrine from the *Sample solution*

r_S = peak response of pseudoephedrine from the *Standard solution*

C_S = concentration of USP Pseudoephedrine Sulfate RS in the *Standard solution* (mg/mL)

C_U = concentration of Pseudoephedrine Sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ▲*USP40*

- **Residue on Ignition** (281): NMT 0.1%

- **Chloride and Sulfate** (221), *Chloride*

Sample: 200 mg of Pseudoephedrine Sulfate

Acceptance criteria: The *Sample* shows no more chloride than corresponds to 0.4 mL of 0.02 N hydrochloric acid (0.14%).

Delete the following:

- * • **Heavy Metals** (231)

Standard solution: 10 ppm of *standard lead solution*

Sample solution: 1.0 g of Pseudoephedrine Sulfate in 20 mL of *diluted alcohol*

Sodium hydroxide solution: 1.0 g of *sodium hydroxide* in 20 mL of *water*

Analysis: Treat the *Sample solution* with 5 mL of *Sodium hydroxide solution* and 5 drops of *sodium sulfide TS*.

Acceptance criteria: The color developed is not darker than that of the *Standard solution* performed simultaneously. • (Official 1-Jan-2018)

Change to read:

- **Organic Impurities**

Procedure: Ordinary Impurities (466)**Standard solution:** Prepare in alcohol.**Sample solution:** Prepare in alcohol.**Eluant:** Alcohol, glacial acetic acid, and water (10:3:1)**Visualization:** Expose the plate for 24 h to iodine vapors in a pre-equilibrated closed chamber, on the bottom of which are iodine crystals.

▲ **Solution A, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the Assay except for the *Injection volume* as indicated below.

Standard solution: 0.5 µg/mL each of USP Pseudoephedrine Sulfate RS and USP Ephedrine Sulfate RS in water**Sample solution:** 1 mg/mL of Pseudoephedrine Sulfate in water**Injection volume:** 10 µL**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 2.0 between ephedrine and pseudoephedrine sulfate, *System suitability solution***Relative standard deviation:** NMT 5%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Inject water after the *Standard solution* to prevent carry over.

Calculate the percentage of ephedrine in the portion of Pseudoephedrine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (2M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of ephedrine from the *Sample solution* r_S = peak response of ephedrine from the *Standard solution* C_S = concentration of USP Ephedrine Sulfate RS in the *Standard solution* (mg/mL) C_U = concentration of Pseudoephedrine Sulfate in the *Sample solution* (mg/mL) M_{r1} = molecular weight of ephedrine, 165.23 M_{r2} = molecular weight of ephedrine sulfate, 428.54

Calculate the percentage of any individual unspecified impurity in the portion of Pseudoephedrine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of each individual unspecified impurity from the *Sample solution* r_S = peak response of pseudoephedrine from the *Standard solution* C_S = concentration of USP Pseudoephedrine Sulfate RS in the *Standard solution* (mg/mL) C_U = concentration of Pseudoephedrine Sulfate in the *Sample solution* (mg/mL)

▲USP40

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ephedrine	0.8	0.2
Pseudoephedrine	1.0	—
Individual unspecified impurity	—	0.10
Total impurities	—	0.3

SPECIFIC TESTS

Delete the following:

▲ ● Melting Range or Temperature, Class I (741)

Acceptance criteria: 174°–179°, but the range between beginning and end of melting is NMT 2° ▲*USP40*

● **Optical Rotation (781S), Procedures, Specific Rotation**

Sample solution: 50 mg/mL in water

Acceptance criteria: +56.0° to +59.0°

● **pH (791)**

Sample solution: 50 mg/mL

Acceptance criteria: 5.0–6.5

● **Loss on Drying (731)**

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

● **USP Reference Standards (11)**

▲ **USP Ephedrine Sulfate RS** ▲*USP40*

USP Pseudoephedrine Sulfate RS

BRIEFING

Riboflavin Tablets, USP 38 page 5165. It is proposed to revise the monograph to be consistent with the recent revision to *Riboflavin Assay (481)*, also published in this issue of *PF*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(NBDS: N. Davydova.)

Correspondence Number—C165267

Comment deadline: January 31, 2016

Riboflavin Tablets

DEFINITION

Riboflavin Tablets contain NLT 95.0% and NMT 115.0% of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$).

ASSAY

Change to read:

- **Riboflavin Assay** (481),
▲ *Chemical Methods, Procedure 1* ▲*USP40*

Assay preparation:

▲ **Sample solution:** ▲*USP40*

Transfer a portion of the powder from NLT 20 finely powdered Tablets, equivalent to 20 mg of riboflavin, to a 250-mL flask, and add 150 mL of 0.1 N *hydrochloric acid*. Shake vigorously, and wash down the sides of the flask with sufficient 0.1 N *hydrochloric acid* to ensure that the pH remains below 1.5 during the subsequent period of heating. Heat the mixture on a steam bath, with frequent agitation, until the riboflavin has dissolved, or in an autoclave at 121° for 30 min. Cool, and with vigorous agitation, adjust the mixture with 1 N *sodium hydroxide* to a pH of 5–6. Transfer to a 1000-mL volumetric flask, and dilute with water to volume. If the solution is not clear, filter through paper known not to adsorb riboflavin. Dilute an aliquot of the clear solution with water to a final volume that contains 0.1 µg/mL of riboflavin.

Analysis: Proceed as directed in the chapter.

Calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of Tablets taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = corrected fluorescence value from the ~~Assay Preparation:~~

▲ *Sample solution* ▲*USP40*

I_S = corrected fluorescence value from the ~~Standard Preparation:~~

▲ *Standard solution* ▲*USP40*

C_S = concentration of USP Riboflavin RS in the ~~Standard Preparation:~~

▲ *Standard solution* ▲*USP40*
(µg/mL)

C_U = nominal concentration of riboflavin in the ~~Assay Preparation:~~

▲ *Sample solution* ▲*USP40*
(µg/mL)

Acceptance criteria: 95.0%–115.0%

PERFORMANCE TESTS

- **Dissolution** (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: Known concentration of USP Riboflavin RS in *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

Mobile phase: A mixture of *methanol*, *glacial acetic acid*, and water (27:1:73) containing 1.40 mg/mL of *sodium 1-hexanesulfonate*

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V/L) \times 100$$

r_U = peak area of riboflavin from the *Sample solution*

r_S = peak area of riboflavin from the *Standard solution*

C_S = concentration of USP Riboflavin RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) is dissolved.

- **Uniformity of Dosage Units** <905>: Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** <11>

USP Riboflavin RS

BRIEFING

Ringer's Injection, *USP 38* page 5182. In preparation for the omission of the flame tests

from *Identification Tests—General* (191), proposed in PF 41(2) [Mar.–Apr. 2015], it is proposed to delete the references to *Sodium* and *Potassium* under *Identification* test A and include a complete description of each flame test in the monograph.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CMP: J. Sun.)

Correspondence Number—C164384

Comment deadline: January 31, 2016

Ringer's Injection

DEFINITION

Ringer's Injection is a sterile solution of Sodium Chloride, Potassium Chloride, and Calcium Chloride in Water for Injection. It contains, in each 100 mL, NLT 323.0 mg and NMT 354.0 mg of sodium (Na) [equivalent to NLT 820.0 mg and NMT 900.0 mg of sodium chloride (NaCl)]; NLT 14.9 mg and NMT 16.5 mg of potassium (K) [equivalent to NLT 28.5 mg and NMT 31.5 mg of potassium chloride (KCl)]; NLT 8.20 mg and NMT 9.80 mg of calcium (Ca) [equivalent to NLT 30.0 mg and NMT 36.0 mg of calcium chloride (CaCl₂·2H₂O)]; and NLT 523.0 mg and NMT 580.0 mg of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride (CaCl₂·2H₂O)]. It contains no antimicrobial agents.

[Note—The calcium, chloride, potassium, and sodium ion contents of Ringer's Injection are approximately 4.5, 156, 4, and 147.5 mEq/L, respectively.]

Sodium Chloride	8.6 g
Potassium Chloride	0.3 g
Calcium Chloride	0.33 g
Water for Injection, a sufficient quantity to make	1000 mL

Dissolve the three salts in the *Water for Injection*, filter until clear, place in suitable containers, and sterilize.

IDENTIFICATION

Change to read:

- **A. Identification Tests—General** (191), ~~*Sodium* (flame test), *Potassium* (flame test),~~

▲▲*USP40*

Calcium and *Chloride*: Meets the requirements of the ammonium oxalate test for *Calcium*, and the test for *Chloride*

Add the following:

- ▲● **B. Sodium:** A sample imparts an intense yellow color to a nonluminous flame. ▲*USP40*

Add the following:

- ▲● **C. Potassium:** A sample imparts a violet color to a nonluminous flame. The presence of

small quantities of sodium masks the color unless the yellow color produced by sodium is screened out by viewing through a blue filter that blocks the emission at 589 nm (sodium). It is transparent to emission at 404 nm (potassium). [Note—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]▲USP40

ASSAY

• Calcium

[Note—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Lanthanum chloride solution: Transfer 17.69 g of lanthanum chloride to a 200-mL volumetric flask, add 100 mL of water, and carefully add 50 mL of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Calcium stock solution: 1 mg/mL of calcium (Ca) prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of diluted hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium (Ca) prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Lanthanum chloride solution*, add 1.0, 1.5, and 2.0 mL, respectively, of *Calcium stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 20.0 mL of Injection, equivalent to 1.8 mg of calcium (Ca), to a 100-mL volumetric flask containing 5.0 mL of *Lanthanum chloride solution*. Dilute with water to volume.

Blank: Transfer 5.0 mL of *Lanthanum chloride solution* to a 100-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometer

Emission wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in mg/mL, of calcium, and draw the straight line best fitting the three plotted points. From the graph, determine the concentration (*C*), in mg/mL, of calcium in the *Sample solution*.

Calculate the quantity (mg) of calcium (Ca) in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of calcium in the *Sample solution*, as determined from the graph (mg/mL)

D = dilution factor of the *Sample solution*, 5

F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 8.20–9.80 mg of calcium (Ca) in each 100 mL

- **Potassium**

Solution A: Suitable wetting agent (1 in 500)

Solution B: 10.93 mg/mL of sodium chloride in water

Standard stock solution: 100 µg/mL of potassium prepared as follows. Dissolve 190.7 mg of potassium chloride, previously dried at 105° for 2 h, in 50 mL of water, transfer to a 1-L volumetric flask, and dilute with water to volume.

Standard solutions: 0.005, 0.01, 0.015, and 0.020 mg/mL of potassium prepared as follows. Transfer 10 mL of *Solution B* to each of four 100-mL volumetric flasks containing 10.0 mL of *Solution A*. To each flask add, 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*, respectively, and dilute with water to volume.

Sample solution: Transfer 10 mL of Injection to a 100-mL volumetric flask. Add 10.0 mL of *Solution A*. Dilute with water to volume.

Blank: Transfer 10 mL of *Solution B* to a 100-mL volumetric flask containing 10.0 mL of *Solution A*. Dilute with water to volume.

Instrumental conditions

Mode: Atomic emission spectrophotometry

Emission wavelength: 766 nm

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set the flame photometer for maximum transmittance at a wavelength of 766 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of potassium. Read the percentage transmittance of the *Sample solution*. Calculate the quantity (mg) of potassium (K) in each 100 mL of Injection.

Acceptance criteria: 14.9–16.5 mg of potassium (K) in each 100 mL

- **Sodium**

Solution A: Suitable nonionic wetting agent (1 in 500)

Standard stock solution: 100 µg/mL of sodium in water prepared as follows. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer the resulting solution to a 1-L volumetric flask, and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium prepared as follows. Transfer 10 mL of *Solution A* to each of four 100-mL volumetric flasks. To each flask add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*, respectively, and dilute with water to volume.

Sample solution: Transfer 5 mL of Injection into a 1-L volumetric flask containing 100 mL of *Solution A*. Dilute with water to volume.

Blank: Transfer 10 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Atomic emission spectrophotometry

Emission wavelength: 589 nm

Analysis

Samples: *Standard solutions, Sample solution, and Blank*

Set the flame photometer for maximum transmittance at a wavelength of 589 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of sodium. Read the percentage transmittance of the *Sample solution*. Calculate the quantity (mg) of sodium (Na) in each 100 mL of Injection.

Acceptance criteria: 323.0–354.0 mg of sodium (Na) in each 100 mL

• Chloride

Sample solution: Transfer 10 mL of Injection into a conical flask. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint. Each mL of *Titrant* is equivalent to 3.545 mg of chloride (Cl). Calculate the quantity (mg) of chloride (Cl) in each 100 mL of Injection.

Acceptance criteria: 523.0–580.0 mg of chloride (Cl) in each 100 mL

IMPURITIES

Delete the following:

- ~~• **Heavy Metals** (231): Evaporate 67 mL to a volume of 20 mL, add 2 mL of 1-N acetic acid, and dilute with water to 25 mL: NMT 0.3 ppm. (Official 1-Jan-2018)~~

SPECIFIC TESTS

- **Bacterial Endotoxins Test** (85): It contains NMT 0.5 USP Endotoxin Unit/mL
- **pH** (791): 5.0–7.5
- **Other Requirements:** It meets the requirements under *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **Labeling:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in

mOsmol/mL.

- **USP Reference Standards** (11)
USP Endotoxin RS

BRIEFING

Secobarbital Sodium Injection, *USP 38* page 5269. It is proposed to omit this monograph for the following reasons:

1. No drug products formulated as defined under Secobarbital Sodium Injection are currently marketed in the United States.
2. The drug product is currently not used in veterinary medicine in the United States.

(CHM4: R. Ravichandran.)

Correspondence Number—C162943

Comment deadline: January 31, 2016

Delete the following:

▲ Secobarbital Sodium Injection

» Secobarbital Sodium Injection is a sterile solution of Secobarbital Sodium in a suitable solvent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{12}H_{17}N_2NaO_3$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light, in a refrigerator.

Labeling—The label indicates that the Injection is not to be used if it contains a precipitate.

~~USP Reference standards (11)—~~

~~USP Secobarbital RS~~

~~USP Endotoxin RS~~

Identification—

A: Transfer a volume of Injection, equivalent to about 100 mg of secobarbital sodium, to a separator containing 15 mL of water. Render the mixture distinctly acid to litmus with hydrochloric acid, extract the liberated secobarbital with 25 mL of ether, collect the ether extract in a separator, and wash with 10 mL of water. Discard the water solution. Filter the ether extract into a beaker, and evaporate on a steam bath with the aid of a current of air just to dryness. Dissolve the residue in 3 mL of alcohol, and evaporate to dryness. Repeat the dissolution and evaporation with 3 mL of alcohol, and dry the residue at 100° for 2 hours: the IR absorption spectrum of a solution prepared by dissolving the residue of secobarbital so obtained in chloroform to a concentration of about 50 mg per mL, 0.1-mm sodium chloride cells being used and chloroform being used as the blank, exhibits maxima only at the same wavelengths as that of a similar preparation of *USP Secobarbital RS*.

B: It responds to the flame test for *Sodium* (191).

~~Bacterial endotoxins (85)—~~It contains not more than 0.9 USP Endotoxin Unit per mg of secobarbital sodium.

~~pH (791): between 9.0 and 10.5.~~

~~**Other requirements**—It meets the requirements under *Injections* (1).~~

~~**Assay**~~

~~*Buffer solution*— Dissolve 6.19 g of boric acid and 14.91 g of potassium chloride in water, dilute with water to 200 mL, and mix. After 24 hours, filter if necessary to obtain a clear solution.~~

~~*Standard preparation 1*— Dissolve a suitable quantity of *USP Secobarbital RS*, accurately weighed, in 0.5 N sodium hydroxide to obtain a solution having a known concentration of about 23 µg per mL.~~

~~*Standard preparation 2*— Mix 5.0 mL of *Standard preparation 1* with 5.0 mL of *Buffer solution*.~~

~~*Assay preparation 1*— Transfer an accurately measured volume of *Injection*, equivalent to about 50 mg of secobarbital sodium, to a 100 mL volumetric flask, dilute with 0.5 N sodium hydroxide to volume, and mix. Pipet 5 mL of this solution into a 100 mL volumetric flask, add 0.5 N sodium hydroxide to volume, and mix.~~

~~*Assay preparation 2*— Mix 5.0 mL of *Assay preparation 1* with 5.0 mL of *Buffer solution*.~~

~~*Procedure*— Concomitantly determine the absorbances of *Assay preparation 1* and *Standard preparation 1* in 1-cm cells at 260 nm, with a suitable spectrophotometer, using 0.5 N sodium hydroxide as the blank. Similarly determine the absorbances of *Assay preparation 2* and *Standard preparation 2*, using as the blank a mixture of equal volumes of 0.5 N sodium hydroxide and *Buffer solution*. Calculate the quantity, in mg, of $C_{12}H_{17}N_2NaO_3$ in the volume of *Injection* taken by the formula:~~

$$1.092(2C)(A_U - 2a_U) / (A_S - 2a_S)$$

~~in which 1.092 is the ratio of the molecular weight of sodium secobarbital to that of secobarbital, C is the concentration, in µg per mL, of *USP Secobarbital RS* in *Standard preparation 1*, A_U and A_S are the absorbances of *Assay preparation 1* and *Standard preparation 1*, respectively, and a_U and a_S are the absorbances of *Assay preparation 2* and *Standard preparation 2*, respectively. ▲*USP40*~~

BRIEFING

Secobarbital Sodium for Injection, USP 38 page 5270. It is proposed to omit this monograph for the following reasons:

1. No drug products formulated as defined under Secobarbital Sodium for Injection are currently marketed in the United States.
2. The drug product is currently not used in veterinary medicine in the United States.

(CHM4: R. Ravichandran.)

Correspondence Number—C162944

Comment deadline: January 31, 2016

Delete the following:

▲ **Secobarbital Sodium for Injection**

» ~~Secobarbital Sodium for Injection is Secobarbital Sodium suitable for parenteral use. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of secobarbital sodium ($C_{12}H_{17}N_2NaO_3$).~~

Packaging and storage—~~Preserve in Containers for Sterile Solids as described under Injections (1).~~

~~USP Reference standards (11)—~~

~~USP Secobarbital RS~~

~~USP Endotoxin RS~~

Constituted solution—~~At the time of use, it meets the requirements for Constituted Solutions under Injections (1).~~

~~Bacterial endotoxins (85)—It contains not more than 0.9 USP Endotoxin Unit per mg of secobarbital sodium.~~

Other requirements—~~It conforms to the Definition, responds to the Identification tests, and meets the requirements for pH, Completeness of solution, Loss on drying, Heavy metals, and Assay under Secobarbital Sodium. It meets also the requirements for Sterility Tests (71), Uniformity of Dosage Units (905), and Labeling under Injections (1). ▲USP40~~

BRIEFING

Secobarbital Sodium and Amobarbital Sodium Capsules, USP 38 page 5270. It is proposed to omit this monograph for the following reasons:

1. No drug products formulated as defined under Secobarbital Sodium and Amobarbital Sodium Capsules are currently marketed in the United States.
2. The drug product is currently not used in veterinary medicine in the United States.

(CHM4: R. Ravichandran.)

Correspondence Number—C162945

Comment deadline: January 31, 2016

Delete the following:

▲ **Secobarbital Sodium and Amobarbital Sodium Capsules**

» ~~Secobarbital Sodium and Amobarbital Sodium Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of secobarbital sodium ($C_{12}H_{17}N_2NaO_3$) and amobarbital sodium ($C_{11}H_{17}N_2NaO_3$).~~

Packaging and storage—~~Preserve in well-closed containers.~~

~~USP Reference standards (11)—~~

~~USP Amobarbital RS~~

~~USP Secobarbital RS~~

Identification—

A: ~~Suspend the contents of 1 Capsule in 10 mL of water, and filter: the filtrate responds to the~~

flame test for *Sodium* (191):

B: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those of the *Standard preparation* obtained in the *Assay*.

Dissolution (711):

Medium: water; 500 mL.

Apparatus 1: 100 rpm.

Time: 60 minutes.

Procedure— Determine the total amount of $C_{12}H_{17}N_2NaO_3$ and $C_{11}H_{17}N_2NaO_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 239 nm of filtered portions of the solution under test, suitably diluted with 0.1 N sodium hydroxide, in comparison with a Standard solution having known concentrations of about 7.5 µg each per mL, of *USP Secobarbital RS* and *USP Amobarbital RS* in the same medium. An amount of alcohol not to exceed 1% of the total volume of the Standard solution may be used to dissolve the Reference Standards prior to dilution with water and 0.1 N sodium hydroxide.

Tolerances— Not less than 60% (Q) of the labeled total amount of $C_{12}H_{17}N_2NaO_3$ and $C_{11}H_{17}N_2NaO_3$ is dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements for *Content Uniformity* with respect to secobarbital sodium and to amobarbital sodium.

Assay—

Internal standard solution— Dissolve aprobarbital in chloroform to obtain a solution having a concentration of about 0.75 mg per mL.

Standard preparation— Transfer about 92 mg of *USP Secobarbital RS*, and about 91 mg of *USP Amobarbital RS*, both accurately weighed, to a 100 mL volumetric flask, and dissolve in 50 mL of chloroform. Dilute with chloroform to volume, and mix.

Assay preparation— Remove, as completely as possible, the contents of not less than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of secobarbital sodium, to a separator, add 20 mL of water, 1 mL of hydrochloric acid, and 100.0 mL of chloroform, and shake for 3 minutes. Remove the chloroform layer, and use as directed in the *Procedure*.

Chromatographic system (see *Chromatography* (621))— The gas chromatograph is equipped with a flame ionization detector and contains a 0.6-m × 3.5-mm glass column packed with 3 percent liquid phase G10 on 100 to 120 mesh support S1AB. The column is maintained at about 175°, the injection port at about 235°, the detector block at about 245°, and dry helium is used as the carrier gas at a flow rate of about 55 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2%; the resolution factor between amobarbital and the internal standard is not less than 1.5; the resolution factor between amobarbital and secobarbital is not less than 2.5; and the tailing factor does not exceed 1.5 for any of the three peaks.

Procedure— Mix 5.0 mL of the *Standard preparation* with 5.0 mL of the *Internal standard solution*. Mix 5.0 mL of the *Assay preparation* with 5.0 mL of the *Internal standard solution*. Separately inject equal volumes (about 3 μ L) of the resulting solutions into the chromatograph, and record the chromatograms. Measure the responses for the major peaks. The relative retention times with respect to the internal standard are about 1.3 for amobarbital and 1.8 for secobarbital. Calculate the quantity, in mg, of secobarbital sodium ($C_{12}H_{17}N_2NaO_3$) in the portion of Capsules taken by the formula:

$$(260.27 / 238.28)W(R'_U / R'_S)$$

in which 260.27 and 238.28 are the molecular weights of secobarbital sodium and secobarbital, respectively, W is the weight, in mg, of *USP Secobarbital RS* taken for the *Standard preparation*, and R'_U and R'_S are the ratios of the peak response of secobarbital to that of the internal standard in the *Assay preparation* and the *Standard preparation*, respectively. Similarly calculate the quantity, in mg, of amobarbital sodium ($C_{11}H_{17}N_2NaO_3$) in the portion of Capsules taken by the formula:

$$(248.26 / 226.28)W''(R''_U / R''_S)$$

in which 248.26 and 226.28 are the molecular weights of amobarbital sodium and amobarbital, respectively, W'' is the weight, in mg, of *USP Amobarbital RS* taken for the *Standard preparation*, and R''_U and R''_S are the ratios of the peak response of amobarbital to that of the internal standard obtained from *Assay preparation* and the *Standard preparation*, respectively.

▲USP40

BRIEFING

Selamectin. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods of analysis, is proposed. The HPLC procedures in the *Assay* and in the test for *Organic Impurities* are based on analyses performed with the Waters Nova-Pac C18 brand of L1 column. The typical retention times for selamectin are about 9 min in the *Assay* and about 22 min in the test for *Organic Impurities*.

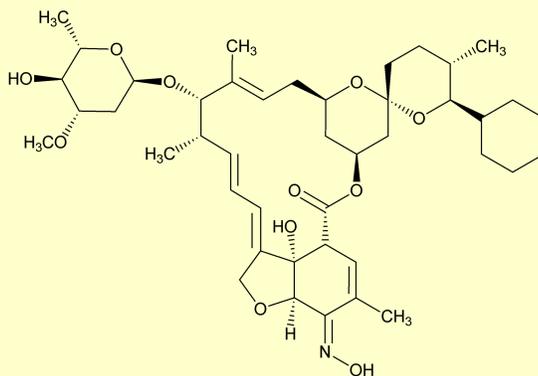
(CHM3: M. Puderbaugh.)

Correspondence Number—C124406

Comment deadline: January 31, 2016

Add the following:

▲ Selamectin



$C_{43}H_{63}NO_{11}$

769.96

25-Cyclohexyl-4'-O-de(2,6-dideoxy-3-O-methyl- α -l-*arabino*-hexopyranosyl)-5-demethoxy-25-de(1-methylpropyl)-22,23-dihydro-5-(hydroxyimino)-avermectin A1a;
 (2aE,4E,5'S,6S,6'S,7S,8E,11R,13R,15S,17aR,20aR,20bS)-6'-Cyclohexyl-7-[(2,6-dideoxy-3-O-methyl- α -l-*arabino*-hexopyranosyl)oxy]-3',4',5',6,6',7,10,11,14,15,20a,20b-dodecahydro-20b-hydroxy-5',6,8,19-tetramethylspiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-17,20(17aH)-dione 20-oxime [220119-17-5].

DEFINITION

Selamectin contains NLT 96.0% and NMT 102.0% of selamectin (C₄₃H₆₃NO₁₁), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. Infrared Absorption** (197)
 [Note—Methods described under (197K), (197M), or (197A) may be used.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• Procedure

Mobile phase: *Acetonitrile* and water (80:20)

Standard solution: 0.2 mg/mL of USP Selamectin RS in *Mobile phase*

Sample solution: 0.2 mg/mL of Selamectin in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 243 nm

Column: 3.9-mm × 15-cm; 4- μ m packing L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of selamectin (C₄₃H₆₃NO₁₁) in the portion of Selamectin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Selamectin RS in the *Standard solution* (mg/mL)

C_U = concentration of Selamectin in the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **Residue on Ignition** (281)

Sample: 1.0 g

Acceptance criteria: NMT 0.1%

- **Organic Impurities**

Solution A: Water

Solution B: *Acetonitrile*

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	40	60
28	40	60
45	20	80

Diluent: *Acetonitrile* and water (60:40)

System suitability solution: 500 µg/mL of USP Selamectin RS in *Diluent*

Standard solution: 2.5 µg/mL of USP Selamectin RS in *Diluent*

Sample solution: 500 µg/mL of Selamectin in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 243 nm

Column: 3.9-mm × 15-cm; 4-µm packing L1

Column temperature: 30°

Flow rate: 2.0 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—USP Selamectin RS contains the impurities didehydroselamectin and selamectin aglycone as minor components. The relative retention times for didehydroselamectin and selamectin aglycone are 0.4 and 0.5, respectively.]

Suitability requirements

Resolution: NLT 4.0 between didehydroselemectin and selamectin aglycone, *System suitability solution*

Tailing factor: NMT 1.6 for the selamectin peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Selamectin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of selamectin from the *Standard solution*

C_S = concentration of USP Selamectin RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of Selamectin in the *Sample solution* ($\mu\text{g/mL}$)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting level for impurities is 0.2%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Hydroxyselamectin ^a	0.2	1.0	2.0
Didehydroselamectin ^b	0.4	1.0	2.0
Selamectin aglycone ^c	0.5	1.0	1.5
Selamectin	1.0	—	—
α -Oleandrosyl selamectin ^d	1.7	0.67	1.5
Any other individual impurity	—	1.0	1.0
Total impurities	—	—	4.0

^a (2*aE*,2'*R*,4*E*,5'*S*,6*S*,6'*S*,7*S*,8*E*,11*R*,13*R*,15*S*,17*aR*,20*aR*,20*bS*)-6'-Cyclohexyl-7-[(2,6-dideoxy-3-*O*-methyl- α -l-*arabino*-hexopyranosyl)oxy]-3',4',5',6,6',7,10,11,14,15,20*a*,20*b*-dodecahydro-4',20*b*-dihydroxy-5',6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2'-[2*H*]pyran]-17,20(17*aH*)-dione 20-oxime.

^b (2*aE*,4*E*,5'*S*,6*S*,6'*S*,7*S*,8*E*,11*R*,13*S*,15*S*,17*aR*,20*aR*,20*bS*)-6'-Cyclohexyl-7-[(2,6-dideoxy-3-*O*-methyl- α -l-*arabino*-hexopyranosyl)oxy]-5',6,6',7,10,11,14,15,20*a*,20*b*-decahydro-20*b*-hydroxy-5',6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2'-[2*H*]pyran]-17,20(17*aH*)-dione 20-oxime.

^c (2*aE*,4*E*,5'*S*,6*S*,6'*S*,7*S*,8*E*,11*R*,13*S*,15*S*,17*aR*,20*aR*,20*bS*)-6'-Cyclohexyl-5',6,6',7,10,11,14,15,20*a*,20*b*-decahydro-7,20*b*-dihydroxy-5',6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2'-[2*H*]pyran]-17,20(17*aH*)-dione 20-oxime.

^d (2*aE*,4*E*,5'*S*,6*S*,6'*S*,7*S*,8*E*,11*R*,13*R*,15*S*,17*aR*,20*aR*,20*bS*)-6'-Cyclohexyl-7-[(4-*O*-(2,6-dideoxy-3-*O*-methyl- α -l-*arabino*-hexopyranosyl)-2,6-dideoxy-3-*O*-methyl- α -l-*arabino*-hexopyranosyl)oxy]-3',4',5',6,6',7,10,11,14,15,20*a*,20*b*-dodecahydro-20*b*-hydroxy-5',6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2'-[2*H*]pyran]-17,20(17*aH*)-dione 20-oxime.

SPECIFIC TESTS

- **Water Determination** (921), *Method Ia*

Sample: 0.20 g

Acceptance criteria: NMT 7.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in a tight container.
- **Labeling:** Label it to indicate that it is for veterinary use only.
- **USP Reference Standards** (11)
USP Selamectin RS

▲USP40

BRIEFING

Sodium Citrate and Citric Acid Oral Solution, *USP 38* page 5317. It is proposed to make the following revisions to the monograph:

1. In preparation for the omission of the flame tests from *Identification Tests—General* (191), proposed in PF 41(2) [Mar.–Apr. 2015], it is proposed to delete the reference to (191) under *Identification* test A, and replace it with a complete description of the flame test. Manufacturers are encouraged to submit replacement wet chemistry or instrumental procedures for consideration by the Expert Committee.
2. Add the ratio of the pyridine and acetic anhydride mixture under *Identification* test D.
3. Revise the equations in the *Assay* for *Sodium*, for *Sodium Citrate*, and for *Citric Acid* to be consistent with the *Definition* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM6: K.K. Seela, S. Ramakrishna.)

Correspondence Number—C162940

Comment deadline: January 31, 2016

Sodium Citrate and Citric Acid Oral Solution

DEFINITION

Sodium Citrate and Citric Acid Oral Solution is a solution of Sodium Citrate and Citric Acid in a suitable aqueous medium. It contains, in each 100 mL, NLT 2.23 g and NMT 2.46 g of sodium (Na), and NLT 6.11 g and NMT 6.75 g of citrate ($C_6H_5O_7$), equivalent to NLT 9.5 g and NMT 10.5 g of sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), and NLT 6.34 g and NMT 7.02 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$).

IDENTIFICATION

Delete the following:

- ▲ ● ~~A. Identification Tests—General (191), Sodium: Meets the requirement of the flame test~~
▲USP40

Add the following:

- ▲ ● A. Sodium Citrate and Citric Acid Oral Solution imparts an intense yellow color to a nonluminous flame. ▲USP40
- B.
Sample solution: 2 mL of Oral Solution
Analysis: Add 2 mL of 15% *potassium carbonate TS* to the *Sample solution*, boil, and cool. Add 4 mL of *potassium pyroantimonate TS*.
Acceptance criteria: A dense precipitate is formed (indicates the presence of sodium).
- C.
Sample solution: A dilution of Oral Solution (1 in 20)
Analysis: Add 5 mL of *sodium cobaltinitrite TS* to 2 mL of *Sample solution*.
Acceptance criteria: A yellow precipitate is not formed immediately (indicates the absence of potassium).

Change to read:

- **D. Identification Tests—General** (191), *Citrate*

Sample solution: 3–5 drops of Oral Solution

Analysis: Add 20 mL of a mixture of *pyridine* and *acetic anhydride*

▲ (3:1) ▲*USP40*

to the *Sample solution*.

Acceptance criteria: Meets the requirements

ASSAY**Change to read:**

- **Sodium**

Sodium stock solution: 58.44 mg/mL of *sodium chloride* prepared as follows. Transfer 14.61 g of *sodium chloride*, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with *water* to volume.

Potassium stock solution: 74.56 mg/mL of *potassium chloride* prepared as follows. Transfer 18.64 g of *potassium chloride*, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with *water* to volume.

Diluent: 1.04 mg/mL of *lithium nitrate* in *water* (equivalent to 15 mEq per 1000 mL of lithium) with a suitable nonionic surfactant

Standard stock solution: Pipet 50 mL of the *Sodium stock solution* and 50 mL of the *Potassium stock solution* into a 500-mL volumetric flask and dilute with *water* to volume.

Standard solution: Dilute 50 µL of the *Standard stock solution* with *Diluent* to 10 mL.

Sample stock solution: Transfer a volume of Oral Solution, equivalent to 1 g of sodium citrate dihydrate, to a 100-mL volumetric flask and dilute with *water* to volume.

Sample solution: Dilute 50 µL of the *Sample stock solution* with *Diluent* to 10 mL.

Blank: *Diluent*

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: Sodium emission line at 589 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Adjust the flame photometer to read zero with the *Diluent*. Concomitantly determine the sodium flame emission readings for the *Standard solution* and the *Sample solution*.

Calculate the quantity, in g, of Na in each mL of Oral Solution:

$$\text{Result} = (14.61/25V) \times (22.99/58.44) \times (R_{U,Na}/R_{S,Na})$$

V = volume of the Oral Solution taken to prepare the *Sample stock solution* (mL)

$R_{U,Na}$ = sodium emission reading of the *Sample solution*

$R_{S,Na}$ = sodium emission reading of the *Standard solution*

▲ Calculate the quantity (g) of sodium (Na) in 100 mL of Oral Solution taken:

$$\text{Result} = (R_{U, Na} / R_{S, Na}) \times (W/V) \times F \times (A_r / M_r) \times F_1$$

$R_{U, Na}$ = sodium emission reading of the *Sample solution*

$R_{S, Na}$ = sodium emission reading of the *Standard solution*

W = weight of sodium chloride in the *Sodium stock solution* (g)

V = volume of Oral Solution taken (mL)

F = ratio of the dilution factor of the *Sample solution* to the *Standard solution*, 0.04

A_r = atomic weight of sodium, 22.99

M_r = molecular weight of sodium chloride, 58.44

F_1 = conversion factor for each 100 mL of Oral Solution, 100 mL \blacktriangle USP40

Acceptance criteria: 2.23–2.46 g of sodium (Na) in each 100 mL of Oral Solution

Change to read:

• Sodium Citrate

Cation-exchange column: Mix 10 g of *styrene-divinylbenzene cation-exchange resin* with 50 mL of *water* in a suitable beaker. Allow the resin to settle and decant the supernatant until a slurry of resin remains. Pour the slurry into a 15-mm × 30-cm glass chromatographic tube (having a sealed-in, coarse-porosity fritted disk and fitted with a stopcock), and allow to settle as a homogeneous bed. Wash the resin bed with about 100 mL of *water*, closing the stopcock when the *water* level is about 2 mm above the resin bed.

Sample solution: Transfer a volume of Oral Solution, equivalent to 1 g of sodium citrate dihydrate, to a 100-mL volumetric flask and dilute with *water* to volume.

Titrimetric system

Mode: Direct titration

Titrant: 0.02 N sodium hydroxide VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Pipet 5 mL of the *Sample solution* carefully onto the top of the resin bed in the *Cation-exchange column*. Place a 250-mL conical flask below the column, open the stopcock, and allow to flow until the solution has entered the resin bed. Elute the column with 60 mL of *water* at a flow rate of about 5 mL/min, collecting about 65 mL of the eluate. Add 5 drops of *phenolphthalein TS* to the eluate, swirl the flask, and titrate with *Titrant*. Record the buret reading and calculate the volume of *Titrant* consumed.

Calculate the quantity (mg) of sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) in each mL of the Oral Solution:

$$\text{Result} = [1.961 \times B \times (20/V)] \div [(294.10/210.14) \times C]$$

B = volume of Titrant

V = volume of the Oral Solution taken (mL)

~~C = concentration of citric acid monohydrate from the Oral Suspension in the Sample solution, as obtained in the Assay for Citric Acid (mg/mL)~~

- ▲ Calculate the quantity (g) of sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) in 100 mL of the Oral Solution taken:

$$\text{Result} = \{[V \times N \times F \times (D/V_S)] - [(M_{r1}/M_{r2}) \times C]\} \times F_1 \times F_2$$

V = volume of Titrant consumed by the Sample solution (mL)

N = actual normality of the Titrant (mEq/mL)

F = equivalency factor, 98.05 mg/mEq for sodium citrate dihydrate

D = dilution factor of the Sample solution, 20

V_S = volume of Oral Solution taken (mL)

M_{r1} = molecular weight of sodium citrate dihydrate, 294.10

M_{r2} = molecular weight of citric acid monohydrate, 210.14

C = concentration of citric acid monohydrate in the Sample solution, as obtained in the Assay for Citric Acid (mg/mL)

F_1 = conversion factor from mg to g, 0.001

F_2 = conversion factor for each 100 mL of Oral Solution, 100 mL ▲USP40

Acceptance criteria: 9.5–10.5 g of sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) in each 100 mL of Oral Solution

Change to read:

● Citric Acid

Sample solution: Transfer a volume of Oral Solution, equivalent to 0.67 g of citric acid monohydrate, to a 100-mL volumetric flask and dilute with water to volume.

Titrimetric system

Mode: Direct titration

Titrant: 0.02 N sodium hydroxide VS

Endpoint detection: Visual

Analysis

Sample: Sample solution

Transfer 5 mL of the Sample solution to a suitable flask. Add 25 mL of water and 5 drops of phenolphthalein TS. Titrate with Titrant to a pink endpoint. Record the buret reading and calculate the volume of Titrant consumed.

~~Calculate the quantity (mg) of citric acid ($C_6H_8O_7 \cdot H_2O$) in each mL of the Oral Solution:~~

~~$$\text{Result} = 1.401 \times A \times (20/V)$$~~

~~A = volume of Titrant consumed (mL)~~

~~V = volume of Oral Solution taken (mL)~~

- ▲ Calculate the quantity (g) of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) in 100 mL of the Oral Solution taken:

$$\text{Result} = V \times N \times F \times (D/V_S) \times F_1 \times F_2$$

V = volume of *Titrant* consumed by the *Sample solution* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 70.05 mg/mEq for citric acid monohydrate

D = dilution factor of the *Sample solution*, 20

V_S = volume of Oral Solution taken (mL)

F_1 = conversion factor from mg to g, 0.001

F_2 = conversion factor for each 100 mL of Oral Solution, 100 mL \blacktriangle *USP40*

Acceptance criteria: 6.34–7.02 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) in each 100 mL of Oral Solution

PERFORMANCE TESTS

- **Deliverable Volume** (698)

For multiple-unit containers

Acceptance criteria: Meets the requirements

- **Uniformity of Dosage Units** (905)

For single-unit containers

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **pH** (791): 4.0–4.4

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

BRIEFING

Sulfadoxine and Pyrimethamine Tablets, *USP 38* page 5390. On the basis of comments received, it is proposed to revise the system suitability criteria in the *Assay*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM1: S. Shivaprasad.)

Correspondence Number—C110871

Comment deadline: January 31, 2016

Sulfadoxine and Pyrimethamine Tablets

DEFINITION

Sulfadoxine and Pyrimethamine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of sulfadoxine ($C_{12}H_{14}N_4O_4S$) and NLT 90.0% and NMT 110.0% of the labeled amount of pyrimethamine ($C_{12}H_{13}ClN_4$).

IDENTIFICATION

Change to read:

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solutions* for pyrimethamine and sulfadoxine, ~~relative to the internal standard,~~
▲▲USP40
as obtained in the *Assay*.

- **B.**

Diluent: Ammonium hydroxide and methanol (1 in 50)

Standard solution A: 10 mg/mL of USP Sulfadoxine RS in *Diluent*

Standard solution B: 0.5 mg/mL of USP Pyrimethamine RS in *Diluent*

Sample solution: Vigorously shake 700 mg of finely ground Tablet powder with 50 mL of *Diluent* for 3 min, and filter.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Developing solvent system: Heptane, chloroform, solution of methanol in alcohol (1 in 20), and glacial acetic acid (4:4:4:1)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Allow the solvent front to move about two-thirds of the length of the plate, remove the plate, dry, and examine under short-wavelength UV light.

Acceptance criteria: The R_F values of the principal spots from the *Sample solution* correspond to the R_F values of the principal spots from the corresponding *Standard solutions*.

ASSAY**Change to read:**

- **Procedure**

Solution A: 1 mL of phosphoric acid in 1000 mL of water

Mobile phase: Acetonitrile and *Solution A* (17:83)

Standard solution: 0.4 mg/mL of USP Sulfadoxine RS and 0.02 mg/mL of USP Pyrimethamine RS prepared as follows. Transfer suitable amounts of USP Sulfadoxine RS and USP Pyrimethamine RS to a suitable volumetric flask. Dissolve in acetonitrile using about 17% of the final flask volume, then dilute with *Solution A* to volume.

Sample stock solution: Transfer an equivalent of about 200 mg sulfadoxine and 10 mg pyrimethamine from NLT 10 finely powdered Tablets to a 100-mL volumetric flask. Add about 28 mL of acetonitrile and sonicate for about 30 min. Allow to cool and dilute with *Solution A* to volume.

Sample solution: Nominally 0.4 mg/mL of sulfadoxine and 0.02 mg/mL of pyrimethamine in

Mobile phase from *Sample stock solution*. Pass through a PVDF filter of 0.45- μ m pore size. Discard the first 5 mL and use the remaining filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 2.0-mm \times 10-cm; 3- μ m packing L11

Flow rate: 0.3 mL/min

Injection volume: 5 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: ~~NLT 8~~

▲ NLT 1.5 ▲ USP40

between pyrimethamine and sulfadoxine

Tailing factor: NMT 1.6 for sulfadoxine; NMT 2.0 for pyrimethamine

Relative standard deviation: NMT 2.0%

▲ for both sulfadoxine and pyrimethamine ▲ USP40

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of sulfadoxine ($C_{12}H_{14}N_4O_4S$) and pyrimethamine ($C_{12}H_{13}ClN_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sulfadoxine or pyrimethamine from the *Sample solution*

r_S = peak response of sulfadoxine or pyrimethamine from the *Standard solution*

C_S = concentration of USP Sulfadoxine RS or USP Pyrimethamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of sulfadoxine or pyrimethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each for sulfadoxine and pyrimethamine

PERFORMANCE TESTS

• Dissolution (711)

Medium: pH 6.8 Phosphate Buffer, prepared as directed in *Reagents, Indicators, and Solutions—Buffer Solutions*; 1000 mL

Apparatus 2: 75 rpm

Time: 30 min

Analysis: Determine the percentage of the labeled amounts of sulfadoxine ($C_{12}H_{14}N_4O_4S$) and pyrimethamine ($C_{12}H_{13}ClN_4$) dissolved, using the procedure set forth in the *Assay*,

making any necessary modifications.

Tolerances: NLT 60% (Q) of the labeled amounts each of sulfadoxine ($C_{12}H_{14}N_4O_4S$) and pyrimethamine ($C_{12}H_{13}ClN_4$) is dissolved.

- **Uniformity of Dosage Units** (905), *Content Uniformity*: Meet the requirements with respect to sulfadoxine and to pyrimethamine

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
- **USP Reference Standards** (11)
 - USP Pyrimethamine RS
 - USP Sulfadoxine RS

BRIEFING

Testosterone Topical Gel. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is proposed.

1. The liquid chromatographic procedure in the *Assay* was validated with the Nucleosil C18 brand of L1 column. The typical retention time for testosterone is about 7.5 min.
2. The liquid chromatographic procedure in the test for *Organic Impurities* was validated with the YMC-Pack ODS-AQ brand of L1 column and the Phenomenex Security Standard C18 brand of L1 guard column. The typical retention time for testosterone is about 13 min.

(CHM5: M. Koleček.)

Correspondence Number—C134203

Comment deadline: January 31, 2016

Add the following:

▲ Testosterone Topical Gel

DEFINITION

Testosterone Topical Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of testosterone ($C_{19}H_{28}O_2$).

IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Mobile phase: *Acetonitrile* and water (50:50)

System suitability stock solution: 400 µg/mL of USP Testosterone Related Compound A RS in *acetonitrile*

Standard stock solution: 0.4 mg/mL of USP Testosterone RS in *acetonitrile*

System suitability solution: 16 µg/mL each of USP Testosterone RS and USP Testosterone Related Compound A RS from the *Standard stock solution* and the *System suitability stock solution*, respectively, in *Mobile phase*

Standard solution: 0.04 mg/mL of USP Testosterone RS from the *Standard stock solution* in *Mobile phase*

Sample solution: Transfer 200 mg of the Gel to a 100-mL volumetric flask and dilute with *Mobile phase* to volume. Sonicate to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 245 nm. For *Identification* test A, use a diode-array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm column; 3-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 2 times the retention time of testosterone

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for testosterone and testosterone related compound A are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 1.5 between testosterone and testosterone related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of testosterone (C₁₉H₂₈O₂) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of testosterone from the *Sample solution*

r_S = peak response of testosterone from the *Standard solution*

C_S = concentration of USP Testosterone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of Testosterone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Uniformity of Dosage Units** (905): Meets the requirements for gels packaged in single-dose containers

IMPURITIES

- **Organic Impurities**

Mobile phase: *Acetonitrile, methanol, and water (40:10:50)*

System suitability solution: 0.4 µg/mL each of USP Testosterone Related Compound A RS and USP Testosterone Related Compound C RS in *Mobile phase*

Standard stock solution: 0.2 mg/mL of USP Testosterone RS in *acetonitrile*

Standard solution: 0.4 µg/mL of USP Testosterone RS from the *Standard stock solution* in *Mobile phase*

Sample solution: Transfer 1 g of the Gel to a 50-mL volumetric flask and dilute with *Mobile phase* to volume. Sonicate to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 15-cm column; 3-µm packing *L1*
[Note—A suitable guard column may be used.]

Flow rate: 0.8 mL/min

Injection volume: 20 µL

Run time: NLT 1.5 times the retention time of testosterone

System suitability

Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between testosterone related compound A and testosterone related compound C, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of testosterone related compound A in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 1/T \times 100$$

r_U = peak response of testosterone related compound A from the *Sample solution*

r_S = peak response of testosterone from the *Standard solution*

C_S = concentration of USP Testosterone RS in *Standard solution* (mg/mL)

C_U = nominal concentration of testosterone in the *Sample solution* (mg/mL)

T = amount of testosterone determined in the *Assay* (g/100g of gel)

Calculate the percentage of any individual unspecified degradation product in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak response of the individual unspecified degradation product from the *Sample solution*

r_S = peak response of testosterone from the *Sample solution*

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Testosterone related compound I ^{a,b}	0.82	—
Testosterone	1.0	—
Testosterone related compound A	1.3	0.5
Testosterone related compound C ^a	1.4	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	1.5

^a This is a process impurity and is included in the table for identification only. It is not to be reported or included in the total degradation products.

^b Testosterone-6-ene; 17 β -Hydroxyandrosta-4,6-dien-3-one.

SPECIFIC TESTS

- **Viscosity—Rotational Methods** (912)

Sample: Transfer 100 mL of the Gel to a 100-mL beaker and adjust the temperature of the Gel to $20 \pm 2^\circ$.

Analysis: Use a suitable rotational viscometer with a spindle having a disk that is 1.46 cm in diameter and 0.16 cm in height, with an immersion depth of 4.92 cm. Allow the spindle to rotate in the Gel at 10 rpm for 30 s, and then observe the scale reading. Convert the scale reading to centipoises by multiplying by the constant for the spindle and speed used.

Acceptance criteria: 9000–33,000 centipoises

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.

- **USP Reference Standards** (11)

USP Testosterone RS

USP Testosterone Related Compound A RS

Androstenedione

Androst-4-ene-3,17-dione.

C₁₉H₂₆O₂ 286.41USP Testosterone Related Compound C RS

Epitestosterone

17 α -Hydroxyandrost-4-en-3-one.C₁₉H₂₈O₂ 288.42

▲USP40

BRIEFING

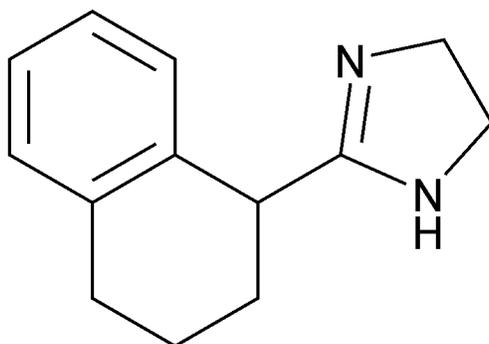
Tetrahydrozoline Hydrochloride, *USP 38* page 5520. As part of the USP monograph modernization effort, it is proposed to make the following changes:

1. *Identification* test *A* is revised to include *Infrared Absorption* (197A).
2. The titration procedure in the *Assay* is replaced with a validated HPLC procedure based on analyses performed with the Waters XBridge C18 brand of L1 column. The typical retention time for tetrahydrozoline is about 4.5 min.
3. The acceptance criteria in the *Definition* is revised from 98.0%–100.5% to 98.0%–102.0%, which is typical for HPLC procedures.
4. The UV procedure in *Identification* test *B* is replaced with a retention time agreement based on the *Assay*.
5. The nonspecific test for *Ordinary Impurities* is replaced with a GC procedure in the test for *Organic Impurities* based on the current monograph for *Tetrahydrozoline Hydrochloride* in the *European Pharmacopoeia*. The GC procedure in the test for *Organic Impurities* is based on analyses performed with the Agilent HP-1 brand of G1 column. The typical retention time for tetrahydrozoline is about 12 min.
6. The storage temperature is added in the *Packaging and Storage* section.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(CHM6: F. Mao.)

Correspondence Number—C157405

Comment deadline: January 31, 2016**Tetrahydrozoline Hydrochloride**

• HCl

C₁₃H₁₆N₂·HCl 236.741*H*-Imidazole, 4,5-dihydro-2-(1,2,3,4-tetrahydro-1-naphthalenyl)-, monohydrochloride;

2-(1,2,3,4-Tetrahydro-1-naphthyl)-2-imidazoline monohydrochloride [522-48-5].

DEFINITION

Change to read:

Tetrahydrozoline Hydrochloride contains NLT 98.0% and NMT ~~100.5%~~

~~▲ 102.0%~~▲*USP40*

of tetrahydrozoline hydrochloride (C₁₃H₁₆N₂·HCl), calculated on the dried basis.

IDENTIFICATION

Change to read:

● A. ~~Infrared Absorption~~ ~~(197K)~~

~~▲ Infrared Absorption (197):~~ [Note—Methods described in ~~(197K)~~ or ~~(197A)~~ may be used.]

~~▲*USP40*~~

Delete the following:

~~▲ ● B. Ultraviolet Absorption~~ ~~(197U)~~

~~Analytical wavelengths:~~ 264 and 271 nm

~~Sample solution:~~ 250 µg/mL in water

~~Acceptance criteria:~~ Absorptivities differs by NMT 4.0%, calculated on the dried basis.

~~▲*USP40*~~

Add the following:

~~▲ ● B.~~ The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲*USP40*

● C. Identification Tests—General (191), Chloride

Sample solution: 5 mg/mL

Acceptance criteria: Meets the requirements

ASSAY

Change to read:

● Procedure

~~**Sample solution:** Transfer 400 mg of tetrahydrozoline hydrochloride to a 250 mL beaker, and dissolve in 60 mL of glacial acetic acid, heating if necessary.~~

~~**Analysis:** To *Sample solution*, add 5 mL of acetic anhydride, 5 mL of mercuric acetate TS, and 3 drops of quinaldine red TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 23.67 mg of C₁₃H₁₆N₂·HCl.~~

~~**Acceptance criteria:** 98.0%–100.5% on the dried basis~~

~~▲ **Buffer:** 2.64 g/L of dibasic ammonium phosphate in water, adjusted with ammonium hydroxide to a pH of 9.0~~

~~**Mobile phase:** Acetonitrile and Buffer (15:85)~~

~~**Standard solution:** 0.025 mg/mL of USP Tetrahydrozoline Hydrochloride RS in water~~

Sample solution: 0.025 mg/mL of Tetrahydrozoline Hydrochloride in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Column temperature: 37°

Flow rate: 1.2 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tetrahydrozoline hydrochloride ($C_{13}H_{16}N_2 \cdot HCl$) in the portion of Tetrahydrozoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Tetrahydrozoline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Tetrahydrozoline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ▲*USP40*

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

Delete the following:

- **Heavy Metals** (231)

Sample: 0.40 g

Analysis: Dissolve the *Sample* in 23 mL of water, and add 2 mL of 1 N acetic acid.

Acceptance criteria: NMT 50 ppm ● (Official 1-Jan-2018)

Delete the following:

- ▲ ● **Ordinary Impurities** (466)

Sample solution: Methanol

Standard solution: Methanol

Eluant: ~~Methanol, glacial acetic acid, and water (8:1:1)~~

Visualization: ~~3, followed by overspraying with hydrogen peroxide TS.~~

~~[Note—Cover the thin-layer chromatographic plate with a glass plate to slow fading of the spots.]~~

▲USP40

Add the following:

▲ ● **Organic Impurities**

Diluent: *Methanol and 1 M sodium hydroxide (75:25)*

Standard solution: 0.1 mg/mL of USP Tetrahydrozoline Hydrochloride RS in *Diluent*

Sample solution: 100 mg/mL of Tetrahydrozoline Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 25-m × 0.32-mm; 1.0-μm coating of *G1*

Temperatures

Injection port: 220°

Detector: 220°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
160	0	160	8
160	20	220	4

Carrier gas: Helium

Flow rate: 2.5 mL/min

Injection volume: 1 μL

Injection type: Split, 40:1

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3%

Signal-to-noise ratio: NLT 50

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of each impurity in the portion of Tetrahydrozoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of tetrahydrozoline from the *Standard solution*

C_S = concentration of USP Tetrahydrozoline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Tetrahydrozoline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
α -Cyanotetraline ^a	0.5	0.1
Tetrahydrozoline ^b	1.0	—
Any individual unspecified impurity	—	0.1
Total impurities	—	0.2
^a 1,2,3,4-Tetrahydronaphthalene-1-carbonitrile. ^b 2-(1,2,3,4-Tetrahydro-1-naphthyl)-2-imidazoline.		

▲USP40

SPECIFIC TESTS

- **Loss on Drying** (731)

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in tight containers.

▲ Store at room temperature. ▲USP40

- **USP Reference Standards** (11)

USP Tetrahydrozoline Hydrochloride RS

BRIEFING

Thiamine Hydrochloride Tablets, USP 38 page 5539. It is proposed to revise the monograph to be consistent with the recent revision to *Thiamine Assay* (531), which is published elsewhere in this issue of *PF*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(NBDS: N. Davydova.)

Correspondence Number—C164839

Comment deadline: January 31, 2016

Thiamine Hydrochloride Tablets

DEFINITION

Thiamine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$).

IDENTIFICATION• **A.**

Sample solution: Triturate a quantity of powdered Tablets, equivalent to 10 mg of thiamine hydrochloride, with 10 mL of 0.5 N *sodium hydroxide*, and filter.

Analysis: To 5 mL of the *Sample solution* add 0.5 mL of *potassium ferricyanide TS* and 5 mL of *isobutyl alcohol*, shake the mixture vigorously for 2 min, and allow the liquid layers to separate. Illuminate from above by a vertical beam of UV light, and observe the air-liquid meniscus at a right angle to this beam.

Acceptance criteria: The air-liquid meniscus shows a vivid blue fluorescence, which disappears when the mixture is slightly acidified, but reappears when it is again made alkaline.

• **B.**

Sample solution: Triturate a quantity of powdered Tablets, equivalent to 10 mg of thiamine hydrochloride, with 10 mL of water, and filter.

Analysis 1: To 2 mL of the *Sample solution* add *iodine TS*.

Acceptance criteria 1: A red-brown precipitate is formed.

Analysis 2: To 2 mL of the *Sample solution* add *mercuric chloride TS*.

Acceptance criteria 2: A white precipitate is formed.

Analysis 3: *Identification Tests—General* (191), *Chloride*.

Acceptance criteria 3: Meet the requirements

• **C.**

Sample solution: Use the remainder of the *Sample solution* from *Identification* test *B*.

Analysis: Add 1 mL of lead acetate TS and 1 mL of 2.5 N sodium hydroxide.

Acceptance criteria: A yellow color is produced. Heat the mixture for several min on a steam bath: the color changes to brown, and, on standing, a precipitate of lead sulfide separates.

ASSAY**Change to read:**• **Thiamine Assay** (531),

▲ *Chemical Methods, Procedure 1* ▲ *USP40*

Assay preparation:

▲ **Sample solution:** ▲ *USP40*

Place NLT 20 Tablets in a flask of suitable size, half fill the flask with 0.2 N *hydrochloric acid*, and heat on a steam bath, with frequent agitation, until the Tablets have dissolved or have disintegrated so that a uniform dispersion is obtained. Cool, transfer the contents of the flask to a volumetric flask, and dilute with 0.2 N *hydrochloric acid* to volume. If the

mixture is not clear, either centrifuge it or filter it through paper known not to adsorb thiamine. Dilute a portion of the clear solution with 0.2 N *hydrochloric acid* to obtain a 0.2- $\mu\text{g}/\text{mL}$ solution of thiamine hydrochloride.

Analysis: Proceed as directed in the chapter.

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Dissolution** (711), *Procedure, Apparatus 1 and 2, Procedure for a Pooled Sample*

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: A known concentration of USP Thiamine Hydrochloride RS in *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

Mobile phase: A mixture of methanol, *glacial acetic acid*, and water (27:1:73) containing 1.40 mg/mL of *sodium 1-hexanesulfonate*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of thiamine hydrochloride ($\text{C}_{12}\text{H}_{17}\text{ClN}_4\text{OS}\cdot\text{HCl}$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V/L) \times 100$$

r_U = peak area of thiamine from the *Sample solution*

r_S = peak area of thiamine from the *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*

V = volume of *Medium*, 900 mL

L = labeled claim of thiamine hydrochloride (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) is dissolved.

- **Uniformity of Dosage Units** (905): Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** (11)
USP Thiamine Hydrochloride RS

BRIEFING

Citrulline. Because there is no existing *USP* monograph for this dietary ingredient, a new monograph is being proposed. The liquid chromatographic procedures in the *Assay* and the test for *Related Compounds* are based on analyses performed with the GL Sciences Inertsil ODS-3V brand of L1 column. The typical retention times for citrulline and *N*-acetyl-leucine are 3.8 and 9.3 min, respectively.

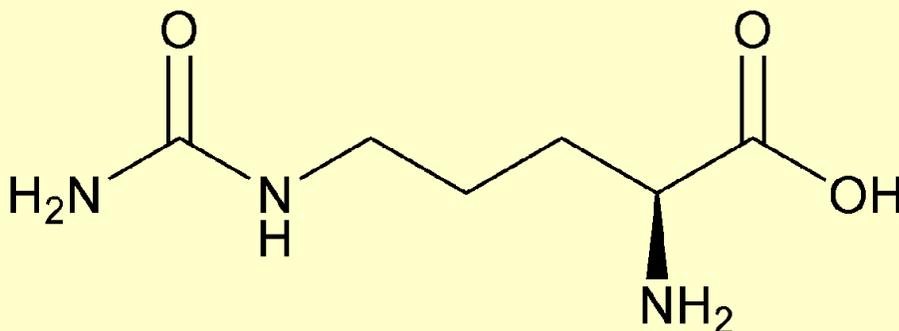
(NBDS: H. Dinh.)

Correspondence Number—C158693

Comment deadline: January 31, 2016

Add the following:

▲ Citrulline



$C_6H_{13}N_3O_3$ 175.19

(*S*)-2-Amino-5-ureidopentanoic acid;
L-Citrulline [372-75-8].

DEFINITION

Citrulline contains NLT 98.0% and NMT 102.0% of L-citrulline ($C_6H_{13}N_3O_3$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)

- **B.** It meets the requirements in *Specific Tests for Optical Rotation* (781S), *Procedures, Specific Rotation*.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Solution A: 10 mM octanesulfonic acid sodium salt in water. Adjust with dilute phosphoric acid (1 in 10) to a pH of 2.5.

Solution B: Acetonitrile

Mobile phase: Gradient elution. See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
4	85	15
15	70	30
20	85	15
25	85	15

System suitability solution: 0.1 mg/mL of USP L-Citrulline RS and 0.05 mg/mL of USP N-Acetyl-L-Leucine RS in water

Standard solution: 0.1 mg/mL of USP L-Citrulline RS in water

Sample solution: 0.1 mg/mL of Citrulline in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 15-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for L-citrulline and N-acetyl-L-leucine are 1.0 and 2.4, respectively.]

Suitability requirements

Resolution: NLT 10.0 between L-citrulline and N-acetyl-L-leucine peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of L-citrulline ($C_6H_{13}N_3O_3$) in the portion of Citrulline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP L-Citrulline RS in the *Standard solution* (mg/mL)

C_U = concentration of Citrulline in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

- **Chloride and Sulfate** (221), *Chloride*

Standard solution: 0.10 mL of 0.020 N *hydrochloric acid*

Sample: 0.36 g of Citrulline

Acceptance criteria: NMT 0.02%

- **Chloride and Sulfate** (221), *Sulfate*

Standard solution: 0.10 mL of 0.020 N *sulfuric acid*

Sample: 0.48 g of Citrulline

Acceptance criteria: NMT 0.02%

- **Related Compounds**

Mobile phase, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Sample solution: 0.5 mg/mL of Citrulline in water

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Citrulline taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria

Individual impurity: NMT 0.1%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **Optical Rotation** (781S), *Procedures, Specific Rotation*

Sample solution: 80 mg/mL in 6 N hydrochloric acid

Acceptance criteria: +24.5° to +26.5°

- **Loss on Drying** (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

- **USP Reference Standards** (11)

USP *N*-Acetyl-L-Leucine RS

Acetyl-L-leucine.

C₈H₁₅NO₃ 173.21

USP L-Citrulline RS

▲USP40

BRIEFING

Creatine. Because there is no existing *USP* monograph for this dietary ingredient, a new monograph is being proposed. The liquid chromatographic procedures in the *Assay* and in the test for *Related Compounds* are based on analyses performed with the Agilent Zorbax SiL brand of L3 column. Typical retention times for creatinine and creatine are 3.8 and 13.4 min, respectively.

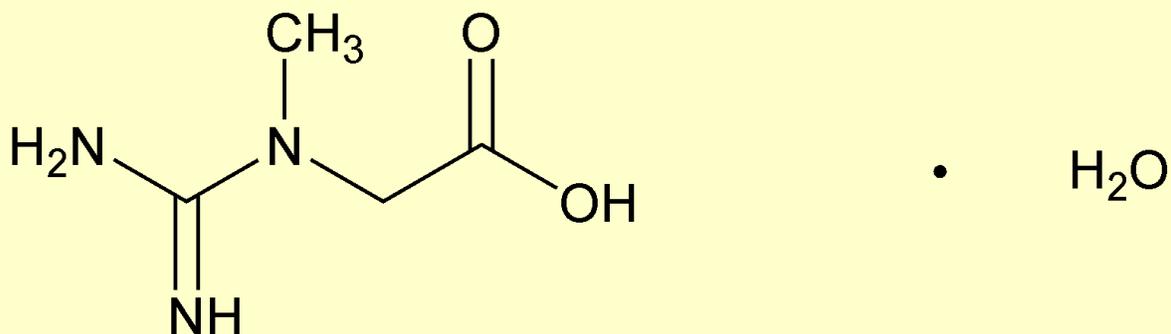
(NBDS: H. Dinh.)

Correspondence Number—C159414

Comment deadline: January 31, 2016

Add the following:

▲ Creatine



C₄H₉N₃O₂·H₂O 149.15

2-[Carbamimidoyl(methyl)amino]acetic acid;

Hydrate [6020-87-7].C₄H₉N₃O₂ 131.13

Anhydrous [57-00-1].

DEFINITION

Creatine contains one molecule of water of hydration. It contains NLT 98.0% and NMT 102.0% of creatine ($C_4H_9N_3O_2$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer solution: 10 mM *monobasic sodium phosphate* in water

Mobile phase: Acetonitrile and *Buffer solution* (83:17)

Diluent: Acetonitrile and water (50:50)

System suitability solution: 20 µg/mL of USP Creatine RS and 2 µg/mL of USP Creatinine RS in *Diluent*

Standard solution: 0.2 mg/mL of USP Creatine RS in *Diluent*

Sample solution: 0.2 mg/mL of Creatine in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 208 nm

Column: 4.6-mm × 15-cm; 5-µm packing *L3*

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for creatinine and creatine are 0.3 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 10 between creatine and creatinine, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of creatine in the portion of Creatine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Creatine RS in the *Standard solution* (mg/mL)

C_U = concentration of Creatine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** (281): NMT 0.1%

- **Chloride and Sulfate** (221), *Sulfate*

Standard solution: 0.10 mL of 0.020 N sulfuric acid

Sample: 100 mg of Creatine

Acceptance criteria: NMT 0.1%

- **Related Compounds**

Mobile phase, Diluent, System suitability solution, Chromatographic system, and

System suitability: Proceed as directed in the *Assay*.

Sample solution: 0.2 mg/mL of Creatine in *Diluent*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Creatine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria

Individual impurity: NMT 0.1%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **Loss on Drying** (731)

Analysis: Dry at 105° for 2 h.

Acceptance criteria: 10.5%–12.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

- **USP Reference Standards** (11)

USP Creatine RS

USP Creatinine RS

BRIEFING

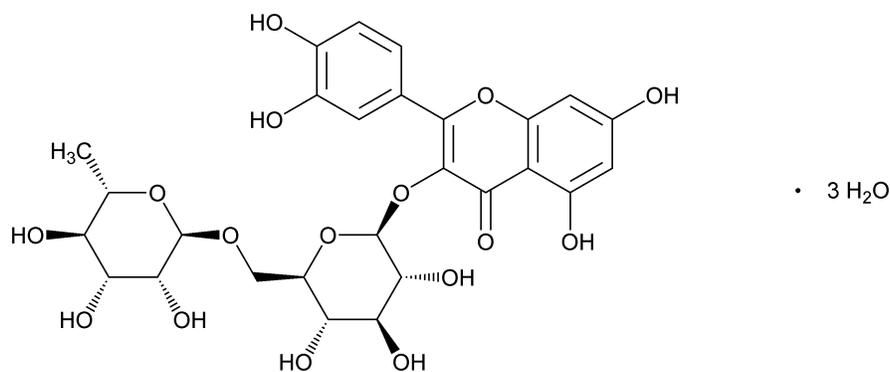
Rutin, USP 38 page 6204. On the basis of comments received, it is proposed to modify the gradient elution in *Table 1* in order to improve the robustness of the *Quercetin and Other Related Compounds* test. Typical retention times for rutin, kaempferol 3-rutinoside, isoquercitroside, and quercetin are 7.5, 8.4, 9.1, and 18.5 min, respectively.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(NBDS: H. Dinh.)

Correspondence Number—C163643

Comment deadline: January 31, 2016

Rutin

$C_{27}H_{30}O_{16} \cdot 3H_2O$ 664.56

3-Rhamnoglucoside of 5,7,3',4'-tetrahydroxyflavonol;
2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4*H*-chromen-4-one-3-yl 6-*O*- α -L-rhamnopyranosyl- β -D-glucoside [250249-75-3].

DEFINITION

Rutin contains NLT 95.0% and NMT 101.0% of rutin ($C_{27}H_{30}O_{16}$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** (197K)

- **B. Ultraviolet Absorption** (197U)

Analytical wavelength: 210–450 nm

Sample solution: 20 μ g/mL of Rutin in *methanol*. Filter if necessary.

Acceptance criteria: The spectrum exhibits two absorption maxima at 257 nm and 358 nm. The absorptivity at the maximum at 358 nm is 30.5–33.0, calculated on the anhydrous basis.

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the

Standard solution, as obtained in the test for *Quercetin and Other Related Compounds*.

ASSAY

• Procedure

Sample: 200 mg of Rutin

Blank: 20 mL of *dimethylformamide*

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: *Tetrabutylammonium hydroxide*

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 20 mL of *dimethylformamide*, and titrate with *Titrant*. Perform the *Blank* determination and make corrections if necessary.

Calculate the percentage of rutin (C₂₇H₃₀O₁₆) in the portion of the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

M = actual *Titrant* molarity (mmol/mL)

F = equivalency factor, 305.3 mg/mmol

W = *Sample* weight (mg)

Acceptance criteria: 95.0%–101.0% on the anhydrous basis

IMPURITIES

• **Residue on Ignition** (281): NMT 0.1%

• Light Absorbing Substances

Analytical wavelength: 450–800 nm

Sample solution: Dissolve 0.200 g of Rutin in 40 mL of *2-propanol*. Stir for 15 min, dilute with *2-propanol* to 50.0 mL, and filter.

Acceptance criteria: The absorbance of any substance is NMT 0.10.

• Methanol Insoluble Substances

Sample: 2.5 g

Analysis: Shake the *Sample* for 15 min in 50 mL of *methanol* at 20°–25°. Filter under reduced pressure through a sintered-glass filter previously dried for 15 min at 100°–105°, cooled in a desiccator, and tared. Wash the filter three times with 20 mL of *methanol*. Dry the filter for 30 min at 105°. Allow to cool, and weigh.

Acceptance criteria: The residue weighs NMT 75 mg (3%).

Delete the following:

• **Heavy Metals**, *Method II* (231): NMT 20 µg/g (Official 1-Jan-2018)

Change to read:

- **Quercetin and Other Related Compounds**

Solution A: Mix 50 mL of *tetrahydrofuran* with 950 mL of a 15.6 mg/mL solution of *monobasic sodium phosphate*. Adjust with *phosphoric acid* to a pH of 3.0.

Solution B: Mix 400 mL of *tetrahydrofuran* with 600 mL of a 15.6 mg/mL solution of *monobasic sodium phosphate*. Adjust with *phosphoric acid* to a pH of 3.0.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	50	50
10	0	100
15 ▲ 20 ▲ USP40	0	100
16 ▲ 21 ▲ USP40	50	50
20 ▲ 25 ▲ USP40	50	50

Standard solution A: Transfer 10 mg of USP Rutin RS into a 10-mL volumetric flask, add 2 mL of *methanol*, and mix to dissolve. Dilute with *Solution B* to volume.

Standard solution B: 20 µg/mL of USP Rutin RS in *Solution B*, prepared from the dilution of *Standard solution A* with *Solution B*

Standard solution C: 20 µg/mL of USP Quercetin RS in *Solution B*

Sample solution: Transfer 100 mg of Rutin into a 100-mL volumetric flask, add 20 mL of *methanol*, and mix to dissolve. Dilute with *Solution B* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4-mm × 25-cm; 5-µm packing *L7*

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution A*

[Note—The relative retention times for rutin, kaempferol 3-rutinoside, isoquercitroside, and quercetin are 1.0, 1.1, 1.2, and 2.5, respectively.]

Suitability requirements

Chromatographic similarity: The chromatogram from *Standard solution A* is similar to the reference chromatogram provided with the USP Rutin RS being used.

Resolution: NLT 1.5, between the rutin and kaempferol 3-rutinoside peaks

Analysis

Samples: *Standard solution B, Standard solution C, and Sample solution*

Calculate the percentage of quercetin¹ in the portion of Rutin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of quercetin from the *Sample solution*

r_S = peak response of quercetin from *Standard solution C*

C_S = concentration of USP Quercetin RS in *Standard solution C* (mg/mL)

C_U = concentration of Rutin in the *Sample solution* (mg/mL)

Calculate the percentage of each impurity in the portion of Rutin taken: [Note—Disregard any impurity less than 0.1%.]

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of rutin from *Standard solution B*

C_S = concentration of USP Rutin RS in *Standard solution B* (mg/mL)

C_U = concentration of Rutin in the *Sample solution* (mg/mL)

F = correction factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Correction Factor (F)	Acceptance Criteria, NMT (%)
Kaempferol 3-Rutinoside ^a	1.1	1	2.0
Isoquercitroside ^b	1.2	0.8	2.0
Quercetin	—	—	2.0
Any other impurity	—	1	1.0
Total impurities	—	—	4.0
^a 3-[[6-O-(6-Deoxy- α -l-mannopyranosyl)- β -d-glucopyranosyl]oxy]-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one. ^b 2-(3,4-Dihydroxyphenyl)-3-(β -d-glucofuranosyloxy)-5,7-dihydroxy-4H-1-benzopyran-4-one.			

SPECIFIC TESTS

- **Water Determination** (921), *Method Ia*

Sample: 100 mg

Acceptance criteria: 7.5%–9.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, tight, light-resistant containers.

- **USP Reference Standards** (11)

USP Quercetin RS USP Rutin RS

¹ 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one.

BRIEFING

Sodium Ferrous Citrate. Because there is no existing *USP* monograph for this dietary ingredient, a new monograph is being proposed.

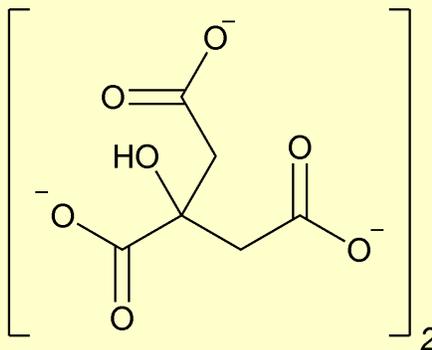
(NBDS: H. Dinh.)

Correspondence Number—C162405

Comment deadline: January 31, 2016

Add the following:

▲ Sodium Ferrous Citrate


 $\text{C}_{12}\text{H}_{10}\text{FeNa}_4\text{O}_{14} \quad 526.00$

Tetrasodium biscitrato iron (II) [43160-25-4].

DEFINITION

Sodium Ferrous Citrate contains NLT 10.0% and NMT 11.0% of iron (Fe), calculated on the as-is basis.

IDENTIFICATION

- **A. Sodium**

Sample: Ignite 3 g of Sodium Ferrous Citrate at 500°–600° for 3 h.

Acceptance criteria: The residue obtained from the ignition imparts an intense yellow color to a nonluminous flame.

- **B. Identification Tests—General** (191), *Iron, Ferrous salts:* A solution of 10 mg/mL of Sodium Ferrous Citrate in 5% *hydrochloric acid* meets the requirements.

- **C. Citrate**

Sample: 0.5 g of Sodium Ferrous Citrate

Analysis: Transfer the *Sample* to an appropriate container. Add 5 mL of water and 10 mL of 4% *potassium hydroxide* solution. Heat the solution in a water bath for 10 min while stirring well. Allow the solution to cool to room temperature and filter. Neutralize a portion of the filtrate with 50% *acetic acid*, add an excessive amount of 7.5% *calcium chloride* solution and boil. A white crystalline precipitate is formed. Collect the precipitate.

Acceptance criteria: The precipitate formed does not dissolve upon addition of 4% *sodium hydroxide* solution; the precipitate does dissolve in 25% *hydrochloric acid* solution.

- **D. Complex Salt of Ferrous Iron and Citric Acids**

Sample solution: 10 mg/mL of Sodium Ferrous Citrate

Analysis: Add 2 mL of *stronger ammonia water* to 5 mL of the *Sample solution*.

Acceptance criteria: A red-brown color develops with no precipitation.

ASSAY

- **Content of Iron**

Sample: 1 g of Sodium Ferrous Citrate

Blank: Proceed as directed in the *Analysis* without the *Sample*.

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N *sodium thiosulfate VS*

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a flask with a ground-glass stopper. Carefully add 25 mL of 5% *sulfuric acid* and 2 mL of *nitric acid* to the flask, and boil the mixture for 10 min. Allow the mixture to cool to room temperature. Add 20 mL of water and 4 g of *potassium iodide*, and immediately stopper the flask tightly. Allow the mixture to stand in the dark for 15 min, then add 100 mL of water. Titrate the liberated iodine with *Titrant* (indicator: *starch TS*). Perform a blank determination.

Calculate the percentage of iron (Fe) in the portion of the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual *Titrant* normality (mEq/mL)

F = equivalency factor, 55.85 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 10.0%–11.0% on the as-is basis

IMPURITIES

- **Chloride and Sulfate** (221), *Chloride*

Standard solution: 0.10 mL of 0.020 N *hydrochloric acid*

Sample: 73 mg of Sodium Ferrous Citrate

Acceptance criteria: NMT 0.1%

- **Chloride and Sulfate** (221), *Sulfate*

Standard solution: 1.0 mL of 0.20 N *sulfuric acid*

Sample: 200 mg of Sodium Ferrous Citrate

Acceptance criteria: NMT 0.5%

- **Ferric Iron**

Sample: 2.0 g of Sodium Ferrous Citrate

Analysis: Transfer the *Sample* to an appropriate flask with a ground-glass stopper, dissolve in 5 mL of *hydrochloric acid*, and dilute with water to 30 mL. Add 4 g of *potassium iodide* and close the flask with the stopper. Allow the mixture to stand in the dark for 15 min. Add 2 mL of *starch TS*, and mix well.

Acceptance criteria: A color develops and disappears upon addition of 1.0 mL of 0.1 N *sodium thiosulfate VS* to the solution.

- **Elemental Impurities—Procedures** (233)

Acceptance criteria

Arsenic: NMT 4.0 µg/g

Lead: NMT 10 µg/g

Mercury: NMT 3.0 µg/g

SPECIFIC TESTS

- **Tartrate**

Sample: 1.0 g of Sodium Ferrous Citrate

Analysis: Transfer the *Sample* into an appropriate container. Carefully add 5 mL of water and 10 mL of 6.7% (w/v) *potassium hydroxide* solution. Heat the mixture in a water bath for 10 min while stirring well. Allow the mixture to cool to room temperature and filter. To 5 mL of the filtrate, add 25% *acetic acid* solution to make the mixture weakly acidic, and 2 mL of *acetic acid*. Allow the solution to stand for 24 h.

Acceptance criteria: No white, crystalline precipitate is formed.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **Labeling:** Label it to indicate that it is not to be used if it is coated with brownish yellow basic ferric sulfate. ▲*USP40*

BRIEFING

Tienchi Ginseng Root and Rhizome Dry Extract Capsules. A new monograph is proposed. The liquid chromatographic procedures in the test for *Content of Ginsenosides* use the Phenomenex Kinetex brand of L1 column. The typical retention times for notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd are about 2.1, 2.7, 2.9, 4.7, and 5.2 min, respectively.

(BDSHM: N. Davydova.)

Correspondence Number—C143401

Comment deadline: January 31, 2016

Add the following:

▲ Tienchi Ginseng Root and Rhizome Dry Extract Capsules

DEFINITION

Tienchi Ginseng Root and Rhizome Dry Extract Capsules contain Tienchi Ginseng Root and Rhizome Dry Extract. They contain NLT 90.0% and NMT 110.0% of the labeled amount of ginsenosides calculated as the sum of notoginsenoside R1 ($C_{47}H_{80}O_{18}$), ginsenoside Rg1 ($C_{42}H_{72}O_{14}$), ginsenoside Re ($C_{48}H_{82}O_{18}$), ginsenoside Rb1 ($C_{54}H_{92}O_{23}$), and ginsenoside Rd ($C_{48}H_{82}O_{18}$).

IDENTIFICATION

• **A. HPTLC for Articles of Botanical Origin** (203)

Standard solution A: 0.5 mg/mL of USP Ginsenoside Rg1 RS in methanol

Standard solution B: 5 mg/mL of USP *Panax notoginseng* Root and Rhizome Dry Extract RS in methanol. Sonicate for about 10 min, centrifuge, and use the supernatant.

Sample solution: Transfer a portion of the contents of the Capsules, equivalent to 50 mg of Tienchi Ginseng Root and Rhizome Dry Extract, to a conical flask, add 10 mL of methanol, mix and sonicate for 20 min, centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 μ m (HPTLC plates)

Application volume: 4 μ L, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Developing solvent system: A mixture of dichloromethane, dehydrated alcohol, and water (60: 45: 6.5)

Developing distance: 6 cm

Derivatization reagent: 10% Sulfuric acid in alcohol. Prepare fresh. Keep alcohol cold over ice. Carefully and gradually add sulfuric acid.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate, and dry in air. Develop the chromatograms in a saturated chamber, remove the plate from the chamber, and dry. Treat with *Derivatization reagent*, heat at 105° for 5–10 min, and examine immediately under visible light and UV light at 366 nm.

System suitability

Under visible light: The chromatogram of *Standard solution B* exhibits five main reddish-violet bands. A band in the upper-half section corresponding in R_f to the band of ginsenoside Rg1 in *Standard solution A*; a band in the lower-third section of the

chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower band due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside R1. The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Under UV light at 366 nm: The chromatogram of *Standard solution B* exhibits a pinkish-violet band at an R_f corresponding to the ginsenoside Rg1 band in the chromatogram of *Standard solution A*; a blue fluorescent band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside R1 (the band due to ginsenoside Rd is blue fluorescent, while the other two bands are pinkish-violet). The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Acceptance criteria

Under visible light: The chromatogram of the *Sample solution* exhibits five main reddish-violet bands corresponding in R_f to similar bands in the chromatogram of *Standard solution B*: a band in the upper-half section corresponding in R_f to the band of ginsenoside Rg1 in *Standard solution A*; a band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower band due to ginsenoside Rd, the middle one due to ginsenoside Re, and the upper due to notoginsenoside R1. The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Under UV light at 366 nm: The chromatogram of the *Sample solution* exhibits the bands corresponding in R_f to similar bands in the chromatogram of *Standard solution B*: a pinkish-violet band at an R_f corresponding to the ginsenoside Rg1 band in the chromatogram of *Standard solution A*; a blue fluorescent band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside R1 (the band due to ginsenoside Rd is blue fluorescent, while the other two bands are pinkish-violet). The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

• B. LC

Analysis: Proceed as directed in the test for *Content of Ginsenosides*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits peaks at the retention times corresponding to the peaks due to notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd in the chromatogram of *Standard solution B*. The two most intense peaks in the chromatogram are those of ginsenoside Rg1 and ginsenoside Rb1.

STRENGTH

• Content of Ginsenosides

Extraction solvent: Methanol and water (7:3)

Solution A: 0.03% Phosphoric acid in water (v/v)

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	83	17
2.4	80	20
3.5	70	30
4.2	69	31
5.0	58	42
5.1	0	100
6.0	0	100
6.1	83	17
7.5	83	17

Standard solution A: 0.04 mg/mL of USP Ginsenoside Rg1 RS in methanol. Sonicate to dissolve, if necessary.

Standard solution B: 3.0 mg/mL of USP *Panax notoginseng* Root and Rhizome Dry Extract RS in *Extraction solvent*. Sonicate for about 10 min, centrifuge, and use the supernatant. Before injection, pass through a polytetrafluoroethylene (PTFE) membrane filter of 0.2- μ m pore size and discard the first portion of the filtrate.

Sample solution: Determine the total weight of 20 Capsules. Open the Capsules and combine their contents in an appropriate container. Weigh the empty Capsule shells and calculate the average fill weight per Capsule. Transfer a portion of the Capsule contents, equivalent to 35 mg of ginsenosides (sum of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd) into a 25-mL volumetric flask. Add 20 mL of *Extraction solvent* and sonicate for 30 min with occasional shaking. Cool to room temperature, dilute with *Extraction solvent* to volume, mix well, and centrifuge. Before injection, pass through a PTFE membrane filter of 0.2- μ m pore size and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 203 nm

Column: 2.1-mm \times 5-cm; 1.7- μ m packing L1

Column temperature: 30°

Flow rate: 0.8 mL/min

Injection volume: 5 μ L

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the

reference chromatogram provided with the lot of USP *Panax notoginseng* Root and Rhizome Dry Extract RS being used.

Resolution: NLT 1.5 between the ginsenoside Rg1 and ginsenoside Re peaks, *Standard solution B*

Relative standard deviation: NMT 2.0%, determined from the ginsenoside Rg1 peak in repeated injections, *Standard solution A*

Analysis

Samples: *Standard solution A, Standard solution B, and Sample solution*

Using the chromatograms of *Standard solution A, Standard solution B,* and the reference chromatogram provided with the lot of USP *Panax notoginseng* Root and Rhizome Dry Extract RS being used, identify the peaks corresponding to notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd in the *Sample solution* chromatogram. Measure the areas of the analyte peaks.

Calculate the quantity, in mg, each of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd in each Capsule:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times F \times (W_{av}/W)$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of ginsenoside Rg1 from *Standard solution A*

C_S = concentration of USP Ginsenoside Rg1 RS in *Standard solution A* (mg/mL)

V = volume of the solvent taken for preparation of the *Sample solution* (mL)

F = response factor for analytes (see *Table 2*)

W_{av} = average fill weight per Capsule (mg)

W = weight of the sample taken for preparation of the *Sample solution* (mg)

Table 2

Analyte	Response Factor
Notoginsenoside R1	1.09
Ginsenoside Rg1	1.00
Ginsenoside Re	1.02
Ginsenoside Rb1	1.26
Ginsenoside Rd	1.03

Calculate the percentage of the labeled amount of ginsenosides as the sum of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd in each Capsule:

$$\text{Result} = (\Sigma Q_i/L) \times 100$$

ΣQ_i = sum of the quantities of ginsenosides as determined above (mg)

L = labeled amount of ginsenosides (mg)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Disintegration and Dissolution** (2040), *Disintegration*: Meet the requirements
- **Weight Variation** (2091): Meet the requirements

CONTAMINANTS

- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage**: Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling**: The label states the Latin binomial and the official name. The label states the amount of ginsenosides as the sum of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd; and the amount of Tienchi Ginseng Root and Rhizome Dry Extract in mg/Capsule.
- **USP Reference Standards** (11)
USP Ginsenoside Rg1 RS
USP *Panax notoginseng* Root and Rhizome Dry Extract RS

▲USP40

BRIEFING

Tienchi Ginseng Root and Rhizome Dry Extract Tablets. A new monograph is proposed. The liquid chromatographic procedures in the test for *Content of Ginsenosides* use the Phenomenex Kinetex brand of C18 column. The typical retention times for notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd are about 2.1, 2.7, 2.9, 4.7, and 5.2 min, respectively

(BDSHM: N. Davydova.)

Correspondence Number—C143402

Comment deadline: January 31, 2016**Add the following:**▲ **Tienchi Ginseng Root and Rhizome Dry Extract Tablets****DEFINITION**

Tienchi Ginseng Root and Rhizome Dry Extract Tablets contain Tienchi Ginseng Root and Rhizome Dry Extract. They contain NLT 90.0% and NMT 110.0% of the labeled amount of ginsenosides calculated as the sum of notoginsenoside R1 ($C_{47}H_{80}O_{18}$), ginsenoside Rg1 ($C_{42}H_{72}O_{14}$), ginsenoside Re ($C_{48}H_{82}O_{18}$), ginsenoside Rb1 ($C_{54}H_{92}O_{23}$), and ginsenoside Rd ($C_{48}H_{82}O_{18}$).

IDENTIFICATION

- **A. HPTLC for Articles of Botanical Origin** (203)

Standard solution A: 0.5 mg/mL of USP Ginsenoside Rg1 RS in methanol

Standard solution B: 5 mg/mL of USP *Panax notoginseng* Root and Rhizome Dry Extract RS in methanol. Sonicate for about 10 min, centrifuge, and use the supernatant.

Sample solution: Transfer a portion of the powdered Tablets, equivalent to 50 mg of Tienchi Ginseng Root and Rhizome Dry Extract, to a conical flask, add 10 mL of methanol, mix and sonicate for 20 min, centrifuge, and filter.

Chromatographic system

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 μm (HPTLC plates)

Application volume: 4 μL , as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Developing solvent system: A mixture of dichloromethane, dehydrated alcohol, and water (60: 45: 6.5)

Developing distance: 6 cm

Derivatization reagent: 10% Sulfuric acid in alcohol. Prepare fresh. Keep alcohol cold over ice. Carefully and gradually add sulfuric acid.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate, and dry in air. Develop the chromatograms in a saturated chamber, remove the plate from the chamber, and dry. Treat with *Derivatization reagent*, heat at 105° for 5–10 min, and examine immediately under visible light and UV light at 366 nm.

System suitability

Under visible light: The chromatogram of *Standard solution B* exhibits five main reddish-violet bands. A band in the upper-half section corresponding in R_f to the band of ginsenoside Rg1 in *Standard solution A*; a band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower band due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside R1. The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Under UV light at 366 nm: The chromatogram of *Standard solution B* exhibits a pinkish-violet band at an R_f corresponding to the ginsenoside Rg1 band in the chromatogram of *Standard solution A*; a blue fluorescent band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside R1 (the band due to ginsenoside Rd is blue fluorescent, while the other two bands are pinkish-violet). The two most intense bands in the chromatogram are those of

ginsenoside Rb1 and ginsenoside Rg1.

Acceptance criteria

Under visible light: The chromatogram of the *Sample solution* exhibits five main reddish-violet bands corresponding in R_f to similar bands in the chromatogram of *Standard solution B*: a band in the upper-half section corresponding in R_f to the band of ginsenoside Rg1 in *Standard solution A*; a band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower band due to ginsenoside Rd, the middle one due to ginsenoside Re, and the upper due to notoginsenoside R1. The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Under UV light at 366 nm: The chromatogram of the *Sample solution* exhibits the bands corresponding in R_f to similar bands in the chromatogram of *Standard solution B*: a pinkish-violet band at an R_f corresponding to the ginsenoside Rg1 band in the chromatogram of *Standard solution A*; a blue fluorescent band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside R1 (the band due to ginsenoside Rd is blue fluorescent, while the other two bands are pinkish-violet). The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

• B. LC

Analysis: Proceed as directed in the test for *Content of Ginsenosides*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits peaks at the retention times corresponding to the peaks due to notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd in the chromatogram of *Standard solution B*. The two most intense peaks in the chromatogram are those of ginsenoside Rg1 and ginsenoside Rb1.

STRENGTH

• Content of Ginsenosides

Extraction solvent: Methanol and water (7:3)

Solution A: 0.03% Phosphoric acid in water (v/v)

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	83	17
2.4	80	20
3.5	70	30
4.2	69	31
5.0	58	42
5.1	0	100
6.0	0	100
6.1	83	17
7.5	83	17

Standard solution A: 0.04 mg/mL of USP Ginsenoside Rg1 RS in methanol. Sonicate to dissolve, if necessary.

Standard solution B: 3.0 mg/mL of USP *Panax notoginseng* Root and Rhizome Dry Extract RS in *Extraction solvent*. Sonicate for about 10 min, centrifuge, and use the supernatant. Before injection, pass through a polytetrafluoroethylene (PTFE) membrane filter of 0.2- μ m pore size and discard the first portion of the filtrate.

Sample solution: Weigh NLT 20 Tablets, determine the average Tablet weight, and finely powder. Transfer a portion of finely powdered Tablets, nominally equivalent to 35 mg of ginsenosides (sum of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd) into a 25-mL volumetric flask. Add 20 mL of *Extraction solvent* and sonicate for 30 min with occasional shaking. Cool to room temperature, dilute with *Extraction solvent* to volume, mix well, centrifuge, pass the supernatant through a PTFE membrane filter of 0.2- μ m pore size, and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 203 nm

Column: 2.1-mm \times 5-cm; 1.7- μ m packing L1

Column temperature: 30°

Flow rate: 0.8 mL/min

Injection volume: 5 μ L

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of USP *Panax notoginseng* Root and Rhizome Dry Extract RS being used.

Resolution: NLT 1.5 between the ginsenoside Rg1 and ginsenoside Re peaks, *Standard solution B*

Relative standard deviation: NMT 2.0%, determined from the ginsenoside Rg1 peak in repeated injections, *Standard solution A*

Analysis

Samples: *Standard solution A, Standard solution B, and Sample solution*

Using the chromatograms of *Standard solution A, Standard solution B,* and the reference chromatogram provided with the lot of USP *Panax notoginseng* Root and Rhizome Dry Extract RS being used, identify the peaks corresponding to notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd in the *Sample solution* chromatogram. Measure the areas of the analyte peaks.

Calculate the quantity, in mg, each of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd in each Tablet:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times F \times (W_{av}/W)$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of ginsenoside Rg1 from *Standard solution A*

C_S = concentration of USP Ginsenoside Rg1 RS in *Standard solution A* (mg/mL)

V = volume of the solvent taken for preparation of the *Sample solution* (mL)

F = response factor for analytes (see *Table 2*)

W_{av} = average Tablet weight (mg)

W = weight of the sample taken for preparation of the *Sample solution* (mg)

Table 2

Analyte	Response Factor
Notoginsenoside R1	1.09
Ginsenoside Rg1	1.00
Ginsenoside Re	1.02
Ginsenoside Rb1	1.26
Ginsenoside Rd	1.03

Calculate the percentage of the labeled amount of ginsenosides, as the sum of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd, in each Tablet:

$$\text{Result} = (\Sigma Q_i/L) \times 100$$

ΣQ_i = sum of the quantities of ginsenosides as determined above (mg)

L = labeled amount of ginsenosides (mg)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Disintegration and Dissolution** (2040), *Disintegration:* Meet the requirements
- **Weight Variation** (2091): Meet the requirements

CONTAMINANTS

- **Microbial Enumeration Tests** <2021>: The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** <2022>, *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage**: Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling**: The label states the Latin binomial and the official name. The label states the amount of ginsenosides, as the sum of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd; and the amount of Tienchi Ginseng Root and Rhizome Dry Extract in mg/Tablet.
- **USP Reference Standards** <11>
 USP Ginsenoside Rg1 RS
 USP *Panax notoginseng* Root and Rhizome Dry Extract RS

▲USP40

BRIEFING

Tienchi Ginseng Root and Rhizome Powder Capsules. A new monograph is proposed. The liquid chromatographic procedures in the test for *Content of Ginsenosides* use the Phenomenex Kinetex brand of C18 column. The typical retention times for notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd are about 2.1, 2.7, 2.9, 4.7, and 5.2 min, respectively.

(BDSHM: N. Davydova.)

Correspondence Number—C143400

Comment deadline: January 31, 2016

Add the following:

▲ Tienchi Ginseng Root and Rhizome Powder Capsules

DEFINITION

Tienchi Ginseng Root and Rhizome Powder Capsules contain Tienchi Ginseng Root and Rhizome Powder. They contain NLT 5.0% of ginsenosides calculated as the sum of notoginsenoside R1 ($C_{47}H_{80}O_{18}$), ginsenoside Rg1 ($C_{42}H_{72}O_{14}$), ginsenoside Re ($C_{48}H_{82}O_{18}$), ginsenoside Rb1 ($C_{54}H_{92}O_{23}$), and ginsenoside Rd ($C_{48}H_{82}O_{18}$) from the labeled amount of Tienchi Ginseng Root and Rhizome Powder.

IDENTIFICATION

- **A. HPTLC for Articles of Botanical Origin** <203>
Standard solution A: 0.5 mg/mL of USP Ginsenoside Rg1 RS in methanol
Standard solution B: 5 mg/mL of USP *Panax notoginseng* Root and Rhizome Dry Extract RS in methanol. Sonicate for about 10 min, centrifuge, and use the supernatant.

Sample solution: Transfer a finely powdered portion of the contents of the Capsules, equivalent to 0.4 g of Tienchi Ginseng Root and Rhizome Powder, to a suitable container, add 10.0 mL of methanol, and sonicate for 20 min. Centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 μm (HPTLC plates)

Application volume: 4 μL , as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Developing solvent system: A mixture of dichloromethane, dehydrated alcohol, and water (60: 45: 6.5)

Developing distance: 6 cm

Derivatization reagent: 10% Sulfuric acid in alcohol. Prepare fresh. Keep alcohol cold over ice. Carefully and gradually add sulfuric acid.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate, and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Treat the plate with *Derivatization reagent*, heat at 105° for 5 min, and examine immediately under visible light and UV light at 366 nm.

System suitability

Under visible light: The chromatogram of *Standard solution B* exhibits five main reddish-violet bands. A band in the upper-half section corresponding in R_f to the band of ginsenoside Rg1 in *Standard solution A*; a band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower band due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside R1. The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Under UV light at 366 nm: The chromatogram of *Standard solution B* exhibits a pinkish-violet band at an R_f corresponding to the ginsenoside Rg1 band in the chromatogram of *Standard solution A*; a blue fluorescent band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside R1 (the band due to ginsenoside Rd is blue fluorescent, while the other two bands are pinkish-violet). The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Acceptance criteria

Under visible light: The chromatogram of the *Sample solution* exhibits five main reddish-violet bands corresponding in R_f to similar bands in the chromatogram of *Standard solution B*: a band in the upper-half section corresponding in R_f to the band of

ginsenoside Rg1 in *Standard solution A*; a band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower band due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside R1. The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Under UV light at 366 nm: The chromatogram of the *Sample solution* exhibits the bands corresponding in R_f to similar bands in the chromatogram of *Standard solution B*: a pinkish-violet band at an R_f corresponding to the ginsenoside Rg1 band in the chromatogram of *Standard solution A*; a blue fluorescent band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside R1 (the band due to ginsenoside Rd is blue fluorescent, while the other two bands are pinkish-violet). The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

- **B. LC**

Analysis: Proceed as directed in the test for *Content of Ginsenosides*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits peaks at the retention times corresponding to the peaks due to notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd in the chromatogram of *Standard solution B*. The two most intense peaks in the chromatogram are those of ginsenoside Rg1 and ginsenoside Rb1.

STRENGTH

- **Content of Ginsenosides**

Extraction solvent: Methanol and water (7:3)

Solution A: 0.03% Phosphoric acid in water (v/v)

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	83	17
2.4	80	20
3.5	70	30
4.2	69	31
5.0	58	42
5.1	0	100
6.0	0	100
6.1	83	17
7.5	83	17

Standard solution A: 0.04 mg/mL of USP Ginsenoside Rg1 RS in methanol. Sonicate to

dissolve, if necessary.

Standard solution B: 3.0 mg/mL of USP *Panax notoginseng* Root and Rhizome Dry Extract RS in *Extraction solvent*. Sonicate for about 10 min, centrifuge, and use the supernatant. Before injection, pass through a polytetrafluoroethylene (PTFE) membrane filter of 0.2- μ m pore size, and discard the first portion of the filtrate.

Sample solution: Determine the total weight of 20 Capsules. Open the Capsules and combine their contents in an appropriate container. Weigh the empty Capsule shells and calculate the average fill weight per Capsule. Thoroughly mix and finely powder the contents of the Capsules. Transfer a portion of the Capsule contents, equivalent to 0.3 g of Tienchi Ginseng Root and Rhizome Powder to a 50-mL centrifuge tube. Add 10 mL of *Extraction solvent*, and sonicate for 20 min. Centrifuge, and transfer the supernatant to a 25-mL volumetric flask. Repeat this extraction with 10 mL of *Extraction solvent*, and sonicate for 10 min. Combine the extracts in a volumetric flask, dilute with *Extraction solvent* to volume, and mix. Before injection, pass through a PTFE membrane filter of 0.2- μ m pore size, and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 203 nm

Column: 2.1-mm \times 5-cm; 1.7- μ m packing L1

Column temperature: 30°

Flow rate: 0.8 mL/min

Injection volume: 5 μ L

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of USP *Panax notoginseng* Root and Rhizome Dry Extract RS being used.

Resolution: NLT 1.5 between the ginsenoside Rg1 and ginsenoside Re peaks, *Standard solution B*

Relative standard deviation: NMT 2.0%, determined from the ginsenoside Rg1 peak in repeated injections, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatograms of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of USP *Panax notoginseng* Root and Rhizome Dry Extract RS being used, identify the peaks corresponding to notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd in the *Sample solution* chromatogram. Measure the areas of the analyte peaks.

Calculate the quantity, in mg, each of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd, in each Capsule:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times F \times (W_{av}/W)$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of ginsenoside Rg1 from *Standard solution A*

C_S = concentration of USP Ginsenoside Rg1 RS in *Standard solution A* (mg/mL)

V = volume of the solvent taken for preparation of the *Sample solution* (mL)

F = response factor for analytes (see *Table 2*)

W_{av} = average Capsule fill weight (mg)

W = weight of the sample taken for preparation of the *Sample solution* (mg)

Table 2

Analyte	Response Factor
Notoginsenoside R1	1.09
Ginsenoside Rg1	1.00
Ginsenoside Re	1.02
Ginsenoside Rb1	1.26
Ginsenoside Rd	1.03

Calculate the percentage of ginsenosides, as the sum of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd, of the labeled amount of Tienchi Ginseng Root and Rhizome Powder in each Capsule:

$$\text{Result} = (\Sigma Q_i/L) \times 100$$

ΣQ_i = sum of the quantities of ginsenosides as determined above (mg)

L = labeled amount of Tienchi Ginseng Root and Rhizome Powder (mg)

Acceptance criteria: NLT 5.0%

PERFORMANCE TESTS

- **Disintegration and Dissolution** (2040), *Disintegration*: Meet the requirements
- **Weight Variation** (2091): Meet the requirements

CONTAMINANTS

- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling:** The label states the Latin binomial and the official name. The label states the amount of Tienchi Ginseng Root and Rhizome Powder in mg/Capsule.

- **USP Reference Standards** <11>

USP Ginsenoside Rg1 RS USP *Panax notoginseng* Root and Rhizome Dry Extract RS

▲USP40

BRIEFING

Tienchi Ginseng Root and Rhizome Powder Tablets. A new monograph is proposed. The liquid chromatographic procedures in the test for *Content of Ginsenosides* use the Phenomenex Kinetex brand of C18 column. The typical retention times for notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd are about 2.1, 2.7, 2.9, 4.7, and 5.2 min, respectively.

(BDSHM: N. Davydova.)

Correspondence Number—C143403

Comment deadline: January 31, 2016**Add the following:**▲ **Tienchi Ginseng Root and Rhizome Powder Tablets****DEFINITION**

Tienchi Ginseng Root and Rhizome Powder Tablets contain Tienchi Ginseng Root and Rhizome Powder. They contain NLT 5.0% of ginsenosides calculated as the sum of notoginsenoside R1 ($C_{47}H_{80}O_{18}$), ginsenoside Rg1 ($C_{42}H_{72}O_{14}$), ginsenoside Re ($C_{48}H_{82}O_{18}$), ginsenoside Rb1 ($C_{54}H_{92}O_{23}$), and ginsenoside Rd ($C_{48}H_{82}O_{18}$) from the labeled amount of Tienchi Ginseng Root and Rhizome Powder.

IDENTIFICATION

- **A. HPTLC for Articles of Botanical Origin** <203>

Standard solution A: 0.5 mg/mL of USP Ginsenoside Rg1 RS in methanol

Standard solution B: 5 mg/mL of USP *Panax notoginseng* Root and Rhizome Dry Extract RS in methanol. Sonicate for about 10 min, centrifuge, and use the supernatant.

Sample solution: Transfer a portion of the finely powdered Tablets, equivalent to 0.4 g of Tienchi Ginseng Root and Rhizome Powder, to a suitable container, add 10.0 mL of methanol, and sonicate for 20 min. Centrifuge and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 μ m (HPTLC plates)

Application volume: 4 μ L, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Developing solvent system: A mixture of dichloromethane, dehydrated alcohol, and water (60: 45: 6.5)

Developing distance: 6 cm

Derivatization reagent: 10% Sulfuric acid in alcohol. Prepare fresh. Keep alcohol cold over ice. Carefully and gradually add sulfuric acid.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate, and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Treat the plate with *Derivatization reagent*, heat at 105° for 5 min, and examine immediately under visible light and UV light at 366 nm.

System suitability

Under visible light: The chromatogram of *Standard solution B* exhibits five main reddish-violet bands. A band in the upper-half section corresponding in R_f to the band of ginsenoside Rg1 in *Standard solution A*; a band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower band due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside R1. The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Under UV light at 366 nm: The chromatogram of *Standard solution B* exhibits a pinkish-violet band at an R_f corresponding to the ginsenoside Rg1 band in the chromatogram of *Standard solution A*; a blue fluorescent band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside R1 (the band due to ginsenoside Rd is blue fluorescent, while the other two bands are pinkish-violet). The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Acceptance criteria

Under visible light: The chromatogram of the *Sample solution* exhibits five main reddish-violet bands corresponding in R_f to similar bands in the chromatogram of *Standard solution B*: a band in the upper-half section corresponding in R_f to the band of ginsenoside Rg1 in *Standard solution A*; a band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower band due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside R1. The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Under UV light at 366 nm: The chromatogram of the *Sample solution* exhibits the bands corresponding in R_f to similar bands in the chromatogram of *Standard solution B*: a pinkish-violet band at an R_f corresponding to the ginsenoside Rg1 band in the chromatogram of *Standard solution A*; a blue fluorescent band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated between the bands of ginsenoside Rg1 and ginsenoside Rb1—the lower due to ginsenoside Rd, the middle due to ginsenoside Re, and the upper due to notoginsenoside

R1 (the band due to ginsenoside Rd is blue fluorescent, while the other two bands are pinkish-violet). The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

- **B. LC**

Analysis: Proceed as directed in the test for *Content of Ginsenosides*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits peaks at the retention times corresponding to the peaks due to notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd in the chromatogram of *Standard solution B*. The two most intense peaks in the chromatogram are those of ginsenoside Rg1 and ginsenoside Rb1.

STRENGTH

- **Content of Ginsenosides**

Extraction solvent: Methanol and water (7:3)

Solution A: 0.03% Phosphoric acid in water (v/v)

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	83	17
2.4	80	20
3.5	70	30
4.2	69	31
5.0	58	42
5.1	0	100
6.0	0	100
6.1	83	17
7.5	83	17

Standard solution A: 0.04 mg/mL of USP Ginsenoside Rg1 RS in methanol. Sonicate to dissolve, if necessary.

Standard solution B: 2.0 mg/mL of USP *Panax notoginseng* Root and Rhizome Dry Extract RS in *Extraction solvent*. Sonicate for about 10 min, centrifuge, and use the supernatant. Before injection, pass through a polytetrafluoroethylene (PTFE) membrane filter of 0.2- μ m pore size and discard the first portion of the filtrate.

Sample solution: Weigh NLT 20 Tablets, determine the average Tablet weight, and finely powder. Transfer a portion of finely powdered Tablets, equivalent to 0.3 g of Tienchi Ginseng Root and Rhizome Powder to a 50-mL centrifuge tube. Add 10 mL of *Extraction solvent* and sonicate for 20 min. Centrifuge, and transfer the supernatant to a 25-mL volumetric flask. Repeat this extraction with 10 mL of *Extraction solvent*, sonicate for 10 min. Combine the extracts in a volumetric flask, dilute with *Extraction solvent* to volume, and mix. Before injection, pass through a PTFE membrane filter of 0.2- μ m pore size, and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 203 nm

Column: 2.1-mm × 5-cm; 1.7-μm packing L1

Column temperature: 30°

Flow rate: 0.8 mL/min

Injection volume: 5 μL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of USP *Panax notoginseng* Root and Rhizome Dry Extract RS being used.

Resolution: NLT 1.5 between the ginsenoside Rg1 and ginsenoside Re peaks, *Standard solution B*

Relative standard deviation: NMT 2.0%, determined from the ginsenoside Rg1 peak in repeated injections, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatograms of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of USP *Panax notoginseng* Root and Rhizome Dry Extract RS being used, identify the peaks corresponding to notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd in the *Sample solution*.

Calculate the quantity, in mg, each of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd in each Tablet:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times F \times (W_{av}/W)$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of ginsenoside Rg1 from *Standard solution A*

C_S = concentration of USP Ginsenoside Rg1 RS in *Standard solution A* (mg/mL)

V = volume of the solvent taken for preparation of the *Sample solution* (mL)

F = response factor for analytes (see *Table 2*)

W_{av} = average Tablet weight (mg)

W = weight of the sample taken for preparation of the *Sample solution* (mg)

Table 2

Analyte	Response Factor
Notoginsenoside R1	1.09
Ginsenoside Rg1	1.00
Ginsenoside Re	1.02
Ginsenoside Rb1	1.26
Ginsenoside Rd	1.03

Calculate the percentage of ginsenosides, as the sum of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd, of the labeled amount of Tienchi Ginseng Root and Rhizome Powder in each Tablet:

$$\text{Result} = (\Sigma Q_i/L) \times 100$$

ΣQ_i = sum of the quantities of ginsenosides as determined above (mg)

L = labeled amount of Tienchi Ginseng Root and Rhizome Powder (mg)

Acceptance criteria: NLT 5.0%

PERFORMANCE TESTS

- **Disintegration and Dissolution** (2040), *Disintegration*: Meet the requirements
- **Weight Variation** (2091): Meet the requirements

CONTAMINANTS

- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** (2022), *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage**: Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling**: The label states the Latin binomial and the official name. The label states the amount of Tienchi Ginseng Root and Rhizome Powder in mg/Tablet.
- **USP Reference Standards** (11)
 - USP Ginsenoside Rg1 RS
 - USP *Panax notoginseng* Root and Rhizome Dry Extract RS

▲USP40

BRIEFING

Tribasic Calcium Phosphate, NF 33 page 6559. On the basis of comments received, the following changes are proposed:

1. Modify the *Analysis* section in *Identification* test A by changing the volume of *ammonium molybdate TS* added from 0.5 mL to 2 mL to improve the test performance.
2. In preparation for the omission of the flame tests from *Identification Tests—General*

(191), proposed in *PF* 41(2) [Mar.–Apr. 2015], it is proposed to replace the flame test for calcium with a calcium precipitation test.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(NBDS: H. Dinh.)

Correspondence Number—C164267

Comment deadline: January 31, 2016

Tribasic Calcium Phosphate

$\text{Ca}_5(\text{OH})(\text{PO}_4)_3$ 502.31

Calcium hydroxide phosphate [12167-74-7].

DEFINITION

Tribasic Calcium Phosphate consists of a variable mixture of calcium phosphates with the approximate composition ($10\text{CaO}\cdot 3\text{P}_2\text{O}_5\cdot \text{H}_2\text{O}$). It contains NLT 34.0% and NMT 40.0% of calcium (Ca).

IDENTIFICATION

Change to read:

- **A.**

Sample solution: Dissolve 100 mg in 5 mL of *diluted nitric acid*.

Analysis: Warm the *Sample solution*, and add ~~0.5 mL~~

▲ 2 mL ▲*NF35*

of *ammonium molybdate TS*.

Acceptance criteria: A yellow precipitate is formed.

Delete the following:

▲ ● **~~B. Identification Tests—General, Calcium (191):~~** Meets the requirements of the flame test ▲*NF35*

Add the following:

- ▲ ● **B.**

Sample: 100 mg of Tribasic Calcium Phosphate

Analysis: Dissolve the *Sample* by warming in 10 mL of 2 N *hydrochloric acid*. Add 2.5 mL of *ammonia TS* dropwise, with shaking, and then add 5 mL of *ammonium oxalate TS*.

Acceptance criteria: A white precipitate is formed. ▲*NF35*

ASSAY

Change to read:

- **Procedure**

Sample: 150 mg of Tribasic Calcium Phosphate

• (ERR 1-Dec-2014)

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample*, with the aid of gentle heat if necessary, in a mixture of *hydrochloric acid* and *water* (5:3) contained in a 250-mL beaker equipped with a magnetic stirrer, and cautiously add 125 mL of *water*. With constant stirring, add in the following order: 0.5 mL of *triethanolamine*, 300 mg of *hydroxy naphthol blue*, and from the titration buret, about 23 mL of *Titrant*. Add *sodium hydroxide* solution (45 in 100) until the initial red color changes to clear blue. Continue to add it dropwise until the color changes to violet, and add an additional 0.5 mL. The pH is 12.3–12.5. Continue the titration dropwise with *Titrant* to the appearance of a clear blue endpoint that persists for NLT 60 s. Calculate the percentage of calcium (Ca) in the portion of the *Sample* taken:

$$\text{Result} = [(V_S \times M \times F)/W] \times 100 \quad \bullet \text{(ERR 1-Dec-2014)}$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

• (ERR 1-Dec-2014)

M = actual molarity of the *Titrant* (mmol/mL)

F = equivalency factor, 40.08 mg/mmol

W = *Sample* weight (mg)

Acceptance criteria: 34.0%–40.0%

IMPURITIES

- **Chloride and Sulfate** (221), *Chloride*

Standard: 1.0 mL of 0.020 N *hydrochloric acid*

Sample: 0.500 g of Tribasic Calcium Phosphate

Analysis: Dissolve the *Sample* in 25 mL of 2 N *nitric acid*, and add 1 mL of *silver nitrate TS*.

Acceptance criteria: NMT 0.14%

- **Chloride and Sulfate** (221), *Sulfate*

Standard: 1.0 mL of 0.020 N *sulfuric acid*

Sample: 0.500 g of Tribasic Calcium Phosphate

Analysis: Dissolve the *Sample* in the smallest possible amount of 3 N *hydrochloric acid*. Dilute with *water* to 100 mL, and filter, if necessary. To 25 mL of the filtrate add 1 mL of *barium chloride TS*.

Acceptance criteria: NMT 0.8%

- **Arsenic** (211), *Method I*

Test preparation: Dissolve 1.0 g in just sufficient 3 N *hydrochloric acid*.

Acceptance criteria: NMT 3 ppm

- **Barium**

Sample: 500 mg of Tribasic Calcium Phosphate

Analysis: Mix the *Sample* with 10 mL of *water*, heat, add *hydrochloric acid*, dropwise, until solution is effected, and then add 2 drops of the acid in excess. Filter, and add to the filtrate 1 mL of *potassium sulfate TS*.

Acceptance criteria: No turbidity appears within 15 min.

Delete the following:

- ~~**Heavy Metals**, *Method I* (231)~~

~~**Test preparation:** Mix 1.3 g with 9 mL of 3 N *hydrochloric acid*, dilute with *water* to 50 mL, and heat to boiling. Cool to room temperature, and filter. [Note—Filter the mixture after the pH adjustment.]~~

~~**Acceptance criteria:** NMT 30 ppm (Official 1-Jan-2018)~~

- **Carbonate**

Sample: 2 g of Tribasic Calcium Phosphate

Analysis: Mix the *Sample* with 20 mL of *water*, and add 3 N *hydrochloric acid*, dropwise, to effect solution.

Acceptance criteria: No effervescence is produced.

- **Acid-Insoluble Substances**

Analysis: If an insoluble residue remains in the test for *Carbonate*, boil the solution, filter, wash the residue well with hot *water* until the last washing is free from chloride, and ignite the residue to constant weight.

Acceptance criteria: NMT 0.2%; the weight of the residue is NMT 4 mg.

Change to read:

- **Dibasic Salt and Calcium Oxide**

Sample: 1.5 g of Tribasic Calcium Phosphate

● (ERR 1-Dec-2014)

Titrimetric system

(See *Titrimetry* (541).)

Mode: Residual titration

Titrant: 1 N *hydrochloric acid VS*

Back-titrant: 0.1 N *sodium hydroxide VS*

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* by warming with 25.0 mL of *Titrant*. Cool, and slowly titrate the excess of *Titrant*, while agitating constantly, with the *Back-titrant* to a pH of 4.0.

Acceptance criteria: 13.0–14.3 mL of 1 N *hydrochloric acid* is consumed for each g of salt, calculated on the ignited basis.

- **Limit of Fluoride**

[Note—Prepare and store all solutions in plastic containers.]

Buffer solution: 294 mg/mL of *sodium citrate dihydrate* in *water*

Standard stock solution: 1.1052 mg/mL of USP Sodium Fluoride RS in *water*

Standard solution: Transfer 20.0 mL of *Standard stock solution* to a 100-mL volumetric flask containing 50.0 mL of *Buffer solution*, dilute with *water* to volume, and mix. Each mL of this solution contains 100 µg of fluoride ion.

Sample solution: Transfer 2.0 g of Tribasic Calcium Phosphate to a beaker containing a plastic-coated stirring bar. Add 20 mL of *water* and 3.0 mL of *hydrochloric acid*, and stir until dissolved. Add 50.0 mL of *Buffer solution* and sufficient *water* to make 100 mL.

Electrode system: Use a fluoride-specific ion-indicating electrode and a silver-silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see *pH* (791)).

Analysis

Samples: *Standard solution* and *Sample solution*

Standard response line: Transfer 50.0 mL of *Buffer solution* and 3.0 mL of *hydrochloric acid* to a beaker, and add *water* to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, 500, and 500 µL of *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, 1.0, and 1.5 µg/mL) versus potential, in mV.

Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard response line* determine the concentration, *C* (in µg/mL), of fluoride ion in the *Sample solution*.

Calculate the content of fluoride in the portion of Tribasic Calcium Phosphate taken:

$$\text{Result} = (V \times C)/W$$

V = volume of the *Sample solution* (mL)

C = concentration of fluoride ion in the *Sample solution* determined from the *Standard response line* (µg/mL)

W = weight of Tribasic Calcium Phosphate taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 75 ppm

- **Limit of Nitrate**

Sample: 200 mg of Tribasic Calcium Phosphate

Analysis: Mix the *Sample* with 5 mL of *water*, and add just sufficient *hydrochloric acid* to effect solution. Dilute with *water* to 10 mL, add 0.20 mL of *indigo carmine TS*, then add, with stirring, 10 mL of *sulfuric acid*.

Acceptance criteria: The blue color persists for NLT 5 min.

- **Water-Soluble Substances**

Sample: 2 g of Tribasic Calcium Phosphate

Analysis: Digest the *Sample* with 100 mL of *water* on a steam bath for 30 min. Cool, add sufficient *water* to restore the original volume, stir well, and filter. Evaporate 50 mL of the

filtrate in a tared porcelain dish on a steam bath to dryness, and dry the residue at 120° to constant weight.

Acceptance criteria: NMT 0.5%; the weight of the residue is NMT 5 mg.

SPECIFIC TESTS

- **Loss on Ignition** (733)

Analysis: Ignite at 800° for 30 min.

Acceptance criteria: NMT 8.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

- **USP Reference Standards** (11)

USP Sodium Fluoride RS

BRIEFING

Croscarmellose Sodium, *NF 33* page 6630. In preparation for the omission of the flame tests from *Identification Tests—General* (191), proposed in *PF 41(2)* [Mar.–Apr. 2015], the reference to (191) in *Identification test C* is deleted and a complete description of the flame test is included in the monograph. Manufacturers are encouraged to submit a replacement wet chemistry test or an instrumental procedure for Expert Committee consideration.

A statement is added in the beginning of the monograph: "Portions of this monograph that are national *USP* text, and are not part of the harmonized text, are marked with symbols (◆) to specify this fact."

The symbols (◆) are added to the non-harmonized attributes or local requirements—*Identification test C, Sodium Chloride and Sodium Glycolate, Content of Water-Soluble Material, and Microbial Enumeration Tests and Tests for Specified Microorganisms*.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC2: J. Liu.)

Correspondence Number—C164908

Comment deadline: January 31, 2016

Croscarmellose Sodium

Add the following:

▲ Portions of this monograph that are national *USP* text, and are not part of the harmonized text, are marked with symbols (◆) to specify this fact. ▲*NF35*

DEFINITION

Croscarmellose Sodium is the sodium salt of a cross-linked, partly *O*-(carboxymethylated) cellulose.

IDENTIFICATION

- **A.** Mix 1 g with 100 mL of methylene blue solution (1 in 250,000), stir the mixture, and allow it to settle. The Croscarmellose Sodium absorbs the methylene blue and settles as a blue, fibrous mass.
- **B.** Mix 1 g with 50 mL of water. Transfer 1 mL of the mixture to a small test tube, and add 1 mL of water and 5 drops of *1-naphthol TS*. Incline the test tube, and carefully add 2 mL of *sulfuric acid* down the side so that it forms a lower layer: a reddish-violet color develops at the interface.

Change to read:

•

▲◆▲NF35

C. A portion of the mixture of Croscarmellose Sodium with water, prepared as directed in *Identification test B*, ~~meets the requirements of the flame test for Identification Tests—General (191), Sodium.~~

▲ imparts an intense yellow color to a nonluminous flame. ◆▲NF35

IMPURITIES

- **Residue on Ignition (281):** 14.0%–28.0%, calculated on the dried basis. Use 1.0 g for the test, and use sufficient *sulfuric acid* to moisten the entire residue after the initial charring step, and additional *sulfuric acid* if an excessive amount of carbonaceous material remains after the initial complete volatilization of white fumes.

Delete the following:

* • **Heavy Metals (231), Method II:** 10 ppm • (Official 1-Jan-2018)

Change to read:

•

▲◆▲NF35

Sodium Chloride and Sodium Glycolate**Sodium chloride**

Sample: 5 g of Croscarmellose Sodium

Analysis: Transfer the *Sample* to a 250-mL beaker. Add 50 mL of water and 5 mL of 30% *hydrogen peroxide*, and heat on a steam bath for 20 min, stirring occasionally to ensure hydration. Cool, and add 100 mL of water and 10 mL of *nitric acid*. Titrate with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-based indicator electrode and a double-junction reference electrode containing 10% potassium nitrate filling solution in the outer jacket and a standard filling solution in the inner jacket, and stirring constantly (see *Titrimetry (541)*).

Calculate the percentage of sodium chloride in the specimen taken:

$$\text{Result} = (F \times V \times N) / [(100 - b) \times W]$$

F = equivalence factor for sodium chloride, 584.4

V = volume of the silver nitrate (mL)

N = normality of the silver nitrate

b = percentage of *Loss on Drying*, determined separately

W = weight of the specimen (g)

Sodium glycolate

Standard stock solution: Transfer 100 mg of *glycolic acid*, previously dried in a desiccator at room temperature overnight, to a 100-mL volumetric flask. Dissolve in and dilute with water to volume, and mix.

[Note—Use this solution within 30 days.]

Standard solution A: Transfer 1.0 mL of the *Standard stock solution* to a 100-mL volumetric flask. Add water to make 5 mL, then add 5 mL of glacial acetic acid. Dilute with acetone to volume, and mix.

Standard solution B: Transfer 2.0 mL of the *Standard stock solution* to a 100-mL volumetric flask. Add water to make 5 mL, then add 5 mL of glacial acetic acid. Dilute with acetone to volume, and mix.

Standard solution C: Transfer 3.0 mL of the *Standard stock solution* to a 100-mL volumetric flask. Add water to make 5 mL, then add 5 mL of glacial acetic acid. Dilute with acetone to volume, and mix.

Standard solution D: Transfer 4.0 mL of the *Standard stock solution* to a 100-mL volumetric flask. Add water to make 5 mL, then add 5 mL of glacial acetic acid. Dilute with acetone to volume, and mix.

Sample solution: Transfer 500 mg to a 100-mL beaker. Moisten thoroughly with 5 mL of *glacial acetic acid*, followed by 5 mL of water, and stir with a glass rod to ensure proper hydration (usually about 15 min). Slowly add 50 mL of acetone while stirring, then add 1 g of *sodium chloride*, and stir for several min to ensure complete precipitation of the carboxymethylcellulose. Filter through a soft, open-textured paper, previously wetted with a small amount of acetone, and collect the filtrate in a 100-mL volumetric flask. Use an additional 30 mL of acetone to facilitate the transfer of the solids and to wash the filter cake, then dilute with acetone to volume, and mix.

Analysis

Samples: *Standard solution A, Standard solution B, Standard solution C, Standard solution D, and Sample solution*

Transfer 2.0 mL of the *Sample solution* and 2.0 mL of each *Standard solution* to separate 25-mL volumetric flasks, and prepare a blank flask containing 2.0 mL of a solution containing 5% each of glacial acetic acid and water in acetone. Place the uncovered flasks in a boiling water bath for 20 min to remove the acetone. Remove from the bath, and cool. Add to each flask 5.0 mL of *2,7-dihydroxynaphthalene TS*, mix, add an additional 15 mL, and again mix. Cover the mouth of each flask with a small piece of aluminum foil. Place the flasks upright in a boiling water bath for 20 min, then remove from the bath, cool, dilute with sulfuric acid to volume, and mix.

Determine the absorbance of each solution at 540 nm, with a suitable spectrophotometer, against the blank, and prepare a standard curve using the absorbances obtained from the *Standard solutions*.

Calculate the percentage of sodium glycolate in the specimen taken:

$$\text{Result} = (F \times W_1) / [(100 - b) \times W_2]$$

F = factor converting glycolic acid to sodium glycolate, 12.9

W_1 = weight of glycolic acid in the specimen, determined from the standard curve and the absorbance of the *Sample solution* (mg)

b = percentage of *Loss on Drying*, determined separately

W_2 = weight of the specimen taken (g)

Acceptance criteria: The sum of the percentages of sodium chloride and sodium glycolate is NMT 0.5%.

▲◆▲NF35

SPECIFIC TESTS

Change to read:

•

▲◆▲NF35

Content of Water-Soluble Material

Analysis: Disperse 10 g in 800 mL of water, and stir for 1 min every 10 min during the first 30 min. Allow to stand for an additional h, or centrifuge, if necessary. Decant 200 mL of the aqueous slurry onto a rapid-filtering filter paper in a vacuum filtration funnel, apply vacuum, and collect about 150 mL of the filtrate. Pour the filtrate into a tared 250-mL beaker, weigh, and calculate the weight, in g, of the filtrate, W_3 , by difference.

Concentrate on a hot plate to a small volume, but not to dryness; dry at 105° for 4 h; and again weigh.

Calculate, in g, the weight of the residue by difference, W_1 .

Calculate the percentage of water-soluble material in the specimen, on the dried basis, taken:

$$\text{Result} = [100 \times W_1 \times (800 + W_2)] / \{W_2 \times W_3 \times [1 - (0.01 \times b)]\}$$

W_1 = weight of residue by difference (g)

W_2 = weight of the specimen taken (g)

W_3 = weight of the filtrate by difference (g)

b = percentage *Loss on Drying* of the specimen taken

Acceptance criteria: NMT 10.0%

▲◆▲NF35

• Degree of Substitution

Sample: 1 g

Analysis: Transfer the *Sample* to a glass-stoppered, 500-mL conical flask. Add 300 mL of sodium chloride solution (1 in 10), then add 25.0 mL of 0.1 N sodium hydroxide VS. Insert the stopper, and allow to stand for 5 min with intermittent shaking. Add 5 drops of *m-cresol purple TS*, and from a buret add 15 mL of 0.1 N hydrochloric acid VS. Insert the stopper in the flask, and shake. If the solution is violet, add 0.1 N hydrochloric acid VS in 1-mL portions until the solution becomes yellow, shaking after each addition. Titrate with 0.1 N sodium hydroxide VS to a violet endpoint.

Calculate the net number of milliequivalents, M , of base required for the neutralization of 1

g of Croscarmellose Sodium, on the dried basis.

Calculate the degree of acid carboxymethyl substitution, A:

$$\text{Result} = 1150 \times M / [7102 - (412 \times M) - (80 \times C)]$$

M = milliequivalents of base

C = percentage of *Residue on Ignition* of the Croscarmellose Sodium as determined in the test for *Residue on Ignition*

Calculate the degree of sodium carboxymethyl substitution, S:

$$\text{Result} = [162 + (58 \times A)] \times C / [7102 - (80 \times C)]$$

A = degree of acid carboxymethyl substitution, as determined above

C = percentage of *Residue on Ignition* of the Croscarmellose Sodium as determined in the test for *Residue on Ignition*

The degree of substitution is the sum of *A* + *S*.

Acceptance criteria: The degree of substitution is 0.60–0.85 on the dried basis

- **Loss on Drying** (731)

Analysis: Dry at 105° for 6 h.

Acceptance criteria: NMT 10.0%

Change to read:

-



Microbial Enumeration Tests (61) and **Tests for Specified Microorganisms** (62): The total aerobic microbial count does not exceed 1000 cfu/g, and the total combined molds and yeasts count does not exceed 100 cfu/g. It meets the requirements of the tests for absence of *Escherichia coli*.



- **pH** (791)

Analysis: Mix 1 g with 100 mL of water for 5 min.

Acceptance criteria: 5.0–7.0

- **Settling Volume**

Analysis: To 75 mL of water in a 100-mL graduated cylinder, add 1.5 g of it in 0.5-g portions, shaking vigorously after each addition. Add water to make 100 mL, shake again until all of the powder is homogeneously distributed, and allow to stand for 4 h. Note the volume of the settled mass.

Acceptance criteria: The volume of the settled mass is 10.0–30.0 mL.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. No storage requirements specified.

Monosodium Glutamate, *NF 33* page 6766. As part of the USP monograph modernization effort and on the basis of comments received, it is proposed to make the following revisions:

1. Add chemical names, chemical structure, chemical formula, and molecular weight.
2. Replace the current *Identification* tests *A* and *B* based on the wet chemistry procedures with a single identification test, *Infrared Absorption* (197A), based on an attenuated total internal reflection IR spectrometric procedure. This test requires the use of USP Monosodium Glutamate RS. Rename the *Identification* tests accordingly.
3. Add a *USP Reference Standards* section and add USP Monosodium Glutamate RS to the section.

Additional work is in progress to develop and validate an HPLC method that will replace the titrimetric *Assay* and will be employed in a test for organic impurities. The HPLC method will be proposed in a future *PF* publication.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

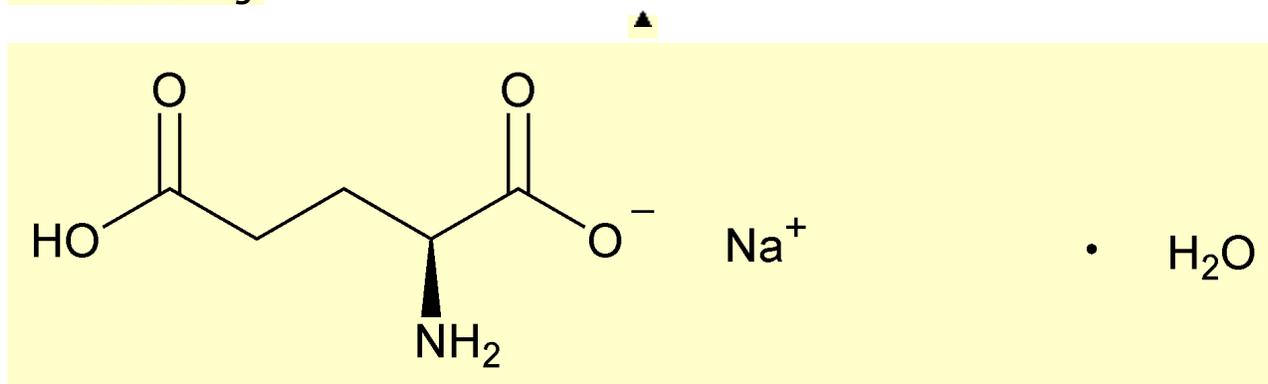
(EXC1: G. Holloway.)

Correspondence Number—C153707

Comment deadline: January 31, 2016

Monosodium Glutamate

Add the following:



$C_5H_8NNaO_4 \cdot H_2O$ 187.13

L-Glutamic acid, sodium salt, hydrate;

Monosodium L-glutamate, hydrate [6106-04-3]. \blacktriangle *NF35*

DEFINITION

Monosodium Glutamate contains NLT 99.0% and NMT 100.5% of monosodium glutamate ($C_5H_8NNaO_4 \cdot H_2O$).

IDENTIFICATION

Delete the following:

\blacktriangle • A^-

Sample solution: 1 in 30

Analysis: To 1 mL of the *Sample solution* add 1 mL of ninhydrin TS and 100 mg of sodium acetate, and heat in a boiling water bath for 10 min.

Acceptance criteria: An intense, violet blue color is formed. ▲NF35

Delete the following:

▲ ● **B.**

Sample solution: 1 in 10

Analysis: To 10 mL of the *Sample solution* add 5.6 mL of 1 N hydrochloric acid.

Acceptance criteria: A white, crystalline precipitate of glutamic acid is formed on standing. Precipitation is promoted by agitation. When 6 mL of 1 N hydrochloric acid is added to the turbid solution, the glutamic acid dissolves on stirring. ▲NF35

Add the following:

▲ ● **A. Infrared Absorption** (197A) ▲NF35

Change to read:

● **C.**

▲ **B.** ▲NF35

Identification Tests—General (191), *Sodium*: It meets the requirements of the pyroantimonate precipitate test.

ASSAY

● **Procedure**

Sample: 250 mg

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Blank: 100 mL of *glacial acetic acid* with a few drops of water

Endpoint detection: Potentiometric

Analysis: Wet the *Sample* with a few drops of water. Dissolve in 100 mL of *glacial acetic acid*. Titrate with 0.1 N perchloric acid VS. Perform a blank determination. Calculate the percentage of monosodium glutamate ($C_5H_8NNaO_4 \cdot H_2O$) in the *Sample* taken:

$$\text{Result} = [(V_S - V_B) \times N_A \times F \times 100] / W$$

V_S = Titrant volume consumed by the *Sample* (mL)

V_B = Titrant volume consumed by the *Blank* (mL)

N_A = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 93.56 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 99.0%–100.5%

IMPURITIES

- **Chloride and Sulfate** (221), *Chloride*: A 280-mg portion shows no more chloride than corresponds to 1.0 mL of 0.020 N *hydrochloric acid* (0.25%).
- **Lead** (251): NMT 10 µg/g

Delete the following:

- ~~**Heavy Metals, Method II** (231): NMT 20 µg/g~~ (Official 1-Jan-2018)

SPECIFIC TESTS

- **Clarity and Color of Solution**

Sample solution: 1.0 g in 10 mL of water

Standard solution: To 0.2 mL of a solution of *sodium chloride* containing 10 µg/mL of chloride ion (Cl), add 20 mL of water and mix. Then add 1 mL of 5 N *nitric acid*, 0.2 mL of *dextrin* solution (1 in 50), and 1 mL of *silver nitrate TS*, and allow to stand for 15 min.

Analysis: Compare the *Sample solution* with the *Standard solution* (see *Nephelometry, Turbidimetry, and Visual Comparison* (855)).

Acceptance criteria: The *Sample solution* is colorless and has no more turbidity than the *Standard solution*.

- **Optical Rotation** (781S), *Procedure, Specific Rotation*

Sample solution: 100 mg/mL in 2 N *hydrochloric acid*

Acceptance criteria: +24.8° to +25.3°, determined at 20°

- **pH** (791): 6.7–7.2, in a solution (1 in 20)

- **Loss on Drying** (731)

Analysis: Dry at 100° for 5 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

Add the following:

- ▲ ● **USP Reference Standards** (11)

USP Monosodium Glutamate RS

▲NF35

BRIEFING

Potassium Hydroxide, *NF 33* page 6835. As part of the USP monograph modernization effort and on the basis of comments and data received, it is proposed to make the following revisions:

1. In the *Definition*, add a requirement for the content of potassium.
2. Add *Identification test B* based on the pH measurement of 0.1 mg/mL of Potassium Hydroxide solution.
3. In the *Assay*, add an atomic absorption procedure for the *Content of Potassium* test.
4. In the *Impurities* section, add a test for the *Limit of Sodium* with an acceptance

criteria of NMT 1.0%.

Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

(EXC1: G. Holloway.)

Correspondence Number—C157337

Comment deadline: January 31, 2016

Potassium Hydroxide

KOH 56.11

Potassium hydroxide [1310-58-3].

DEFINITION

Change to read:

Potassium Hydroxide contains NLT 85.0% of total alkali, calculated as potassium hydroxide (KOH), including NMT 3.5% of potassium carbonate (K_2CO_3).

▲ It also contains NLT 85.0% of potassium. ▲NF35

[**Caution**—Exercise great care in handling Potassium Hydroxide because it rapidly destroys tissues.]

IDENTIFICATION

- **A. Identification Tests—General** (191), *Potassium*: A solution (1 in 25) meets the requirements.

Add the following:

- ▲ ● **B. pH** (791)

Sample solution: 0.1 mg/mL of Potassium Hydroxide

Acceptance criteria: NLT 10.5 ▲NF35

ASSAY

Change to read:

- **Procedure**

▲ **Total Alkali** ▲NF35

Sample: 1.5 g of Potassium Hydroxide

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 1 N sulfuric acid VS

Endpoint detection: Colorimetric

Analysis: Dissolve the *Sample* in 40 mL of carbon dioxide-free water. Cool the solution to 15° and add *phenolphthalein TS*. Titrate with 1 N sulfuric acid VS. At the discharge of the

pink color of the indicator, record the volume of acid solution required, then add *methyl orange TS* and continue the titration to a persistent pink color. Each mL of 1 N sulfuric acid is equivalent to 56.11 mg of total alkali, calculated as potassium hydroxide (KOH), and each mL of acid consumed in the titration with methyl orange is equivalent to 138.2 mg of potassium carbonate (K_2CO_3).

Acceptance criteria: NLT 85.0% of total alkali, calculated as potassium hydroxide (KOH), including NMT 3.5% of potassium carbonate (K_2CO_3)

Add the following:

▲ ● Content of Potassium

Diluent: 1% *Hydrochloric acid* solution

Sodium chloride solution: 0.2 g/mL of *sodium chloride*

Blank solution: Transfer 2.0 mL of the *Sodium chloride solution* to a 100-mL volumetric flask and dilute with *Diluent* to volume.

Standard stock solution: 57.21 $\mu\text{g/mL}$ of *potassium chloride*, previously dried at 105° for 2 h, in water. This solution contains 30 $\mu\text{g/mL}$ of potassium.

Standard solutions: Transfer 2.0-, 4.0-, and 6.0-mL portions of the *Standard stock solution* to separate 100-mL volumetric flasks. To each flask, add 2.0 mL of the *Sodium chloride solution*. Dilute the content of each flask with *Diluent* to volume and mix to obtain solutions with known concentrations of 0.6, 1.2, and 1.8 $\mu\text{g/mL}$ of potassium.

Sample stock solution: 0.5 mg/mL of Potassium Hydroxide

Sample solution: Transfer 1.0 mL of the *Sample stock solution* to a 250-mL volumetric flask. Add 5.0 mL of the *Sodium chloride solution* and dilute with *Diluent* to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 766.5 nm (potassium emission line)

Lamp: Potassium hollow-cathode

Flame: Air-acetylene

Blank: *Blank solution*

Standard curve

Samples: *Standard solutions*

Plot: Absorbance values versus their corresponding concentrations ($\mu\text{g/mL}$) of potassium

Analysis

Sample: *Sample solution*

From the *Standard curve*, determine the concentration of potassium in the *Sample solution*.

Calculate the percentage of potassium in the portion of Potassium Hydroxide taken:

$$\text{Result} = (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

C_S = concentration of potassium in the *Sample solution* from the *Standard curve* ($\mu\text{g/mL}$)

C_U = concentration of Potassium Hydroxide in the *Sample solution* (g/mL)

M_{r1} = molecular weight of potassium hydroxide, 56.11

M_{r2} = molecular weight of potassium, 39.09

Acceptance criteria: NLT 85.0% \blacktriangle NF35

IMPURITIES

Delete the following:

* • Heavy Metals $\bar{(231)}$

Test preparation: ~~0.67 g of Potassium Hydroxide in a mixture of 5 mL of water and 7 mL of 3 N hydrochloric acid. Heat to boiling, cool, and dilute with water to 25 mL.~~

Acceptance criteria: ~~NMT 30 μ g/g~~ * (Official 1-Jan-2018)

Add the following:

\blacktriangle • Limit of Sodium

Diluent: 1% Hydrochloric acid solution

Standard stock solution: 12.71 μ g/mL of sodium chloride, previously dried at 105° for 2 h, in water. This solution contains 5 μ g/mL of sodium.

Standard solutions: Transfer 1.0-, 10.0-, and 20.0-mL portions of the *Standard stock solution* to separate 100-mL volumetric flasks. Dilute the content of each flask with *Diluent* to volume and mix to obtain solutions with known concentrations of 0.05, 0.5, and 1.00 μ g/mL of sodium.

Sample stock solution: 0.5 mg/mL of Potassium Hydroxide

Sample solution: 50 μ g/mL of Potassium Hydroxide in *Diluent*, prepared from the *Sample stock solution*

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 589.0 nm

Lamp: Sodium hollow-cathode

Flame: Air-acetylene

Blank: *Diluent*

Standard curve

Samples: *Standard solutions*

Plot: Absorbance values versus their corresponding concentrations (μ g/mL) of sodium

Analysis

Sample: *Sample solution*

From the *Standard curve*, determine the concentration of sodium in the *Sample solution*. Calculate the percentage of sodium in the portion of Potassium Hydroxide taken:

$$\text{Result} = (C_S/C_U) \times 100$$

C_S = concentration of sodium in the *Sample solution* from the *Standard curve* (μ g/mL)

C_U = concentration of Potassium Hydroxide in the *Sample solution* (g/mL)

Acceptance criteria: NMT 1.0%▲NF35

SPECIFIC TESTS

- **Insoluble Substances**

Sample solution: 1 g of Potassium Hydroxide in 20 mL of water

Acceptance criteria: The *Sample solution* is complete, clear, and colorless.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

Stage 4 Harmonization

This section contains monographs or chapters undergoing harmonization by the Pharmacopoeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (**Stages 1** through **7**, as defined below). Stage 4 drafts are available for comments. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF*, this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

STIMULI TO THE REVISION PROCESS

Stimuli articles do not necessarily reflect the policies of the USPC or the USP Council of Experts

STIMULI TO THE REVISION PROCESS

This section may contain the following:

- reports or statements of Expert Committees
- original research reports
- evaluations of new and existing pharmacopeial methods
- commentaries
- articles relevant to compendial issues.

These items are published to stimulate discussion and continual review of Pharmacopeial standards. Generally, if an Expert Committee publishes an article on which they are specifically seeking comment, this will be clearly stated in the article. Readers may submit comments on issues raised in this section, but comment is not as critical as that for the *In-Process Revision* section. Readers interested in submitting comments should see *Instructions to Authors*.

Instructions to Authors

Contributions in the form of original research reports, evaluations of new and existing compendial methods, and other commentaries and articles relevant to drug standards or to *USP–NF* revision will be considered for publication in *Pharmacopeial Forum* under the section *Stimuli to the Revision Process*. Manuscripts are received with the explicit understanding that they have not been published previously in any language or medium and that they are not simultaneously under consideration by any other publication. All manuscripts are subject to review by USP headquarters staff, Committee members, or qualified outside referees, and if accepted for publication they will be subject to editing by USP staff. Accepted manuscripts become the property of the USP Convention (USPC) and may not be subsequently published elsewhere without written permission from the USPC. Authors are also responsible for obtaining permission for reprinting any illustrations that have been published elsewhere.

Abstract—Include an abstract of not more than 250 words stating the purpose and the results or conclusions of the article.

Style and Usage—*Stimuli* articles generally follow the current *Chicago Manual of Style* except in scientific usage (numbers, abbreviations, etc.). For the latter, authors should use the current *AMA Manual of Style* or the current *ACS Style Guide*. Authors may usefully consult a current edition of *Pharmacopeial Forum*.

References—Consult the current *AMA Manual of Style*, which is generally consistent with the National Library of Medicine's *Recommended Formats for Bibliographic Citation*. A current edition of *Pharmacopeial Forum* will offer examples of reference formats.

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ICMJE Conflicts of Interest:

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Executive Secretariat, USP

12601 Twinbrook Pkwy.

Rockville, MD 20852

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GUIDELINE FOR ASSIGNING TITLES TO USP DIETARY SUPPLEMENT MONOGRAPHS

USP Dietary Supplement and Herbal Medicines Nomenclature Joint Subcommittee, USP Staff^{a,b}

ABSTRACT The first publication of the *Pharmacopoeia of the United States* in 1820 included quality monographs of articles that would today be considered dietary supplements, for example, vitamins, minerals, and certain botanicals. Titles for such monographs were crafted to be brief and distinct, and in many instances a single word sufficed if that word was expressive and unambiguous. The 1820 volume discussed a guide for developing botanical monograph titles and indicated that USP would adapt a nomenclature that was simple, with the intent that the monograph title would be brief and explicit, expressing the medical meaning and nothing else. This *Stimuli* article presents a new guideline for formulating titles of dietary supplement monographs. The intent of this article is to initiate a discussion on this new proposed guideline, to solicit public comments, and to invite the participation of interested parties in USP's efforts to develop a nomenclature guideline for dietary supplement monographs. The goal of this effort is to eventually bring existing monograph titles into alignment with a uniform naming convention.

INTRODUCTION

The first publication of the *United States Pharmacopoeia (USP)* in 1820 included quality monographs of articles that today are referred to as dietary supplements (DSs), including vitamins, minerals, and certain botanicals. Monographs for some of these articles have been included in the compendium since the 1820 edition. In 1993, in response to the Nutrition Labeling and Education Act (NLEA) of 1990, a separate compendium section titled *Nutritional Supplements* was created to contain monographs for vitamins and mineral combinations. DS monographs were started in 1995 in response to the Dietary Supplement Health and Education Act of 1994 (DSHEA) and included some monographs for botanicals that were originally placed in the *National Formulary (NF)*. The *Nutritional Supplements* section was active through the publication of *USP 26-NF 21* in 2003. In 2004, a new section, *Dietary Supplements*, was introduced into *USP 27-NF 22* to replace the *Nutritional Supplements* section and included monographs for ingredients and dosage forms of DSs as defined by DSHEA. Monographs for botanical DSs originally in the *NF* were also moved to this new DS section. This new DS section is currently published in the *USP-NF*, which combines all of the dietary supplement monographs from the two compendia.

It should be noted, however, that other botanical articles in the *USP-NF* are not necessarily DSs because they are classified as drugs, excipients, or medical devices. For example, *Aloe*, *Elm*, *Ipecac*, *Psyllium*, and *Senna* remain in the *USP* section of the *USP-NF*. There is a *USP* monograph for *Gutta Percha*, which is used as a medical device material, e.g., for endodontic (root canal) treatment. *Belladonna*, *Digitalis*, *Opium*, *Podophyllum*, and *Rauwolfia serpentina* are monograph examples currently included in the *USP* as prescription drugs or sources of prescription drugs. Other articles, such as flavors, fragrances, and other excipients, were placed in the *NF*.

Crafting monograph titles for vitamins and minerals has always been more straightforward than

it is for botanicals, as the former are mostly comprised of single ingredients with titles largely formulated in a manner similar to those for drugs. A guide for developing botanical monograph titles was discussed in the first *USP* (published as the *Pharmacopeia of the United States*) in 1820 to adopt a nomenclature to "...be conformable to the present language of science, divested of as much of its prolixity as can be done consistently with clearness and distinctness." The intent was for the monograph title to "...expresses the medicine, and nothing else; ...needed to be short and explicit, and does not require to be mutilated in practical use, as long names will inevitably be" (1). Thus, a monograph title was to be brief and distinct; a single word sufficed if that word was expressive and unambiguous. Plant parts were not included in monograph titles except where multiple monographs were developed for different plant parts of the same species, in which case the plant part was included to distinguish the monographs from each other by title. The USP staff followed this format when formulating monograph titles until the enactment of DSHEA in 1994.

DSHEA defines a DS as: "(1) a product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients: (A) a vitamin; (B) a mineral; (C) a herb or other botanical; (D) an amino acid; (E) a dietary substance for use by man to supplement the diet by increasing the total dietary intake; or (F) a concentrate, metabolite, constituent, extract, or combination of any ingredient described in clause (A), (B), (C), (D), or (E)."¹ DSHEA also mandates that the label of a DS must bear a statement of identity that, as stated in the Code of Federal Regulations Title 21 (21 CFR) section 101.4 (h), includes the common or usual names of botanical dietary ingredients (including fungi and algae) and these names must be consistent with the names standardized in *Herbs of Commerce* (2).

In accordance with DSHEA, USP adopted common names of botanicals utilized in North American commerce as monograph titles (3). In some cases, two or more species of plants may have the same standardized common name (SCN) in *Herbs of Commerce* but they can be distinguished from each other in the monograph title—if necessary to meet the requirements of the monograph—by including another common name (OCN) provided in *Herbs of Commerce* (2). For example, "Labrador tea" is the SCN for both *Ledum groenlandicum* [currently accepted name: *Ledum palustre* subsp. *groenlandicum* (Oeder) Hultén] and *Ledum palustre* subsp. *decumbens*, but to have a separate DS monograph for each species, the OCNs "Bog Labrador Tea" and "Marsh Labrador Tea," respectively, could be included in the monograph titles. The Latin binomial or a recognized common name used in commerce is required in the DS monograph title for plants that are not listed in the *Herbs of Commerce* and thus not assigned an SCN in *Herbs of Commerce*, e.g., *Pelargonium sidoides*, or Banaba for *Lagerstroemia speciosa*; or if the SCN applies to more than one species, no OCNs are provided to distinguish those species, and a distinction is necessary for the purposes of the monograph. There are some cases where the SCN provided in *Herbs of Commerce* is not necessarily the most common name in commerce (2), e.g., *Euterpe oleracea* has the SCN "Cabbage Palm" but is now better known as "Açaí Palm". *Siraitia grosvenorii* has the SCN "Luo Han Guo", which is the best known common name for it when used in Traditional Chinese Medicine, but when used in foods and DSs as a natural low-calorie sweetener it may be better known as "Monk Fruit". In these cases, using the Latin binomial in the DS monograph title helps avoid confusion.

Any name in Latin form shall be in accordance with internationally accepted rules on nomenclature, such as those found in the *International Code of Nomenclature for Algae, Fungi, and Plants (Melbourne Code)* (the 2011 edition replaced the document previously known as the *International Code of Botanical Nomenclature*) (4). Normally, Latin binomials and subspecies or variety names are italicized (4).

For brevity, it is not necessary to include the author citation of a Latin binomial in the DS

monograph title; it will be provided in the *Definition* section of the monograph. The citation of the author or authors who validly published the Latin binomial is a key part of the scientific name of an organism. The authors' names follow directly after the Latin binomial, e.g., *Andrographis paniculata* (Burm.f.) Nees. The reason the Latin binomial needs to be followed by the author citation is that the author citation helps in locating the original published plant description, which helps determine the "type species" (from which the original description was created), and the date (priority) of publication of the name; these are key criteria used to determine which name for a particular species is correct. The author citation also identifies the source of the name to prevent confusion over duplicate names and helps trace changes in names. For example, the author citation for the plant name *Andrographis paniculata* (Burm.f.) Nees indicates that Christian Gottfried Daniel Nees von Esenbeck (internationally standardized abbreviation, "Nees") transferred this species to the genus *Andrographis* after re-examining and reclassifying the same type specimen from which was derived the original name *Justicia paniculata* Burm.f. published by Nicolaas Laurens Burman ("f." stands for *filii* because he was the son of another botanist, Johannes Burman, whose abbreviation is "Burm").

A detailed explanation of how authors are cited and the meaning of terms such as "ex" or "in" found within the author citation is provided in Chapter VI of the *International Code of Nomenclature for Algae, Fungi, and Plants* (4). In the context of the details to be provided in the DS monograph *Definition* section, the author citation becomes critical when a positive identification of the article cannot be made in its absence (e.g., see discussion of *Illicium anisatum* below). This would also be an issue on a product label, on a raw material order form, or in a master formula. Thus, the level of detail in the DS monograph *Description* section will be consistent with the labeling regulations that require inclusion of the designation of the author or authors who published the Latin name.

In the DS monograph title, the name of the plant part follows the name (common or Latin binomial) of the article. For example, *Echinacea purpurea Aerial Parts* is a separate monograph from "*Echinacea purpurea* Root". This is consistent with 21 CFR101.4(h)(1), which requires the DS label to list the part of the plant (e.g., root, leaf) from which the dietary ingredient is derived [e.g., "Garlic bulb" or "Garlic (bulb)"]. The name of the part of the plant shall be expressed in English (e.g., "flower" rather than the Latin term "flos" used in some pharmacopeias).

All titles of dietary ingredient monographs and DS monographs are approved by the appropriate Expert Committee (EC), based on USP staff research and the best scientific judgment of the EC. There have been many considerations in naming dietary ingredients and DSs including, but not limited to: USP's historical and scientific practices, industry convention, international similarities and differences, regulatory status, and environmental and agricultural practices. The guideline below was developed with input from the Nomenclature, Safety, and Labeling (NSL) EC and the Monographs—Dietary Supplements and Herbal Medicines (DSHM) EC as a complement to *Nomenclature* (1121). The guideline is intended to provide a systematic approach to developing monograph titles for dietary ingredients and DS dosage forms admitted to the compendium. To minimize the potential for confusion and controversy, the naming of monographs in this guideline is guided by available scientific conventions, the practices of the DS industry, and the labeling requirements of applicable federal regulations.

Some DS monographs define and characterize plant, fungal, algal, animal, and certain bacterial materials. Botanical materials are often processed to some extent, such as by drying and milling (cutting, sifting, particle sizing, and density adjustment). Other DS monographs describe extracts, processed extracts, partially purified natural complexes, or purified substances of botanical materials. There are also monographs for single chemical entities and for finished oral

dosage forms. This guideline indicates how DS monograph titles shall be developed for the different types of articles included below, with examples. It is preferable that existing monograph titles that do not conform to the new approach be revised on an as-needed, case-by-case basis.

DIETARY INGREDIENT MONOGRAPH TITLES

A "dietary ingredient", as defined by DSHEA, is a substance intended for use in the manufacture of DS finished dosage forms. Some of these articles may in fact be raw materials (as described in *Identification of Articles of Botanical Origin* (563)) that are subject to further processing for the manufacture of dietary ingredients, or they may be dietary ingredients when used directly in the manufacture of DSs. Dietary ingredients can be broadly categorized into the following groups: complex articles of botanical origin, complex articles of animal origin, other complex dietary substance articles, and single chemical entities (including vitamins, minerals, amino acids, and other examples that will be provided below).

Titles for Monographs of Complex Articles of Botanical Origin

These articles include plant (or fungal, algal, or certain bacterial) materials such as the whole plant or a specific plant part (e.g., leaf, root, stem, fruiting body of a fungus, thallus of an alga, and others) and plant products, which are substances produced naturally by a plant or plant part that do not require processing beyond pressing or cutting and scraping to be obtained, such as a seed oil, gum, latex, or resin.

Each monograph shall have a title that is consistent with its *Definition* and *Identification* sections. The monograph title shall include the SCN (or OCN in the cases explained above) from *Herbs of Commerce* or the Latin binomial where necessary (in the cases explained above), followed by the name of the botanical part(s) or botanical product, except in the case of single-celled or colonial organisms such as yeasts (e.g., *Saccharomyces cerevisiae*, *Monascus purpureus*), certain algae (e.g., *Chlorella*), and cyanobacteria (also known as blue-green algae, e.g., *Spirulina*, *Nostoc*), which have no parts. The part name is followed, where applicable, by the processed form. The botanical part name, botanical product name, and processed form name shall be written in English and in singular form.

In cases where more than one species of a genus are represented in a monograph, the genus name shall be used followed by the word Species² unless there is one SCN for all the included species and there is no need to distinguish among them for the purposes of the monograph. For example, *Herbs of Commerce* has a separate SCN for each of 11 different species of willow, but in commerce the barks of various species of *Salix* are used alone or mixed to make "willow bark" or "willow bark extract" supplements (2). Due to substantial anatomical and chemical similarities and hybridization between species, distinguishing them by microscopic, chemical, or genetic tests is neither readily feasible nor necessary. Because the use of any one willow SCN in the DS monograph title will not accurately reflect the composition of the article of commerce, a more appropriate title would be "*Salix* Species Bark".

Additional information about the DS article that is the subject of the monograph, e.g., the Latin binomial(s), with their corresponding author(s) and the family, common name(s), identity and strength of solvent(s), range of ratios of crude plant material to extract, and range of concentration of marker compound(s), shall be included under its *Definition*.

Inclusion of the variety or subspecies in the title of a DS monograph depends on whether or not it is relevant to the accurate definition of the article of commerce. Phytochemical differences, safety differences, and traditional use differences at the variety or subspecies level should be

evaluated to determine whether the variety or subspecies should be included in the monograph title. For example, if the fruit with the SCN "Jujube" did not have an SCN, then to accurately reflect the identity of the article used in Traditional Chinese Medicine it would be necessary for the DS monograph title to include the variety name: "*Ziziphus jujuba* var. *spinosa* Seed". When a variety or subspecies is not relevant to the article's definition and characterization, it should not be used in the title of the monograph.

Occasionally, in the absence of an SCN or OCN from *Herbs of Commerce*, the Latin binomial most widely known in commerce will be used in the monograph title even if it does not represent current accepted taxonomic nomenclature. For example, if a DS monograph were to be developed for the fungus *Antrodia camphorata* (which is an article in the *Herbal Medicines Compendium*), the DS monograph title would be *Antrodia camphorata* Fruiting Body, even though the Index Fungorum—Species Fungorum database indicates that the current name should be *Taiwanofungus camphoratus*. The monograph *Description* would give the authors of the Latin binomial, i.e., *Antrodia camphorata* (M. Zang & C.H. Su) Sheng H. Wu, Ryvardeen & T.T. Chang, to clarify that the currently accepted name is *Taiwanofungus camphoratus* (M. Zang & C.H. Su) Sheng H. Wu, Z.H. Yu, Y.C. Dai & C.H. Su, and indicate that another synonym is *Ganoderma camphoratum* M. Zang & C.H. Su. By providing these details in the *Definition* of the monograph, the connection is maintained between the article of commerce and its various Latin binomials which are subject to revision by taxonomists.

Since a synonym may be used in a DS monograph title, it is important to be pragmatic in selecting which other taxonomic synonyms to be included in the monograph text for clarification of the article's identity, since there may be more than one commonly used synonym for an article of commerce. Taxonomic web sites such as The Plant List (developed through a collaboration between the Royal Botanic Gardens, Kew; the Missouri Botanical Garden; and other authoritative institutions) (5) and the U.S. Department of Agriculture, Agricultural Research Service Germplasm Resources Information Network (GRIN) online database (6) can be checked for a reasonably comprehensive list of synonyms, so it will not be necessary to duplicate all of that information in a DS monograph.

The following criteria may be helpful in deciding how to select synonyms for inclusion in the DS monograph *Definition*:

1. If the Latin binomial selected for use in the DS monograph title or associated with the DS monograph title's SCN is a synonym according to the current nomenclature set out in The Plant List (5) or the USDA GRIN database (6), then clarification of the synonymy should be included in the DS monograph *Definition*. For example, *Polygonum multiflorum* Thunb. is the Latin binomial associated with the SCN "Fo-Ti" but it is a synonym for *Reynoutria multiflora* (Thunb.) Moldenke, so both this synonym and the current correct Latin binomial should be included in the monograph for Fo-Ti. As another example, *Garcinia cambogia* (SCN: *Garcinia*) is a synonym used currently as a DS monograph title; both *Garcinia cambogia* (Gaertn.) Desr. and the accepted Latin binomial name *Garcinia gummi-gutta* (L.) Roxb. (except with the author N. Robson, which has since been revised to Roxb.) are included in the *Definition*. To determine which synonyms are well-established in commerce, references to consult include key compendia or pharmacopeias from authoritative sources (e.g., labeling standards or monographs published by the *European Medicines Agency*, or compendia such as the *Food Chemicals Codex*, *European Pharmacopoeia*, *Pharmacopoeia of the People's Republic of China*, or the *Herbal Medicines Compendium*).
2. Where key compendia or pharmacopeias from authoritative sources provide multiple

synonyms, the presence of a synonym in two or more compendia/pharmacopoeias may be evidence that it is well-known enough to cite in the DS monograph *Definition* [e.g., *Momordica grosvenorii* Swingle and *Thladiantha grosvenorii* (Swingle) C. Jeffrey are commonly cited synonyms for *Siraitia grosvenorii* (Swingle) C. Jeffrey ex A.M. Lu & Zhi Y. Zhang]. Setting a criterion that synonyms must be found in two or more references may help avoid unnecessary listings, if the sources give multiple synonyms that are not necessarily used in commerce. This criterion can be revisited if too long a list is obtained in too many cases.

3. If a synonym is not listed in one of these official compendia but other peer-reviewed literature suggests there is a risk of confusion, such as happened in the case of the potentially hazardous confusion between the edible Chinese star anise, *Illicium verum* Hook. f., synonym *Illicium anisatum* Lour., and the toxic Japanese star anise, *Illicium anisatum* L. (only the authors of these last two Latin binomials differ), then this synonymy should be included in the monograph text as it is relevant to safety.

Below are examples indicating how monograph titles shall be developed for the different types of complex dietary ingredients of botanical origin including botanical materials, botanical products, and botanical processed forms.

titles for botanical material monographs

DS plant articles (or fungal, algal, or bacterial material articles) include the whole plant or a specific part of the plant, with the exception noted above for single-celled or colonial organisms. The term "botanical material" is used here to indicate material derived directly from a plant and does not include articles that may be obtained from these materials when processed, such as extracts, juices, oils, and others. The examples provided in *Table 1* illustrate how titles for botanical material monographs will be derived when following the new guideline, compared to how current monograph titles were derived.

Table 1. Current and Proposed Nomenclature Formats for Botanical Material Monograph Titles

Current Examples	Proposed Examples ^a
[{SCN} OR {LATIN BINOMIAL W/O AUTHORITY}] ^b {BOTANICAL PART(S)}	[{SCN} OR {LATIN BINOMIAL W/O AUTHORITY}] [BOTANICAL PART(S)]
<i>Andrographis</i>	Andrographis Stem and Leaf
<i>Asian Ginseng</i>	Asian Ginseng Root
<i>Capsicum</i>	<i>Capsicum</i> Species Fruit
<i>Centella asiatica</i>	Gotu Kola Aerial Parts
<i>Chamomile</i>	Chamomile Flower Head
NA ^c	Chlorella
<i>Ganoderma lucidum</i> Fruiting Body	Reishi Fruiting Body
NA	Kelp Thallus ^d
<i>Rhodiola rosea</i>	<i>Rhodiola rosea</i> Root and Rhizome
<i>Senna</i> Leaf	<i>Senna</i> Leaf
<i>Senna</i> Pods	<i>Senna</i> Pod
<i>Spirulina</i>	<i>Spirulina</i> Species
<i>Valerian</i>	<i>Valerian</i> Rhizome, Root and Stolon

^a Some examples provided are hypothetical, solely to show what the new titles would look like.

^b Items within brackets [] are required, whereas those within braces { } are to be used as appropriate, e.g., one should use {SCN} where an unambiguous SCN is provided in *Herbs of Commerce* but use {Latin binomial} in other cases as explained above.

^c NA, title not available because currently no USP monograph exists for this article.

^d As an example of an unusual case, *Kelp* is the SCN for various species of brown algae: *Alaria marginata*, *Ascophyllum nodosum*, *Laminaria digitata*, *L. hyperborea* (synonym *L. cloustonii*), *L. setchellii*, *L. sinclairii*, and *Macrocystis pyrifera*. Not all of these species have assigned OCNs. Another species, *L. saccharina*, has "Sugar Kelp" as the SCN. *Kelp* is defined in 21CFR172.365 as the dehydrated, ground product prepared from *Macrocystis pyrifera*, *Laminaria digitata*, *Laminaria saccharina*, and *Laminaria cloustoni* [sic] for special dietary and nutritional additives as a source of the essential mineral iodine. Therefore, using *Kelp* as the SCN in a DS monograph title might be interpreted to capture only three of the four species set out in 21CFR172.365, and could include other genera and species not permitted as *Kelp* under the conditions set out in this regulation. To resolve this rare exception to the general approach, a DS monograph entitled "Kelp Thallus" could specify the four species from 21CFR172.365 in the *Definition*, while a monograph for *Ascophyllum nodosum* could have the title "Kelp (*Ascophyllum nodosum*) Thallus" to include the SCN and be distinguishable from the other monograph.

titles for botanical product monographs

Articles referred to as plant (or fungal, algal, or bacterial) products include substances produced naturally by a plant or plant part that do not require processing beyond pressing or cutting and scraping to be obtained, such as seed oil, gum, latex, resin, and others. The examples provided below illustrate how titles for plant product monographs will be derived following the guideline, compared to how current monograph titles were derived.

Table 2. Current and Proposed Nomenclature Formats for Botanical Product Monograph Titles

Current Examples	Proposed Examples
[{SCN} OR {LATIN BINOMIAL W/O AUTHORITY}] {BOTANICAL PART(S)} {BOTANICAL PRODUCT}	[{SCN} OR {LATIN BINOMIAL W/O AUTHORITY}] [{BOTANICAL PART(S)} AND/OR {BOTANICAL PRODUCT}]
<i>Almond Oil</i>	Almond Seed Oil
<i>Castor Oil</i>	Castor Seed Oil
<i>Aromatic Castor Oil</i>	Castor Seed Aromatic Oil ^a
<i>Crypthecodinium cohnii Oil</i>	<i>Crypthecodinium cohnii</i> Oil
<i>Boswellia serrata</i>	<i>Boswellia serrata</i> Oleo-gum-resin
<i>Evening Primrose Oil</i>	Evening Primrose Seed Oil
<i>Flax Seed Oil</i>	Flax Seed Oil
<i>Guggul</i>	Guggul Oleo-gum-resin
<i>Myrrh</i>	Myrrh Oleo-gum-resin
Palm Oil	Palm Fruit Oil
Palm Oil	Palm Kernel Oil
<i>Schizochytrium Oil</i>	Schizochytrium Species Oil
^a An aromatic botanical product article is created by the addition of essential oils as flavoring agents, so the DS monograph title follows the proposed format with the addition of the adjective "Aromatic".	

titles for botanical processed form monographs

Articles referred to as plant (or fungal, algal, or bacterial) processed forms include plant powders, dry extracts, dry juices, liquid articles, and fractions, but do not include isolated pure compounds. The examples provided below illustrate how titles for plant processed form monographs will be derived when following the new guideline, compared to how current monograph titles were derived.

Titles for botanical powder monographs: The term "powder" often indicates that the botanical material has been milled (comminuted) into a powder, but some materials such as spores and pollen occur naturally as powders. Botanical powders (see *Table 3* for examples) include powdered botanical materials but are not meant to include other botanically derived ingredients that may be powdered or present in powder form, such as dry extracts and dry juices.

Table 3. Current and Proposed Nomenclature Formats for Botanical Powders

Current Examples	Proposed Examples
{PROCESS } [{SCN} OR {LATIN BINOMIAL W/O AUTHORITY}] {BOTANICAL PART(S)}	[{SCN} OR {LATIN BINOMIAL W/O AUTHORITY}] [BOTANICAL PART(S)] [{POWDER} OR {SPORE} OR {POLLEN}]
<i>Powdered Andrographis</i>	Andrographis Stem and Leaf Powder
<i>Powdered Ashwagandha Root</i>	Ashwagandha Root Powder
Powdered Asian Ginseng	Asian Ginseng Root Powder
<i>Powdered Black Cohosh</i>	Black Cohosh Rhizome and Root Powder
NA	Clubmoss Spore
<i>Powdered Centella asiatica</i>	Gotu Kola Aerial Parts Powder
<i>Powdered Garlic</i>	Garlic Bulb Powder
Powdered Hawthorn Leaf with Flower	Hawthorn Leaf with Flower Powder
<i>Powdered Horse Chestnut</i>	Horse Chestnut Seed Powder
NA	Pine Pollen

Titles for botanical extract monographs: Extracts obtained from botanical materials are classified and named based on their physical state or consistency, such as liquid (liquid extracts), semisolid (soft extracts), or dry (extracts in solid form, e.g., powders, granules, or flakes) (see *Botanical Extracts* (565)). The examples provided in *Tables 4* and *5* illustrate how titles for botanical extract monographs will be derived when following the new guideline compared to how current monograph titles were derived. For the sake of clarity, in the following tables the format terms: **[{SCN} OR {LATIN BINOMIAL W/O AUTHORITY}]** **[{BOTANICAL PART(S)} AND/OR {BOTANICAL PRODUCT}]** used above to describe botanical materials, products, and powders will be simplified to **[SOURCE MATERIAL NAME]**, which incorporates all of the above terms.

Table 4. Current and Proposed Nomenclature Formats for Botanical Dry Extracts

Current Examples	Proposed Examples
[{PROCESS} {TYPE}] [SOURCE MATERIAL ^a NAME] [{EXTRACT}]	[SOURCE MATERIAL NAME {FRESH} ^b] [{TYPE} ^c DRY EXTRACT]
<i>Powdered Andrographis Extract</i>	Andrographis Stem and Leaf Dry Extract
<i>Powdered Asian Ginseng Extract</i>	Asian Ginseng Root Dry Extract
<i>Powdered Centella asiatica Extract</i>	<i>Centella asiatica</i> Aerial Parts Dry Extract
<i>Powdered Goldenseal Extract</i>	Goldenseal Root and Rhizome Dry Extract
NA	Oat Fresh Seed Dry Extract
NA	Rosemary Leaf Aqueous Dry Extract
<i>Powdered Valerian Extract</i>	Valerian Rhizome, Root and Stolon Dry Extract
Yeast Extract	Yeast Dry Extract

^a Source material refers to the unprocessed botanical material or product used to prepare an extract or other processed botanical materials. Crude herb, or raw material would be synonymous terms the naming of which is exemplified in *Tables 1* and *2*.

^b If fresh plant material is used to prepare the extract, the word "Fresh" is included after the SCN or Latin binomial and before the plant part. Otherwise, dry material is assumed.

^c "TYPE" is an additional term that further identifies the article. The solvent is specified when two articles need to be differentiated based on their chemical profile due to the solvent used. For example, the terms aqueous or hydroalcoholic specify the type of extraction solvent used, which will create a unique article.

Table 5. Current and Proposed Nomenclature Formats for Botanical Soft Extracts

Current Examples	Proposed Examples
[SOURCE MATERIAL NAME] [{EXTRACT}]	[SOURCE MATERIAL NAME {FRESH}] [{TYPE} {OLEORESIN} OR {TYPE} {SOFT EXTRACT}]
<i>Capsicum Oleoresin</i>	<i>Capsicum</i> Species Fruit Oleoresin
NA	Turmeric Rhizome Ethanol Oleoresin
NA	Ginger Rhizome CO ₂ Soft Extract
NA	Lemon Balm Leaf Soft Extract
NA	Valerian Fresh Rhizome, Root and Stolon Soft Extract

The term "botanical liquid articles" is intended to capture a variety of types of extracts, including not only fluid extracts and tinctures, which are described in (565), but also articles such as essential oils, essential oil spirits, and essential oil waters. Examples are provided in *Table 6*.

Table 6. Current and Proposed Nomenclature Formats for Botanical Liquid Articles

Current Examples	Proposed Examples
[SOURCE MATERIAL NAME] [EXTRACT]	[SOURCE MATERIAL NAME {FRESH}] [LIQUID ARTICLE]
<i>Belladonna Tincture</i>	Belladonna Leaf Tincture
<i>Black Cohosh Fluidextract</i>	Black Cohosh Root and Rhizome Fluidextract
<i>Aromatic Cascara Fluidextract</i>	Cascara Sagrada Bark Aromatic Fluidextract ^a
<i>Garlic Fluidextract</i>	Garlic Bulb Fluidextract
<i>Ginger Tincture</i>	Ginger Rhizome Tincture
<i>Licorice Fluidextract</i>	Licorice Root, Rhizome and Stolon Fluidextract
NA	Oat Fresh Seed Tincture
<i>Peppermint Oil</i>	Peppermint Leaf Essential Oil
<i>Peppermint Spirit</i>	Peppermint Leaf Essential Oil Spirit
<i>Peppermint Water</i>	Peppermint Leaf Essential Oil Water
<i>Rhodiola rosea</i> Tincture	<i>Rhodiola rosea</i> Root and Rhizome Tincture
<i>Valerian Tincture</i>	Valerian Rhizome, Root and Stolon Tincture
^a An aromatic botanical product article is created by the addition of essential oils as flavoring agents, so the DS monograph title follows the proposed format with the addition of the adjective "Aromatic".	

Juices are distinguished from other botanical liquid articles because while they are liquid to start, they may be subsequently concentrated or dried to make the article of commerce. In most cases the ability to extract the juice depends upon the plant material being fresh, so {FRESH} can be assumed unless otherwise specified. Examples are provided in *Table 7*.

Table 7. Current and Proposed Nomenclature Formats for Plant Juices

Current Examples	Proposed Examples
[SOURCE MATERIAL NAME] [EXTRACT]	[SOURCE MATERIAL NAME] [JUICE] OR [DRY JUICE]
<i>Cranberry Liquid Preparation</i>	Cranberry Fruit Juice
NA	<i>Echinacea purpurea</i> Aerial Parts Dry Juice
NA	European Elder Fruit Dry Juice

Chapter (565) states that certain botanical extracts may be referred to as "native extracts", which are extracts with no added inert substances and not processed beyond initial extraction. The only DS monographs with the word "native" in the title are *Native Guggul Extract* and *Native Gymnema Extract*, both of which have a contrasting monograph, *Purified Guggul Extract* and *Purified Gymnema Extract*. Other monographs indicate in the *Definition* rather than the title if suitable added substances such as carriers may be added (e.g., *Powdered Holy Basil Leaf Extract* or *Powdered Red Clover Extract*) or if the extract has no added substances (e.g., *Cranberry Liquid Preparation* or *Saw Palmetto Extract*). Another possibility is that the *Definition* may say nothing about the presence or absence of added substances (e.g., *Maritime Pine Extract*, or *Powdered St. John's Wort Extract*).

Some extracts are subject to additional processes that increase the content of characterized constituents, decrease the content of unwanted constituents, or both. The percentage of

characterized or unwanted constituents in a processed extract may vary and will be specified in the *Definition* of the article. For example, the *Powdered Garcinia Hydroxycitrate Extract* monograph specifies NLT 40% (–)-hydroxycitric acid. *Powdered Decaffeinated Green Tea Extract* serves as an example of a monograph for an article with a reduction of the level of a constituent, by its caffeine specification of NMT 0.1%. Another potential example would be “Deglycyrrhizinated Licorice Root Extract” which is processed to remove glycyrrhizin (glycyrrhizic acid or glycyrrhizinic acid).

In other cases, the specification may be for a class of compounds rather than a single characterized constituent, e.g., NLT 90.0% *Centella asiatica* triterpene derivatives in the monograph *Centella asiatica Triterpenes*; NLT 75.0% oligomeric proanthocyanidins in *Grape Seeds Oligomeric Proanthocyanidins*; NLT 90.0% and NMT 110.0% of the labeled amount of the sum of guggulsterones *E* and *Z* calculated as guggulsterone *Z* in *Purified Guggul Extract*; and NLT 90.0% and NMT 110.0% of the labeled amount of the sum of a specific list of isoflavones in *Powdered Soy Isoflavones Extract*.

As a very complex example, the *Saw Palmetto Extract* monograph allows for three types of extraction solvent: hydroalcoholic mixtures to produce a hydrophilic extract; hexane to produce a lipophilic extract; and supercritical carbon dioxide to produce extracts that are also lipophilic, although their composition can be altered by variations in temperature, pressure, time, and other factors. The hydroalcoholic extract contains NLT 0.01% and NMT 0.15% of long-chain alcohols, whereas the lipophilic extract contains NLT 0.15% and NMT 0.35% of long-chain alcohols; all extracts are required to contain NLT 80.0% of fatty acids, NLT 0.2% of sterols, and NLT 0.1% of β -sitosterol, all on the anhydrous basis.

Titles of monographs for extracts that have been processed for specified content ranges of particular constituents are made more precise by identifying the class of compounds whose content in the extract has been increased or decreased, as demonstrated in the examples provided in *Table 8* with comparisons to current monograph titles.

Table 8. Current and Proposed Nomenclature Formats for Additionally Processed Botanical Extracts

Current Examples	Proposed Examples
<p style="text-align: center;">[{PROCESS}] [{TYPE}] [{SOURCE MATERIAL NAME}] [{CONSTITUENT OR CLASS OF COMPOUNDS}] [{EXTRACT}]</p>	<p style="text-align: center;">[{SOURCE MATERIAL NAME}] [{CONSTITUENT OR CLASS OF COMPOUNDS}] [{TYPE}] [{DRY EXTRACT}] OR [{SOFT EXTRACT}] OR [{LIQUID ARTICLE}] OR [{BOTANICAL PRODUCT}]</p>
<i>Powdered Garcinia Hydroxycitrate Extract</i>	Garcinia Pericarp Hydroxycitrate Dry Extract
<i>Centella asiatica Triterpenes</i>	Gotu Kola Aerial Parts Triterpenes Dry Extract
<i>Grape Seed Oligomeric Proanthocyanidins</i>	Grape Seed Oligomeric Proanthocyanidins Dry Extract
<i>Powdered Decaffeinated Green Tea Extract</i>	Green Tea Leaf Decaffeinated Dry Extract
<i>Purified Guggul Extract</i>	Guggul Guggulsterones Dry Extract
<i>Purified Gymnema Extract</i>	Gymnema Leaf Gymnemic Acids Dry Extract
NA	Licorice Root Deglycyrrhizinated Soft Extract
<i>Psyllium Hemicellulose</i>	Psyllium Seed Husk Hemicellulose Dry Extract
<i>Saw Palmetto Extract</i>	Saw Palmetto Fruit Hydroalcoholic ^a Dry Extract
NA	Saw Palmetto Fruit Lipophilic ^a Soft Extract
NA	Saw Palmetto Fruit CO ₂ ^a Soft Extract
<i>Powdered Soy Isoflavones Extract</i>	Soy Seed Isoflavones Dry Extract
<i>Tomato Extract Containing Lycopene</i>	Tomato Fruit Lycopene Dry Extract
	<i>Echinacea angustifolia</i> Root Alkylamides Fluidextract
	Evening Primrose Seed Gamma-Linolenic Acid Oil
<p>^a For some articles the targets of additional processing include several constituents or classes of compounds, e.g., Saw Palmetto Fruit fatty acids, sterols, and long-chain alcohols, with different specifications for different types of extracts, e.g., with respect to the long-chain alcohols content. In such cases, the type of extract may be used in the DS monograph title for brevity and the details of the associated targeted constituents or classes of compounds provided in the <i>Definition</i>.</p>	

Further processing of plant extracts can lead to the production of “partially purified natural complexes”, as opposed to the processed/semi-purified extracts just described. It would be arbitrary to set any numerical concentration threshold to distinguish between a plant extract processed with regard to particular constituents and a partially purified natural complex—they are all complex articles. However, the *Definition* section of currently monographed articles explicitly makes the distinction that partially purified natural complexes are comprised mainly of particular characterized constituents and their closely related congeners, whereas the processed extracts are characterized as fractions of an extract enriched or depleted in a particular substance or group of related substances. In practice, the degree of purification of natural complexes may overlap with that of processed extracts, but the intent of a monograph for a partially purified natural complex is to provide quality specifications for a complex article that is more akin to a single chemical entity than an unprocessed botanical extract.

The format for monograph titles for partially purified natural complexes is simply **[CLASS OF COMPOUNDS]**. For example, *Sennosides* is defined as a partially purified natural complex of

anthraquinone glucosides isolated from senna leaf and/or senna pod, with NLT 90.0% and NMT 110.0% of the labeled amount of sennosides, and the labeled amount should be NLT 60.0% (w/w) of the article. *Curcuminoids* is defined as a partially purified natural complex of diaryl heptanoid derivatives isolated from turmeric, with NLT 95.0% of curcuminoids, calculated on the dried basis as a sum of curcumin (70.0%–80.0%), desmethoxycurcumin (15.0%–25.0%), and bisdesmethoxycurcumin (2.5%–6.5%).

Titles for Monographs of Complex Articles of Animal Origin

Monograph titles for dietary ingredients of animal origin should follow the directives in 21CFR101.4 *Food; designation of ingredients*, which is consistent with DSHEA with respect to the requirement to use common or usual English names where available. Taxonomic details may be provided in the article's *Definition*, e.g., the families of fish that may be used to produce fish oil are provided in the *Definition* because it is not feasible to identify each individual species in the monograph title or *Definition*. The general nomenclature convention for DS monograph titles is **[ANIMAL NAME] {ANIMAL ORGAN(S)} [ANIMAL PRODUCT] {MAJOR CONSTITUENT}**. Examples include *Cod Liver Oil*, *Krill Oil*, "Oyster Shell", and "Shark Cartilage".

As with partially purified natural complexes from plants, some complex articles of animal origin may be comprised mainly of particular characterized constituents and their closely related congeners, so the format of the monograph titles can be similar to those for processed botanical articles (see *Table 9*). An added benefit of including the source material is that greater precision is provided, e.g., fish is not the only commercial source of omega-3 fatty acid triglycerides from which ethyl esters can be made. α -Linolenic acid can be sourced from the oils of flaxseeds, walnuts, or soybeans, and there are DS monographs for docosahexaenoic acid (DHA) from algal oil sources (*Crypthecodinium cohnii* and *Schizochytrium* spp.).

Table 9. Current and Proposed Nomenclature Formats for Processed Animal Products

Current Examples	Proposed Examples
{PROCESS} {TYPE} {SOURCE MATERIAL NAME} [CLASS OF COMPOUNDS]	[SOURCE MATERIAL NAME] [CLASS OF COMPOUNDS]
<i>Fish Oil Containing Omega-3 Acids</i>	Fish Oil Omega-3 Acids
<i>Omega-3 Acids Triglycerides</i>	Fish Oil Omega-3-Acid Triglycerides

The format for monograph titles for partially purified natural complexes from animal source materials, as with that type of botanical monograph title, is simply **[CLASS OF COMPOUNDS]**, e.g., *Pancreatin*, where the source material name (hog or ox) is provided in the *Definition* and can be specified in labeling, e.g., to allow consumers to make informed choices with respect to kosher or halal products.

Titles for Monographs of Other Complex Dietary Substances

In contrast to the examples cited above of cyanobacteria, which resemble algae in their growth form and thus fit within the DSHEA dietary ingredient definition part (C) "a herb or other botanical", monographs for other bacterial articles such as probiotic species better fit the DSHEA definition part (E), "a dietary substance ...". Titles for these monographs should follow the format **[LATIN BINOMIAL W/O AUTHORITY] [STRAIN IDENTIFIER]** e.g., *Bacillus coagulans* GBI-30, 6086; *Lactobacillus rhamnosus* GG; or *Lactobacillus johnsonii* NCC 533.

Titles for Single Chemical Entity Monographs

The nomenclature for single chemical entities (e.g., vitamins, mineral nutrients, amino acids, enzymes, and isolated or synthetic substances) is the same as for drug substances, as outlined in §1121. Some examples include *Alanine*, *Ascorbic Acid*, *N-Acetylglucosamine*, *Chromium Picolinate*, *Cyanocobalamin*, *Ergocalciferol*, *Glutathione*, *Lactase*, *Lycopene*, *Magnesium Sulfate*, *Melatonin*, *Quercetin*, *Rutin*, *S-Adenosyl-L-methionine Disulfate Tosylate*, and *Vinpocetine*.

In some cases, "single" chemical entities may in fact be comprised of isomers or derivatives. For example, the *Phytonadione* monograph contains a purity specification of NLT 97.0% and NMT 103.0%, but it is a mixture of the *E*- and *Z*-isomers, of which it contains NMT 21.0% of the *Z*-isomer. To be compliant with the "Vitamin A" monograph, the article must possess NLT 95.0% of the vitamin A activity declared on the label but it may consist of retinol or esters of retinol formed from edible fatty acids, principally acetic and palmitic acids. The "Vitamin E" article consists of alpha-tocopherol and its alpha-tocopheryl acetate or alpha-tocopheryl acid succinate derivatives, and it may be the *RRR*- (previously referred to as *d*-) isomer or the all-racemic (*d,l*-) form. Other tocopherols and tocotrienols are not included in the "Vitamin E" *Definition*—a potential separate monograph could cover mixed tocopherols and tocotrienols.

The Term "Preparation" in Monograph Titles

The term "Preparation" is used in a number of current DS monograph titles. The original intent was to indicate articles that may be intermediates used in formulating finished dosage forms. In comparison, the *European Pharmacopoeia* distinguishes an "Herbal Drug Preparation" as an article obtained by subjecting the herbal drug to processes such as extraction (e.g., liquid extract/tincture/dry extract/soft extract); the class of extract may be further indicated as standardized or quantified.

With regard to current DS monographs, *Cranberry Liquid Preparation* has cranberry juice and no added substances. *Vitamin E Preparation* combines a single form of vitamin E with one or more inert substances. *Dexpanthenol Preparation* contains dexpanthenol and pantolactone, both of which have Reference Standards (RS). *Lutein Preparation* combines lutein (95.0%–130.0% of the labeled amount of lutein, with NLT 85.0% lutein, NMT 9.0% zeaxanthin) with one or more inert substances. *Lycopene Preparation* combines lycopene with one or more inert substances and suitable antioxidants. *Vitamin A Oral Liquid Preparation* consists of either retinyl acetate or retinyl palmitate in an emulsion, suspension, or solution.

The term "Preparation" will not be used in future DS monograph titles. As discussed above, many complex botanical articles may be used either as raw materials to be processed further in formulating DS finished products, or they may be used directly as dietary ingredients. Many current monographs that do not contain the word "Preparation" in the title include provisions in the *Definition* section that allow for the addition of "suitable added substances" (excipients, e.g., *Powdered Andrographis Extract*). Some allow for the addition of "suitable antioxidants" (e.g., *Schizochytrium Oil*), and many allow for multiple ingredients that each have an RS (e.g., *Vitamin E*).

TITLES FOR DIETARY SUPPLEMENT MONOGRAPHS

Dietary supplements are finished oral dosage forms manufactured to include dietary ingredients. Most commonly, DSs are available as tablets, capsules, liquid extracts (e.g., fluidextracts and tinctures), syrups, teas for infusion, and powders to be reconstituted for ingestion or sprinkled on food. DS dosage form nomenclature typically follows the same rules as those for drug

products (see general chapter *Pharmaceutical Dosage Forms* (1151)).

Some examples are provided below (note that some examples are hypothetical and are provided only to illustrate how titles should be derived). For the sake of clarity, the format terms:

[{SCN} OR {LATIN BINOMIAL W/O AUTHORITY}] [{FRESH}{BOTANICAL PART(S)} AND/OR {BOTANICAL PRODUCT}] [{TYPE} {DRY EXTRACT} OR {OLEORESIN} OR {SOFT EXTRACT} OR {LIQUID ARTICLE} OR {JUICE} OR {DRY JUICE} AND/OR {CONSTITUENT OR CLASS OF COMPOUNDS}] used above to describe botanical materials and products will be simplified to **[DIETARY INGREDIENT NAME]** which incorporates all of the above terms. The general form is as follows: **[DIETARY INGREDIENT NAME] {RELEASE CHARACTERISTIC} [DOSAGE FORM]**

Tablets: Black Cohosh Rhizome and Root Fluidextract Tablets, Cat's Claw Stem Bark Dry Extract Tablets, *Chondroitin Sulfate Sodium Tablets*, *Glucosamine Tablets*, Gymnema Leaf Dry Extract Tablets, *Methylsulfonylmethane Tablets*.

Capsules: Asian Ginseng Root Powder Capsules, *Fish Oil Omega-3 Acids Capsules*, *Cod Liver Oil Capsules*, Milk Thistle Fruit Dry Extract Capsules.

Lozenges: *Zinc and Vitamin C Lozenges*.

Oral solutions: *Ascorbic Acid Oral Solution*, *Cholecalciferol Solution*, *Oil-Soluble Vitamins with Minerals Oral Solution*, *Water-Soluble Vitamins with Minerals Oral Solution*, *Zinc Acetate Oral Solution*.

Oral suspensions: *Calcium Carbonate Oral Suspension*.

Powders for oral suspension: *Psyllium Hydrophilic Mucilloid for Oral Suspension*.

DRUG VERSUS DIETARY SUPPLEMENT NAMES FOR ARTICLES

In the United States, drugs and DSs conform to different standards and require different testing procedures for identity, purity, strength, and composition. Occasionally, the same substance is used in a drug and in a DS. When used in a drug, the substance is given a US Adopted Name (USAN) or an International Nonproprietary Name (INN), but when the same substance is used in a DS, it may be referred to by another scientific, traditional, or *Herbs of Commerce* name (2). Because the articles (drug vs. DS) may have to meet different standards, the use of different names may be important. *Table 10* provides some examples of such multiple names.

Table 10. USAN Names vs. DS Names

USAN Name	DS Name
Ademetionine	S-Adenosylmethionine or SAME
Ubidecarenone	Coenzyme Q ₁₀ , Co-Q ₁₀
Sinecatechins	Green Tea Catechin Extract

GLOSSARY

This glossary does not include terms for plant (or fungal, algal, bacterial, or animal) materials that are defined in standard textbooks. It focuses on terms specific to DS products and ingredients whose definitions are not so readily available elsewhere or that have been defined differently in various sources. Readers are also encouraged to consult (563) and (565) for additional information on terminology that applies to DSs.

Aqueous extract: Articles prepared by extracting materials with water.

Concentrate: Historically, "concentrate" had two meanings. One was simply reflecting a liquid or solid preparation of higher concentration sometimes referred to as "high potency". The other meaning was that the product must be diluted before administration. Not all "high potency" products had to be diluted, so the word "concentrate" lost its definitive meaning and created confusion. The nomenclature committee has recommended that the term "concentrate" be phased out of nomenclature. Instead, the appropriate dosage form terms, e.g., fluidextract or tincture, with the extraction or concentration ratio in the *Definition* and on the label, can be used to indicate potency. If applicable, the statement "must be diluted" should be displayed prominently on the label.

Dry extract: Solid preparations obtained by evaporation of the solvent used in their production.

Dry juice: Dry material obtained by, for example, freeze drying or spray drying juice, often onto a carrier.

Essential oil: Natural aromatic complex mixtures of compounds (there may be 200 or more in one essential oil) belonging mainly to two chemical classes: terpenoids (e.g., monoterpenoid ketones, alcohols, hydrocarbons, and esters such as carvone, menthol, α -pinene, and thymol acetate; sesquiterpenoids such as α -bisabolol and caryophyllene; and less commonly, diterpenoids such as phyllocladene and (+)-kaurene) and phenylpropanoids (e.g., anethole, cinnamaldehyde, coniferyl alcohol). However, there may also be some phenols such as methyl salicylate (oil of wintergreen) or vanillin, sulfur-containing compounds such as allyl isothiocyanate in mustard oil, or aldehydes such as benzaldehyde in bitter almond essential oil. They are liquid at room temperature and generally immiscible in water but are soluble in alcohol or other organic solvents, so they act like oils. They are called "essential" because they represent the "essence" of the plant in terms of fragrance. Since they evaporate when exposed to the air at room temperature, they are also called volatile oils or ethereal oils. They may be present in the leaf, seed, bark, stem, root, flower, and other plant parts, and may be obtained by steam distillation, extraction using various solvents, or other techniques.

Extract: Preparations with liquid, solid, or semisolid consistency obtained from plant material using solvents (such as ethanol, methanol, and others) to separate constituents of interest from the plant material. Types of extracts are namely: *Dry extract*, *Soft extract*, and *Liquid extract*; each is defined in this *Glossary*. Excipients may be included except for native extracts, which contain no constituents that were not native to the plant from which the extract was made.

Fluid extract: A type of *Liquid extract* preparation of plant matter, containing ethanol as a solvent or as a preservative, or both, so made that each 1 mL contains the extracted constituents of 1 g of the crude dry material that it represents, unless otherwise specified (e.g., 1:2) in the individual monograph.

Fraction: Processed extracts that consist of a specific class of compounds. For example sennosides from Senna, oligomeric proanthocyanidins from Grape Seed, and triterpenes from *Centella asiatica*.

Gum: A water-soluble carbohydrate derivative in the form of a hydrocolloid comprised of an anionic or nonionic polysaccharide or salts of polysaccharides, e.g., tragacanth, arabic (also known as acacia), ghatti, guar, karaya, locust bean, or xanthan.

Latin binomial: A system of nomenclature of animals, plants, and other life forms (developed by Linnaeus) that assigns a two-part Latinized name, the generic and specific epithets, to each species, such as *Harpagophytum procumbens* or *Harpagophytum zeyheri* for the two species of Devil's Claw included in the "Harpagophytum Species Root" monograph.

Latin binomial authority: The author of the Latin binomial, i.e. the individual(s) who first

named, or later revised the name of the plant and validly published that binomial. The author information immediately follows the specific epithet, e.g., *Harpagophytum procumbens* (Burch.) DC. ex Meisn. or *Harpagophytum zeyheri* Decne.

Liquid extract: Liquid preparations of plant matter containing ethanol, water, vinegar, vegetable oil, or glycerin (or a mixture, e.g., aqueous ethanol) as a solvent. The term liquid indicates a material that is pourable and conforms to its container at room temperature.

Oleo-gum-resin: A mixture of an oleoresin and a gum, e.g., myrrh.

Plant processed forms: Plant material that has been subjected to processing, e.g., grinding to powder. Examples of processed plant forms include juices, powders, extracts, and fractions, but not isolated pure compounds.

Plant product: Substance produced naturally by a plant or plant part that does not require processing beyond pressing or cutting and scraping to be obtained. Examples include seed oil, gum, latex, resin, and others.

Resin: An amorphous complex mixture of resin acids, resin alcohols, resinotannols, esters and resins, usually hard and transparent or translucent at room temperature, and insoluble in water, e.g., rosin, guaiac, and mastic.

Soft extract: Soft extracts are preparations having consistencies between those of liquid extracts and those of dry extracts, and are obtained by partial evaporation of the solvent (e.g., water, alcohol, or hydroalcoholic mixture) used for extraction.

Tincture: Tinctures are liquid preparations usually prepared by extracting plant materials with alcohol or hydroalcoholic mixtures. Traditionally, tinctures of potent articles of botanical origin represent the activity of 1 g of the drug in each 10 mL of tincture, the strength being adjusted following the test for content of active principles or marker compounds.

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APPENDIX

USP DS and Herbal Medicines Nomenclature Joint Subcommittee (DSHM Nomenclature SC), of the 2010–2015 Monographs—Dietary Supplements and Herbal Medicines Expert Committee (DSHM EC) & Nomenclature, Safety, and Labeling Expert Committee (NSL EC) members were as follows: Robin J. Marles, Ph.D.; Steven J. Dentali, Ph.D. (Subcommittee Chair); Josef A. Brinckmann (Subcommittee Vice-Chair); Richard Ko, Pharm.D.; Joy A. Joseph, M.S., Ph.D.; Dennis K.J. Gorecki, B.S.P., Ph.D.; Paul L. Schiff, Jr., Ph.D.; Gregory A. Pennyroyal; and Kailas Thakker, Ph.D.

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^a See *Appendix* for a list of Expert Committee members and USP staff.

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¹ See § 201(ff) of DSHEA, 108 Stat. 4325, Public Law 103-471, 103d Congress (1994) for additional details of the definition of a dietary supplement. These provisions are now codified in the Federal Food, Drug, and Cosmetic Act at 21 United States Code § 321(ff).

² The Latin term "Species" with an uppercase S has a different meaning in monograph titles of some other currently valid national pharmacopeias, e.g., the pharmacopeias of Austria (*ÖAB*), Switzerland (*PhHelv*), and Hungary (*PhHg*) as well as *Formulae Normales (FoNo)*, wherein the term Species is used as a synonym for the German term *Teegemische*, meaning herbal teas composed of multiple species.

³ The views presented in this article do not necessarily reflect those of the FDA. No official support or endorsement by the Food and Drug Administration is intended or should be inferred.